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Versatile method to measure locomotion in adult *Drosophila*[1]

Taylor Barwell, Sehaj Raina, and Laurent Seroude

Abstract: Many studies require the ability to quantify locomotor behavior over time. The list of tracking softwares and their capabilities are constantly growing. At the 2019 CanFly Conference, we presented preliminary results from an investigation of the effects of expressing polyglutamine repeats in fly muscles on longevity, locomotion, and protein aggregation. Numerous requests have been received regarding our protocol to measure locomotion and how to use the FlyTracker MatLab software. This report describes a versatile locomotion measuring device and custom MatLab scripts for the extraction, analysis, and compilation of FlyTracker data in a format compatible with spreadsheet softwares. The measurement and analysis of multiple genotypes of both sexes across age demonstrates that this method yields reproducible results that confirm that normal aging is associated with a progressive decline in locomotion as indicated by increased immobility and reduced velocity.

Key words: behavior, FlyTracker, MatLab, aging, *Drosophila melanogaster*.

Résumé : Plusieurs études nécessitent la capacité de quantifier le comportement locomoteur dans le temps. La liste des logiciels de suivi des mouvements et leurs capacités sont en constante évolution. Lors de la conférence CanFly 2019, les auteurs ont présenté les résultats préliminaires d'une étude sur les effets de l'expression de répétitions polyglutaminiques dans les muscles des mouches sur leur longévité, leur locomotion et l'agrégation des protéines. De nombreuses demandes ont été reçues au sujet du protocole employé pour mesurer la locomotion et sur l'emploi du logiciel MatLab FlyTracker. Cet article décrit un appareil polyvalent de mesure de la locomotion ainsi que des scripts MatLab écrits sur mesure et conçus pour extraire, analyser et compiler les données de FlyTracker en une forme qui soit compatible avec des tableurs. La mesure et l'analyse de multiples génotypes des deux sexes au cours de leur vie démontre que cette méthode produit des résultats reproductibles et confirme que le vieillissement normal est associé à un déclin progressif de la locomotion, tel qu'indiqué par une immobilité accrue et une vitesse réduite. [Traduit par la Rédaction]

Mots-clés : comportement, FlyTracker, MatLab, vieillissement, *Drosophila melanogaster*.

Introduction

Locomotion is a fundamental behavioral trait involved directly or indirectly with almost all simple or complex behavior activities. It is also integral to studying a wide span of different biological phenomenon such as neurological or muscular pathologies, age associated changes, and physiological responses. Measuring locomotion is challenging because of the many parameters that need to be controlled (Martin 2003). Additionally, the obtainment of large data sets requires an automated system for tracking, data extraction, and analysis.

Drosophila have proven to be a useful model to study some of the most complex behavioral phenotypes including learning, memory (Pitman et al. 2009; Tully 1996), sleep, circadian rhythms (Dubowy and Sehgal 2017), courting, mating (Greenspan and Ferveur 2000), and response to drugs, addiction (Kaun et al. 2012). With advances in technology numerous experimental setups, manual and automated tracking have been developed to describe and quantify fly locomotion (Martin 2003).

Automated tracking relies on softwares that use a digital subtraction method to remove background to distinguish the fly from optical or background artifacts (Ardekani et al. 2013; Dankert et al. 2009; Donelson et al. 2012; Noldus et al. 2002; Ramazani et al. 2007; Valente et al. 2007). Softwares can be used to track several flies at once in two or three dimensions, quantify specific fly behaviors (grooming, fighting, mating), as well as monitor leg coordination of single animals (Ardekani et al. 2013; Branson et al. 2009; Chan et al. 2012; Duistermars et al. 2018; Mendes et al. 2013; Schretter et al. 2018; Straw and Dickinson 2009; Valente et al. 2007; Wosnitza et al. 2013).

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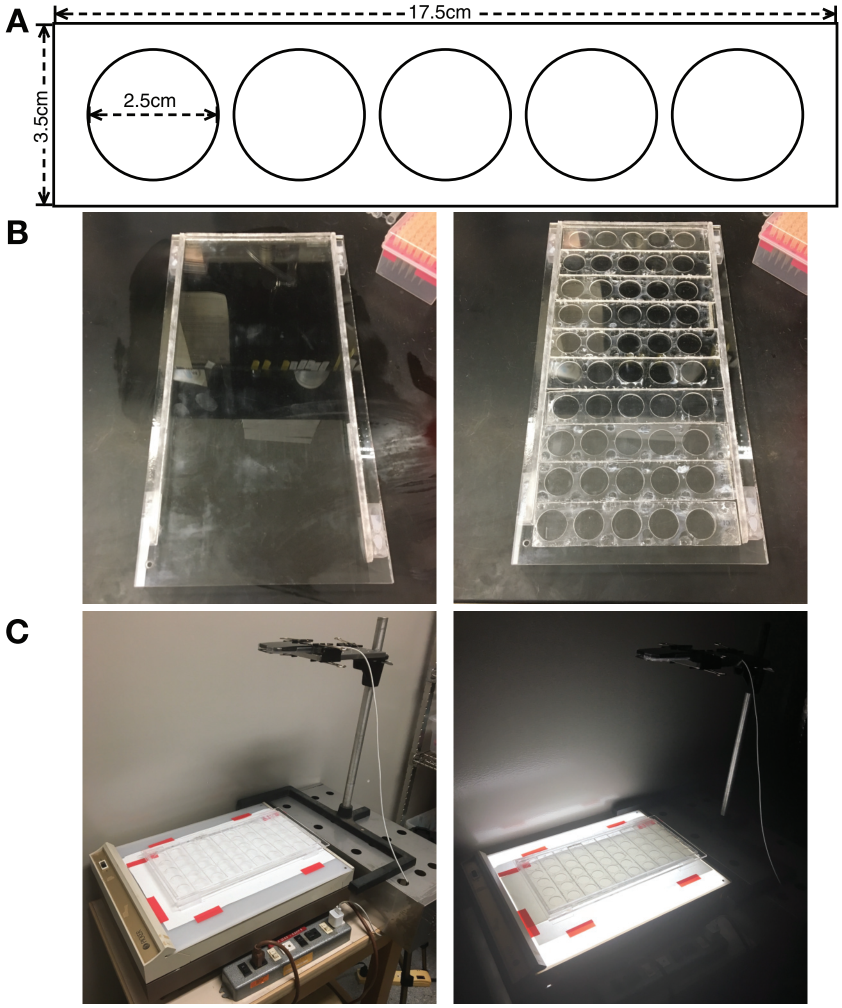
Fig. 1. (A) Dimensions of a 5 chamber strip used in the arena. (B) The base with (right) and without strips (left). (C) Experimental set up. The base with the loaded chambers is positioned atop a white piece of paper taped to a light box to diffuse the light. Recordings were completed with the lights in the room off and the light box on to maximize contrast.



We chose the widely used, multi-platform FlyTracker MatLab software (Duistermars et al. 2018; Eyjolfsdottir et al. 2014; Schretter et al. 2018) that does not require any expensive equipment. Essentially anyone with a camera and a computer can run the software and perform these experiments. FlyTracker detects very accurately multiple flies at once in a video and is able to track the position, orientation, and angle of the wings and legs, as well as distance between the fly and the wall of the chamber housing the fly. At the 2019 CanFly Conference, we presented preliminary results from an investigation of the effects of expressing polyglutamine repeats in fly muscles on longevity, locomotion, and protein aggregation. We received a lot of inquiries about our protocol to measure locomotion and how to use FlyTracker. Here, we report a locomotion measuring device that can accommodate 5–50 flies and custom MatLab scripts for the extraction, analysis, and compilation of FlyTracker data in a format compatible with spreadsheet softwares. Multiple genotypes of both sexes and two experimental replicates across age demonstrate the reproducibility achieved.

Materials and methods

Construction of the arena

A drill press was used to drill 2.5 cm circles into thin (3 mm) clear acrylic sheets in rows of 5. The rows were cut into strips (Fig. 1A). A clear acrylic sheet the same dimensions as the strip was adhered to the back of the strip with liquid cyanoacrylate adhesive. By constructing chambers in strips the arena can be adapted to accommodate experiments requiring 5 chambers and up to 50. A base was made from another acrylic sheet, large enough to fit all 10 strips (Fig. 1B). A small hole was drilled near the edge of the base to load the flies. Three small pieces of acrylic sheet were cut and adhered to the base to use as a guide for positioning the strips consistently. Legs were made for the base out of cuvettes.

Fly husbandry

Crosses were set up in bottles to obtain desired genotypes. Four different wild-type genotypes were obtained by crossing male w^{1118} with UAS-Httex1-Q72-eGFP and UAS-Httex1-Q25-eGFP (Zhang et al. 2010) and female w^{1118}

with DJ694 (Seroude et al. 2002) and MHC-Geneswitch (Osterwalder et al. 2001). After allowing the parents to mate for 2 days, they were then transferred to a second set of bottles to generate a replicate. The second set of bottles was emptied after 2 days. Staged flies (0–2 days) were collected under nitrogen anesthesia. A minimum of four sample vials for each sex of each genotype containing a minimum of 25 flies were collected. This was repeated for the replicate crosses. Flies were allowed to recover for at least 1 day before being recorded. Flies were maintained on standard fly food (0.01% molasses, 8.2% cornmeal, 3.4% yeast, 0.94% agar, 0.18% benzoic acid, 0.66% propionic acid) at 23–26 °C for the duration of the experiment.

Experimental setup

A chamber strip was positioned such that the first chamber was circle-side down over the top of the loading hole of the base. Flies were aspirated from the vial. Once one fly was loaded into the chamber through the loading hole the arena strip was slid such that the second chamber was over top of the loading hole, effectively preventing the fly from escaping. Once all five flies in a strip were loaded, the strip was slid into place using the guides on the base.

The process was repeated until all the chambers were loaded. The base was then placed atop of a light box; the legs provide an air insulation layer that prevents heating from the light bulbs. A piece of white paper was placed on the light box to diffuse the light. An iPod touch® was clamped in a fixed position 40 cm above the light box to capture a single video file encompassing all 50 chambers. Video capture was performed at 23–26 °C with the light table on and the lights in the room off (Fig. 1C).

Flies were acclimatized for 1 min before beginning recording (3 min at 30 frames per second). After recording, flies were aspirated from the arenas out through the hole and returned to their vials. In a given video each genotype was measured in triplicate. Flies were aspirated from one of the four sample vials that were collected, alternating the vial at each recorded time point. The positions of the genotypes in the arena were rearranged each day a recording was done to eliminate bias coming from any specific chamber. Since our genotypes were measured in triplicate, the genotypes shifted by three for each time point. Therefore, the number of possible arena configurations is equal to the number of genotypes tested. Table S3[2] shows an example of the various arena configurations for a 16-genotype experiment.

Software and file requirements

FlyTracker (Eyjolfsdottir et al. 2014) was downloaded from http://www.vision.caltech.edu/Tools/FlyTracker/download.html. The data extraction scripts (File S1[2]) were downloaded and each script (data extraction script and data compilation script) was copied and pasted into MatLab. The data extraction script was annotated (in green text, File S1[2]) and includes instructions (in orange text, File S1[2]) to replace parameters (in red text, File S1[2]) to accommodate any experimental setup. The data extraction script generated spreadsheet files (*.xls and *.csv) that, once grouped in a single folder, can be processed by the data compilation script to obtain an *.xls file combining all experimental data for further analysis, statistical processing, and graphing. A custom arena configuration table was created (Table S3[2]) with the configurations in columns and the chamber numbers in rows. This table was created using spreadsheet software and copied into MatLab.

FlyTracker tracking

FlyTracker requires that the video files are stored in a folder. We organized our video files in folders by date. The path was set in MatLab for FlyTracker. The 'tracker.m' script was run. Specifications for video length, frame rate, and processing options were selected in the FlyTracker interface. In the Calibrator interface, the resolution, number of arenas, number of flies per arena, size and position of arenas, and contrast thresholds for detecting the flies were all selected. Once tracked, all tracking results were verified in the Visualizer interface to ensure accurate tracking. For each video file analyzed, FlyTracker outputs 'feat.mat' and 'track.mat' structure files.

Data analysis

The 'feat.mat' and 'track.mat' files were dragged and dropped into the Workspace in Matlab, and the data extraction script was run to generate spreadsheet files (*.xls and *.csv). The script uses the arena configurations table to assign a genotype according to the chamber number and compiles the distance, the number of frames detecting the presence of the fly, instantaneous velocity, average velocity (calculated from times of fly movement), and percent of time spent immobile (calculated from the number of frames where the fly is immobile). The *.xls files were then moved to a single folder and the data compilation script was used to compile the files into a single *.xls file. Using Microsoft Excel, the values obtained for all three individuals for each of the parameters (distance, velocity, % time immobile) were averaged to give a single time point respectively for each parameter. This was executed for both replicates.

Statistical analysis

Statistical analyses were performed with GraphPad Prism 6 for Mac. Pearson correlation analyses were run between the two replicate cohorts for males and females and all three measured parameters to assess experimental

[2]Supplementary data are available with the article through the journal Web site at http://nrcresearchpress.com/doi/suppl/10.1139/gen-2020-0044.

Fig. 2. Locomotion of male UAS-Httex1-Q25-eGFP/+, UAS-Httex1-Q72-eGFP/Y, DJ694/+, and MHC-Geneswitch/+ flies across age (*x* axis) as measured by percentage of time spent immobile (left), distance traveled (middle), and average velocity during the time of fly movement (right). The blue and orange lines denote each of the independent replicates (each replicate $n \geq 3$). Error bars represent ± SD.

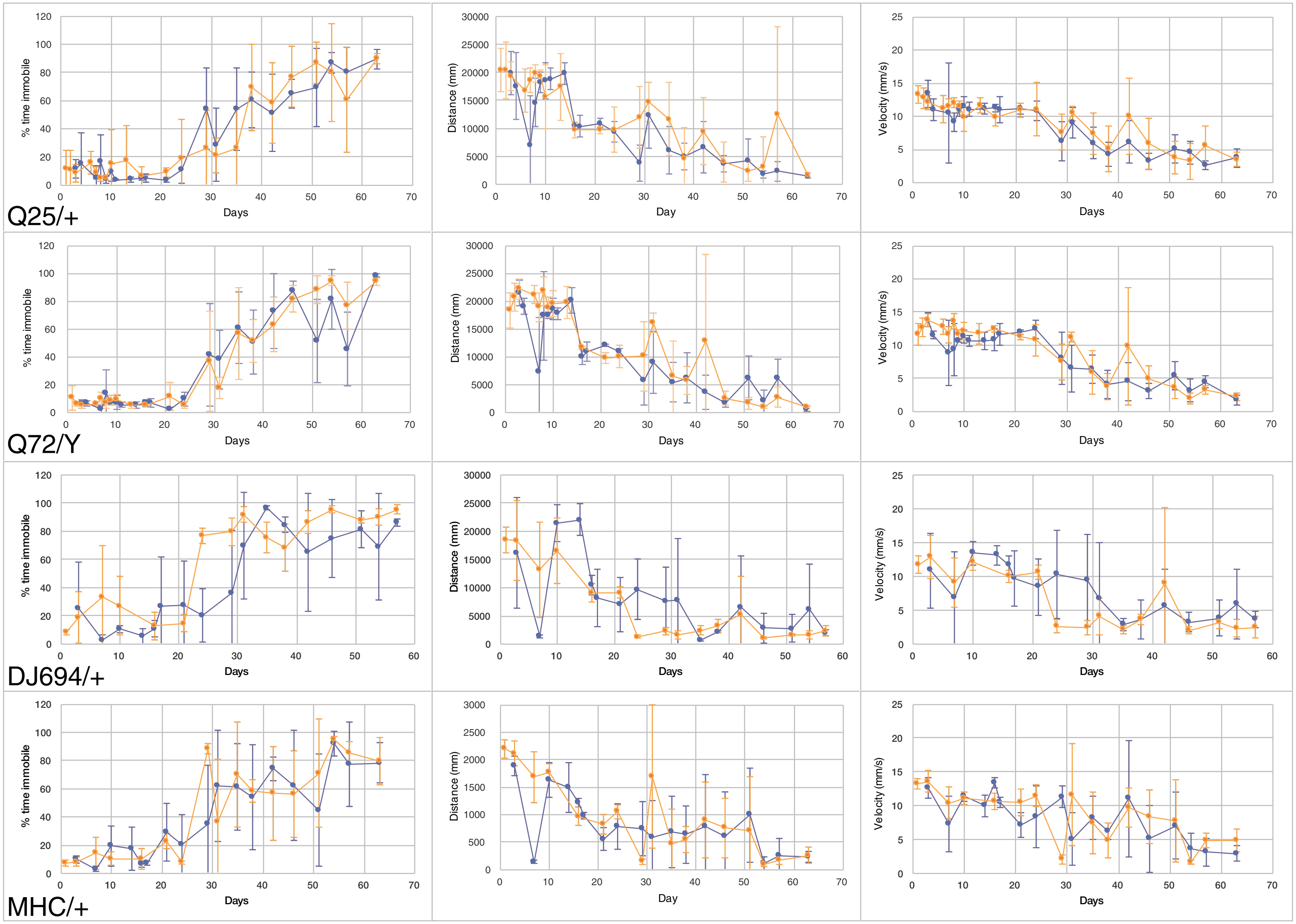


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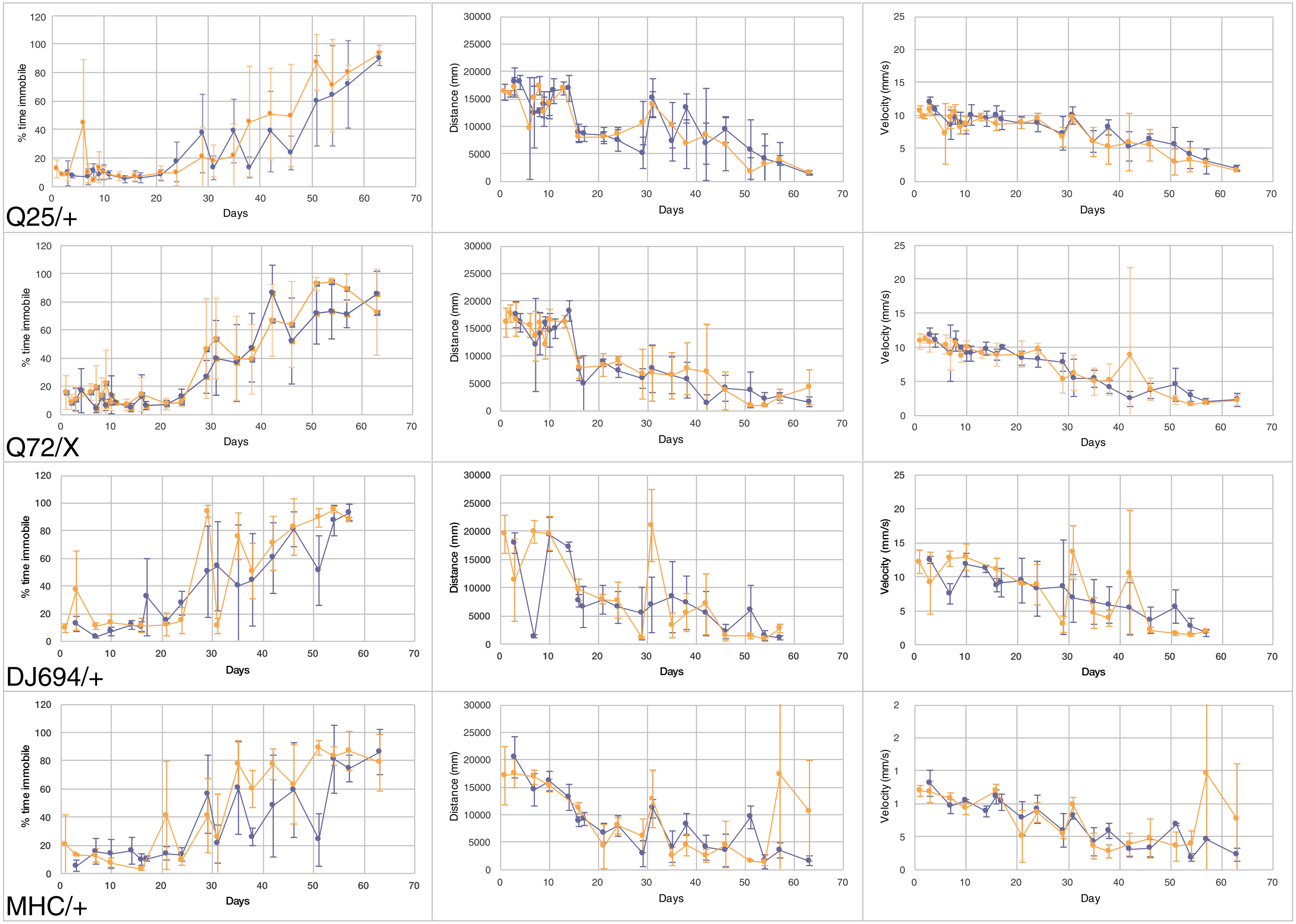
Fig. 3. Locomotion of female UAS-Httex1-Q25-eGFP/+, UAS-Httex1-Q72-eGFP/X, DJ694/+, and MHC-Geneswitch/+ flies across age (*x* axis) as measured by percentage of time spent immobile (left), distance traveled (middle), and average velocity during the time of fly movement (right). The blue and orange lines denote each of the independent replicates (each replicate $n \geq 3$). Error bars represent ±SD.



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reproducibility. One-way ANOVA was run, followed by Dunnett's multiple comparison test to assess declines in locomotion with age.

Results and discussion

Our locomotion measuring device was designed to be adaptable to experiments measuring between 1 and 50 flies (Fig. 1). The device was tested with 15 flies (3 strips) and up to 48 flies (10 strips). The resolution of an HD 1080p camera is not sufficient to be able to use more than 10 strips without losing the ability to accurately track every individual fly.

Four wild-type phenotypes with different genetic backgrounds were recorded for 3 min at multiple time points across age (3–63 days) and at the same time of the day to avoid circadian effects. The resulting video files were tracked and analyzed in MatLab using FlyTracker, and a custom script was designed to calculate the percentage of time spent immobile, instantaneous velocity, and total distance moved. The distance allows to distinguish between flies that moved sporadically versus those that moved consistently the whole duration of the recording, which may influence their velocity.

Figure 2 (Table S1[2]) shows the results of two replicates of four male genotypes: UAS-Httex1-Q25-eGFP/+, UAS-Httex1-Q72-eGFP/Y, DJ694/+, and MHC-Geneswitch/+. The percentage of time spent immobile increased with all genotypes across age in both replicates (blue and orange, Fig. 2). On average, the percentage of time spent immobile remained below 20% for all the genotypes up until day 29 and by late life (50–60 days) the percent immobility reached about 80%. Additionally, the distance traveled and velocity declined with all male genotypes across age in both replicates. On average, the distances traveled begins to decline at day 16 and remains below 10 000 mm into late life. With the velocity, all genotypes started with an average between 10 and 15 mm/s then at day 24–29 declined to below 10 mm/s.

Figure 3 (Table S2[2]) shows the results of two replicates of four female genotypes: UAS-Httex1-Q25-eGFP/+, UAS-Httex1-Q72-eGFP/X, DJ694/+ and MHC-Geneswitch/+. The percentage of time spent immobile increased with all genotypes across age in both replicates (blue and orange, Fig. 3). The female genotypes showed very similar results to that of the males: on average the percentage of time spent immobile remained below 20% and by day 50–60 it reached about 80%. Also, the distance traveled and velocity showed similar results to that of the males: at day 16 the distance declined below 10 000 mm and the velocity decreased below 10 mm/s at day 24–29.

It has been known for a long time that advancing age is correlated with behavioral declines. Declines in negative geotaxis, flight, and locomotion have previously been reported with a variety of different experimental approaches (Arking and Wells 1990; Gargano et al. 2005; Grotewiel et al. 2005; Jones and Grotewiel 2011; Leffelaar and Grigliatti 1983; Martinez et al. 2007; Miller et al. 2008). The method used in this study yields reproducible results (Figs. S1 and S2[2]) that indeed confirm in multiple genotypes and both sexes that normal aging is associated with a progressive decline in locomotion as a result of increased immobility and reduced velocity (Tables S4 and S5[2]).

References

Ardekani, R., Biyani, A., Dalton, J.E., Saltz, J.B., Arbeitman, M.N., Tower, J., et al. 2013. Three-dimensional tracking and behaviour monitoring of multiple fruit flies. J. R. Soc. Interface, **10**(78): 20120547. doi:10.1098/rsif.2012.0547. PMID:23034355.

Arking, R., and Wells, R.A. 1990. Genetic alteration of normal aging processes is responsible for extended longevity in *Drosophila*. Dev. Genet. **11**(2): 141–148. doi:10.1002/dvg.1020110204. PMID:2116251.

Branson, K., Robie, A.A., Bender, J., Perona, P., and Dickinson, M.H. 2009. High-throughput ethomics in large groups of *Drosophila*. Nat. Methods, **6**(6): 451–457. doi:10.1038/nmeth.1328. PMID:19412169.

Chan, K.L., Inan, O., Bhattacharya, S., and Marcu, O. 2012. Estimating the speed of *Drosophila* locomotion using an automated behavior detection and analysis system. Fly (Austin), **6**(3): 205–210. doi:10.4161/fly.20987. PMID:22878427.

Dankert, H., Wang, L., Hoopfer, E.D., Anderson, D.J., and Perona, P. 2009. Automated monitoring and analysis of social behavior in *Drosophila*. Nat. Methods, **6**(4): 297–303. doi:10.1038/nmeth.1310. PMID:19270697.

Donelson, N.C., Kim, E.Z., Slawson, J.B., Vecsey, C.G., Huber, R., and Griffith, L.C. 2012. High-resolution positional tracking for long-term analysis of *Drosophila* sleep and locomotion using the "tracker" program. PLoS ONE, **7**(5): e37250. doi:10.1371/journal.pone.0037250. PMID:22615954.

Dubowy, C., and Sehgal, A. 2017. Circadian rhythms and sleep in *Drosophila melanogaster*. Genetics, **205**(4): 1373–1397. doi:10.1534/genetics.115.185157. PMID:28360128.

Duistermars, B.J., Pfeiffer, B.D., Hoopfer, E.D., and Anderson, D.J. 2018. A brain module for scalable control of complex, multi-motor threat displays. Neuron, **100**(6): 1474–1490.e1474. doi:10.1016/j.neuron.2018.10.027. PMID:30415997.

Eyjolfsdottir, E., Branson, S., Burgos-Artizzu, X.P., Hoopfer, E.D., Schor, J., Anderson, D.J., and Perona, P. 2014. Detecting social actions of fruit flies. *In* Lecture Notes in Computer Science. Vol. 8690. *Edited by* D. Fleet, T. Pajdla, B. Schiele, and T. Tuytelaars. Springer, Cham. pp. 772–787. doi:10.1007/978-3-319-10605-2_50.

Gargano, J.W., Martin, I., Bhandari, P., and Grotewiel, M.S. 2005. Rapid iterative negative geotaxis (RING): a new method for assessing age-related locomotor decline in *Drosophila*. Exp. Gerontol. **40**(5): 386–395. doi:10.1016/j.exger.2005.02.005. PMID:15919590.

Greenspan, R.J., and Ferveur, J.F. 2000. Courtship in *Drosophila*. Annu. Rev. Genet. **34**: 205–232. doi:10.1146/annurev.genet.34.1.205. PMID:11092827.

Grotewiel, M.S., Martin, I., Bhandari, P., and Cook-Wiens, E. 2005. Functional senescence in *Drosophila melanogaster*. Ageing Res. Rev. **4**(3): 372–397. doi:10.1016/j.arr.2005.04.001. PMID:16024299.

Jones, M.A., and Grotewiel, M. 2011. *Drosophila* as a model for age-related impairment in locomotor and other behaviors. Exp. Gerontol. **46**(5): 320–325. doi:10.1016/j.exger.2010.08.012. PMID:20800672.

Kaun, K.R., Devineni, A.V., and Heberlein, U. 2012. *Drosophila melanogaster* as a model to study drug addiction. Hum. Genet. **131**(6): 959–975. doi:10.1007/s00439-012-1146-6. PMID:22350798.

Leffelaar, D., and Grigliatti, T. 1983. Age-dependent behavior loss in adult *Drosophila melanogaster*. Dev. Genet. **4**(3): 211–227. doi:10.1002/dvg.1020040307.

Martin, J.R. 2003. Locomotor activity: a complex behavioural trait to unravel. Behav. Processes, **64**(2): 145–160. doi:10.1016/S0376-6357(03)00132-3. PMID:14556949.

Martinez, V.G., Javadi, C.S., Ngo, E., Ngo, L., Lagow, R.D., and Zhang, B. 2007. Age-related changes in climbing behavior and neural circuit physiology in *Drosophila*. Dev. Neurobiol. **67**(6): 778–791. doi:10.1002/dneu.20388. PMID:17443824.

Mendes, C.S., Bartos, I., Akay, T., Marka, S., and Mann, R.S. 2013. Quantification of gait parameters in freely walking wild type and sensory deprived *Drosophila melanogaster*. Elife, **2**: e00231. doi:10.7554/eLife.00231. PMID:23326642.

Miller, M.S., Lekkas, P., Braddock, J.M., Farman, G.P., Ballif, B.A., Irving, T.C., et al. 2008. Aging enhances indirect flight muscle fiber performance yet decreases flight ability in *Drosophila*. Biophys. J. **95**(5): 2391–2401. doi:10.1529/biophysj.108.130005. PMID:18515368.

Noldus, L.P.J.J., Spink, A.J., and Tegelenbosch, R.A.J. 2002. Computerised video tracking, movement analysis and behaviour recognition in insects. Comput. Electron. Agric. **35**(2–3): 201–227. doi:10.1016/S0168-1699(02)00019-4.

Osterwalder, T., Yoon, K.S., White, B.H., and Keshishian, H. 2001. A conditional tissue-specific transgene expression system using inducible GAL4. Proc. Natl. Acad. Sci. U.S.A. **98**(22): 12596–12601. doi:10.1073/pnas.221303298. PMID:11675495.

Pitman, J.L., DasGupta, S., Krashes, M.J., Leung, B., Perrat, P.N., and Waddell, S. 2009. There are many ways to train a fly. Fly (Austin), **3**(1): 3–9. doi:10.4161/fly.3.1.7726. PMID:19164943.

Ramazani, R.B., Krishnan, H.R., Bergeson, S.E., and Atkinson, N.S. 2007. Computer automated movement detection for the analysis of behavior. J. Neurosci. Methods, **162**(1–2): 171–179. doi:10.1016/j.jneumeth.2007.01.005. PMID:17335906.

Schretter, C.E., Vielmetter, J., Bartos, I., Marka, Z., Marka, S., Argade, S., and Mazmanian, S.K. 2018. A gut microbial factor modulates locomotor behaviour in *Drosophila*. Nature, **563**(7731): 402–406. doi:10.1038/s41586-018-0634-9. PMID:30356215.

Seroude, L., Brummel, T., Kapahi, P., and Benzer, S. 2002. Spatio-temporal analysis of gene expression during aging in *Drosophila melanogaster*. Aging Cell, **1**(1): 47–56. doi:10.1046/j.1474-9728.2002.00007.x. PMID:12882353.

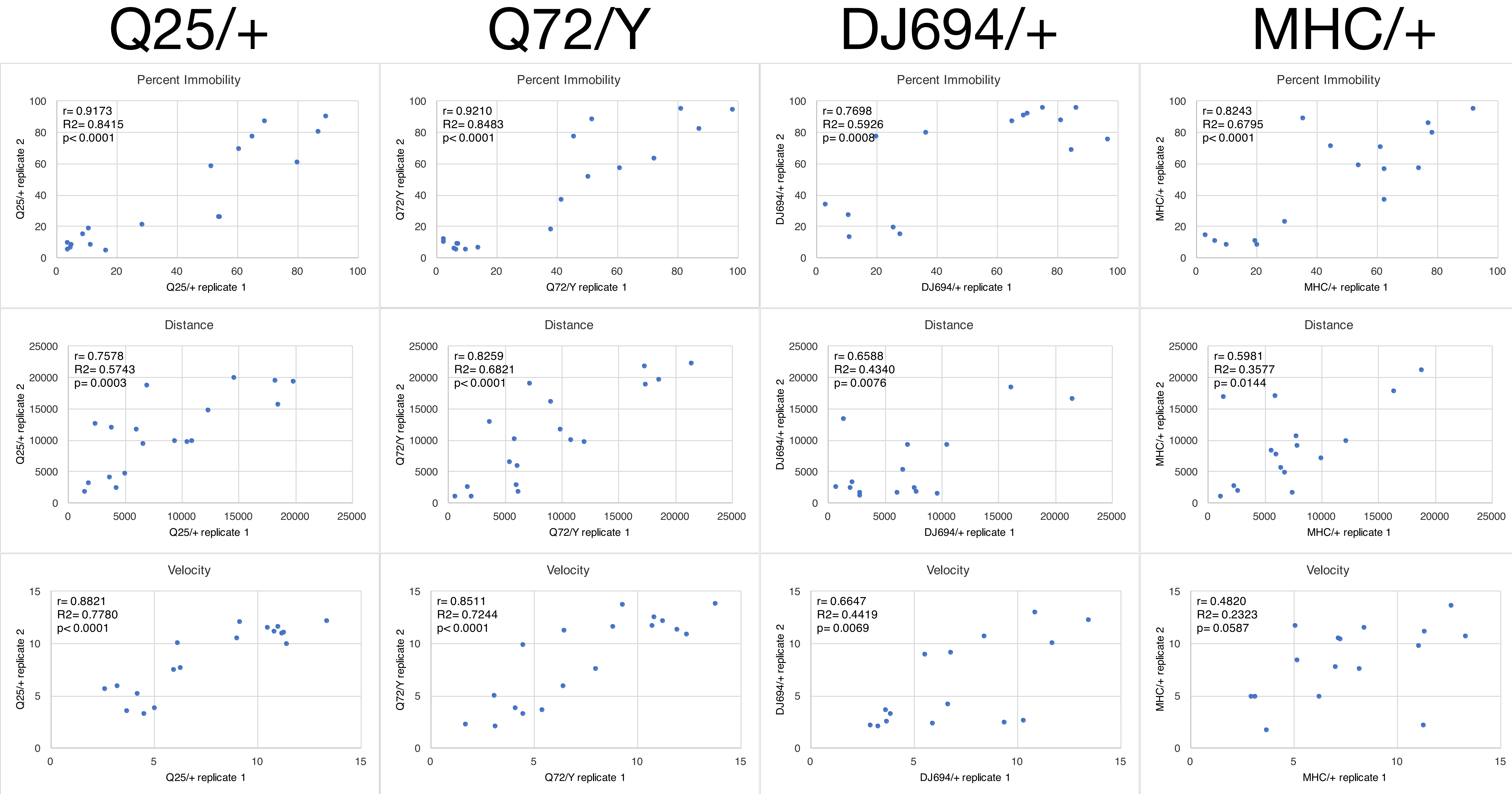
Straw, A.D., and Dickinson, M.H. 2009. Motmot, an open-source toolkit for realtime video acquisition and analysis. Source Code Biol. Med. **4**: 5. doi:10.1186/1751-0473-4-5. PMID:19624853.

Tully, T. 1996. Discovery of genes involved with learning and memory: an experimental synthesis of Hirschian and Benzerian perspectives. Proc. Natl. Acad. Sci. U.S.A. **93**(24): 13460–13467. doi:10.1073/pnas.93.24.13460. PMID:8942957.

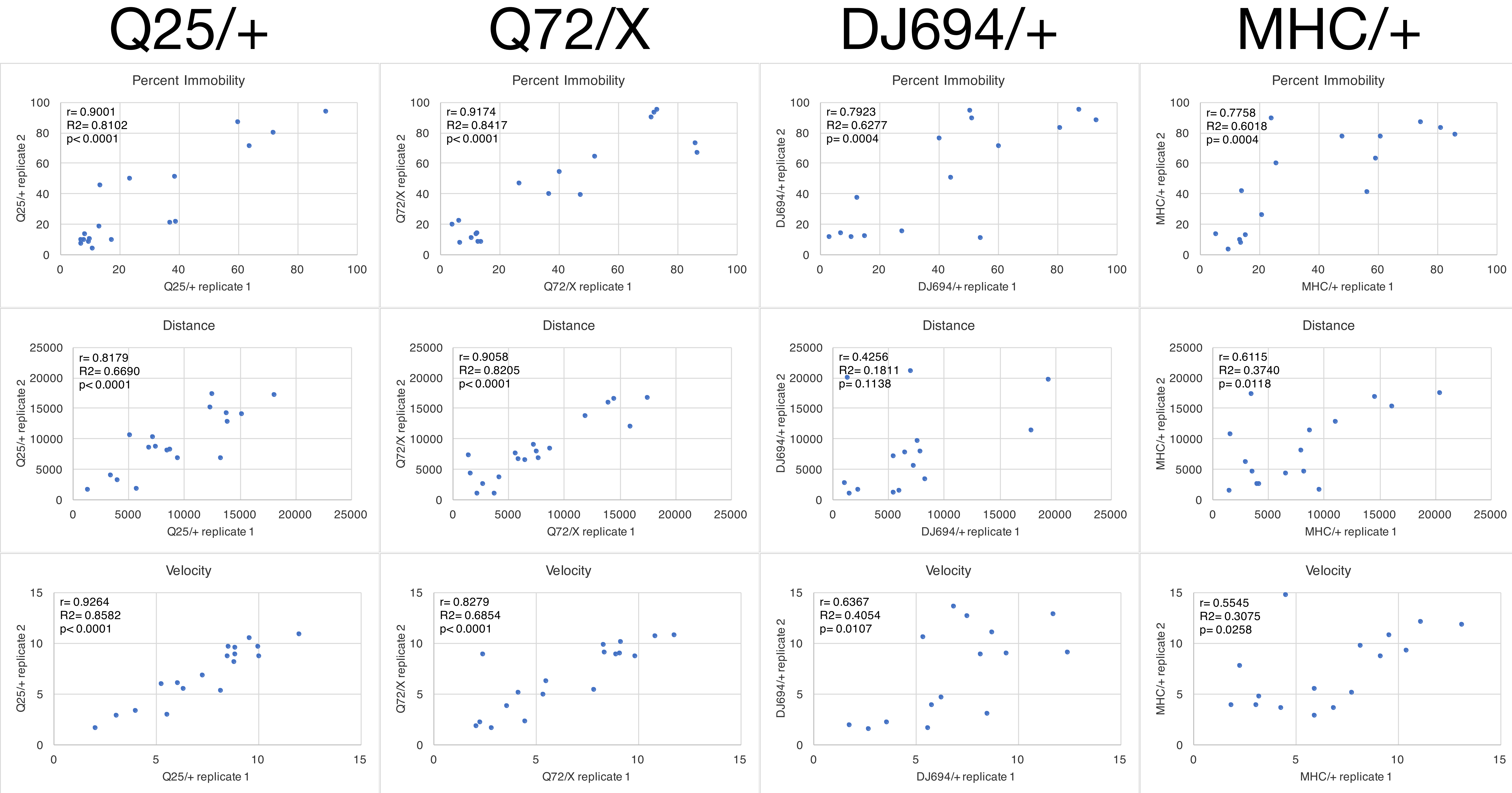
Valente, D., Golani, I., and Mitra, P.P. 2007. Analysis of the trajectory of *Drosophila melanogaster* in a circular open field arena. PLoS ONE, **2**(10): e1083. doi:10.1371/journal.pone.0001083. PMID:17957265.

Wosnitza, A., Bockemuhl, T., Dubbert, M., Scholz, H., and Buschges, A. 2013. Inter-leg coordination in the control of walking speed in *Drosophila*. J. Exp. Biol. **216**(Pt. 3): 480–491. doi:10.1242/jeb.078139. PMID:23038731.

Zhang, S., Binari, R., Zhou, R., and Perrimon, N. 2010. A genome-wide RNA interference screen for modifiers of aggregates formation by mutant Huntingtin in *Drosophila*. Genetics, **184**(4): 1165–1179. doi:10.1534/genetics.109.112516. PMID:20100940.



Supplemental Figure 1: Pearson correlation analysis of male cohorts (replicate 1 and 2) for each measured parameter (percent immobility, distance, average velocity during fly movement). Graphs show replicate 1 plotted as a function of replicate 2. r, R^2, p values are provided in the upper left corner of each graph.



Supplemental Figure 2: Pearson correlation analysis of female cohorts (replicate 1 and 2) for each measured parameter (percent immobility, distance, average velocity during fly movement). Graphs show replicate 1 plotted as a function of replicate 2. r, R^2, p values are provided in the upper left corner of each graph.

Supplemental Table 1: Raw locomotor behaviour data from male genotypes. Each value represents one individual fly that was measured. All individuals of a genotype were averaged for each replicate in the final figures.

Genotype	Age (days)	1	2	3	4	6	7	8	9	10	11	13	14	16	17	21	24	29	31	35	38	42	46	51	54	57	63
	%immobile																										
				7.7	40.7		3.8	4.3	2.1	2.4	4.0		4.3	7.5	4.6	4.4	5.4	29.0	58.4	82.2	75.0	94.1	67.2	22.8	90.7	95.7	86.0
				15.1	3.1		1.9	38.7	2.5	4.9	3.4		1.4	3.8	9.6	2.3	27.1	80.2	15.0	28.0	55.7	29.0	78.3	82.3	79.6	65.3	85.2
				14.6	2.4		1.0	6.7	6.7	5.9	3.3		2.7	4.9	6.6	2.9	14.7	8.2	15.9	10.6	44.1	20.7	53.8	51.4	97.7	52.5	97.4
				3.8			27.7			9.1			7.1	4.3	3.7	7.4	12.1	73.1	63.6	65.6	93.8	73.4	74.9	91.5	78.2	92.3	
	Replicate 1			6.4			6.7			5.1			3.4	3.6	1.1	3.2	2.9	65.4	15.7	81.6	47.9	46.5	59.3	94.3	86.0	94.6	
				24.1			3.2			12.8			3.5	3.5	3.0	2.1	1.8	69.0	2.9	54.5	47.3	43.6	56.0	73.4	89.3	78.9	
				13.7			0.1			4.8			3.0														
				5.3			1.7			14.9			6.1														
				11.2			0.2			20.5			2.6														
		5.3	2.0	3.8		15.9	11.2	6.5	4.7	3.3		4.6		1.6		5.2	5.0	6.2	11.0	15.5	65.9	83.6	77.7	94.6	98.2	94.6	88.7
		4.6	3.6	2.1		23.8	7.5	4.4	6.9	3.3		46.1		4.4		14.9	2.5	6.4	38.7	8.0	86.8	61.0	62.1	98.9	95.6	16.4	94.0
		12.1	26.4	6.9		7.9	15.1	2.9	3.1	7.7		1.9		10.0		8.0	6.7	9.9	6.0	5.4	9.2	74.0	40.9	88.0	96.8	9.6	86.5
		9.6		12.4			13.1			11.7				17.7		8.4	22.5	45.0	25.6	57.6	92.6	84.5	90.9	97.0	94.0	73.1	
	Replicate 2	8.4		8.8			2.4			5.7				1.7		7.0	1.1	15.6	28.6	3.3	75.9	18.2	92.4	60.6	9.5	80.6	
		45.9		7.1			4.3			80.1				1.9		10.2	73.1	73.1	15.7	64.5	84.5	29.0	97.1	81.3	84.7	87.5	
		6.1		5.6			6.9			10.6																	
		4.2		4.0			3.6			3.9																	
		9.7		23.1			9.9			7.1																	
	Distance																										
				22925.0	10603.2		451.8	16241.0	18710.3	21970.5	16632.3		19043.3	10346.7	10236.7	10762.8	11149.7	6224.3	5450.8	1822.2	2878.7	986.3	3122.1	10704.2	1231.7	880.0	1680.0
				18036.0	21227.1		1398.7	9773.0	19794.5	20616.6	20249.7		23303.1	10349.6	7727.5	9946.4	6412.9	1474.7	15599.4	11691.1	6141.2	11753.4	1933.2	2537.1	2563.7	4173.2	1820.8
				18626.5	20696.3		1738.2	17795.7	16192.1	18640.4	19493.0		21625.0	9746.8	8440.5	11233.1	8224.0	9346.6	14937.3	10919.9	7200.0	12238.4	4855.7	7096.4	835.5	4869.5	997.1
				20890.8			14093.7			21497.7			16639.3	10296.9	11520.8	10451.4	9075.3	1727.7	4588.4	3361.0	1145.5	3313.0	2044.7	1189.9	2263.3	1200.8	
	Replicate 1			22685.2			18558.2			19595.5			17774.9	11079.6	12176.7	10807.9	10137.0	2247.8	15024.9	2556.8	6064.9	7259.6	4880.9	1219.8	2184.0	1034.1	
				13502.5			22655.5			13651.3			19909.6	11235.4	12087.2	12285.4	11158.2	1924.8	18389.6	6039.5	6555.5	4146.0	5207.4	2980.0	1688.5	2022.1	
				17424.0			452.1			20361.0			19591.3														
				26779.5			1423.7			17236.8			20134.8														
Q25/+				17908.1			1716.4			13251.9			20094.5														
		20860.8	22902.3	19084.0		13532.7	17910.8	18072.4	20039.1	18666.2		21029.6		11062.3		10621.3	11193.3	17447.9	15596.6	11838.6	4550.7	2441.8	4255.3	1026.0	870.7	1880.4	1632.5
		22151.6	23629.0	23032.4		15520.0	21059.9	20490.1	19859.7	22676.5		10549.1		8732.1		10257.9	11638.4	16847.6	10184.7	15857.6	1735.2	9466.4	6541.8	521.7	1040.0	32355.0	1304.1
		15541.8	14548.2	20294.0		20978.9	16960.6	20878.3	18159.6	12726.0		20694.3		8286.6		9517.9	11274.6	13827.6	20353.0	16362.4	15462.0	14988.5	9718.1	3882.7	744.9	33223.2	2088.1
		23136.6		18689.3			15242.0			16489.4				8322.6		9775.0	10605.6	7645.2	13128.2	5572.8	1103.8	2222.0	1007.2	889.6	725.5	3169.5	
	Replicate 2	20537.6		19107.8			20059.8			16031.5				10350.3		8744.6	12133.7	12743.6	12711.1	18122.0	2361.3	15931.1	1420.6	5021.6	13836.1	1610.0	
		12624.5		18066.4			21874.3			2231.4				11230.2		9349.5	1697.5	3173.2	16456.2	2053.5	2468.5	11363.5	1095.3	2531.1	1492.4	2610.5	
		22498.0		21821.5			16648.4			15557.9																	
		24721.8		19180.8			19922.6			18172.2																	
		21710.2		13802.7			17689.0			17630.7																	
	Velocity																										
				15.0	9.1		20.7	9.1	11.0	12.7	9.8		11.4	11.4	10.8	11.5	12.7	8.8	6.3	3.9	2.8	1.8	2.3	7.3	5.6	1.5	4.0
				12.7	12.2		21.0	7.8	11.9	12.2	11.8		12.6	10.9	8.3	9.7	8.6	5.9	10.4	9.3	6.4	9.5	2.3	5.3	5.0	2.9	5.0
				13.1	11.8		4.4	10.6	10.1	11.3	11.5		11.5	10.4	9.2	11.2	9.7	10.6	10.0	7.0	5.3	8.9	3.7	7.6	1.6	2.7	2.2
				13.1			10.7			13.3			10.2	11.2	12.2	11.6	10.5	4.7	5.8	2.7	1.4	5.8	1.9	3.3	3.5	3.1	
	Replicate 1			14.8			10.9			11.7			10.2	11.7	12.7	11.0	11.6	5.2	10.4	5.9	4.9	7.7	4.8	4.3	5.4	2.4	
				10.4			13.2			8.7			10.7	12.0	12.8	12.1	11.7	2.5	11.1	7.0	4.5	3.2	4.2	2.5	6.1	3.3	
				12.0			0.2			12.1			11.4														
				17.2			11.7			11.4			11.5														
				12.1			1.5			9.2			10.7														
		12.9	13.2	11.2		8.9	11.4	11.2	11.9	10.9		12.5		11.5		12.0	12.0	10.2	10.1	6.4	6.5	4.0	10.3	3.3	1.5	1.7	4.3
		13.8	14.1	13.7		11.4	12.9	12.7	12.1	13.3		10.4		9.2		12.1	12.2	10.0	9.3	9.7	3.1	10.9	8.8	1.5	4.0	8.9	3.9
		10.3	11.0	12.7		13.0	11.2	12.2	10.6	7.6		12.0		9.0		10.4	12.5	8.5	12.3	10.1	9.9	19.5	8.7	2.6	1.4	8.4	2.3
		14.6		12.3			9.8			10.5				12.0		11.3	13.8	5.6	10.1	5.1	1.5	4.8	2.4	1.7	2.0	5.2	
	Replicate 2	12.7		12.2			11.6			9.5				12.6		9.4	12.6	8.0	10.0	10.5	2.1	12.5	3.2	7.9	8.3	3.1	
		13.1		11.3			13.2			5.6				11.8		10.4	3.1	3.3	11.2	2.9	7.5	8.2	1.7	5.4	2.1	6.4	
		13.5		13.3			10.5			9.8																	
		14.7		11.6			11.7			10.8																	
		13.7		10.1			11.1			10.8																	

Supplemental Table 1: Raw locomotor behaviour data from male genotypes. Each value represents one individual fly that was measured. All individuals of a genotype were averaged for each replicate in the final figures.

Genotype	Age (days)	1	2	3	4	6	7	8	9	10	11	13	14	16	17	21	24	29	31	35	38	42	46	51	54	57	63
	%immobile																										
				3.1	4.6		1.1	32.7	7.7	4.3	7.6		1.8	7.4	9.7	3.0	3.9	10.9	58.0	55.7	66.2	58.1	91.7	14.6	95.1	31.4	99.1
				6.3	7.2		0.7	5.6	5.2	5.0	4.2		3.6	9.1	6.6	4.0	10.9	68.1	51.1	54.1	60.0	97.9	80.1	26.6	82.9	13.8	99.0
				1.7	7.5		0.2	3.7	7.7	6.5	4.9		4.4	3.4	8.3	2.4	10.6	6.3	34.1	81.6	60.0	89.1	90.5	38.6	93.6	58.6	96.9
				9.0			4.6			4.3			5.6	5.4	9.7	2.0	17.4	74.4	56.9	53.4	60.5	58.7	97.4	93.1	40.1	82.9	
	Replicate 1			4.7			5.3			20.3			5.3	5.3	3.9	1.7	4.9	82.2	6.6	97.1	50.7	98.6	85.7	66.5	98.7	40.9	
				2.1			8.1			8.6			9.9	9.7	1.6	2.1	10.6	7.2	21.5	23.2	4.6	32.3	77.5	70.1	77.0		
				9.3			1.7			5.4			5.7														
				10.6			0.5			4.5			7.4														
				7.0			0.4			5.7			2.3														
		31.7	7.7	1.6		6.0	10.6	2.4	12.1	16.7		7.1		7.7		5.4	2.5	17.1	19.9	76.0	75.9	86.3	88.2	92.2	88.4	70.2	91.1
		6.5	6.5	3.4		7.4	7.5	9.2	8.9	7.5		2.7		6.0		11.5	1.8	89.7	11.5	72.1	45.5	67.9	76.6	68.7	96.1		94.9
		10.4	4.1	8.5		4.7	4.1	7.7	5.1	9.5		5.8		4.2		4.3	5.8	5.8	15.2	25.6	49.8	36.6	95.2	85.0	96.7		96.1
		3.0		5.2			19.9			4.7				4.7		31.6	7.9	17.2	17.2	68.0	31.7	80.8	68.3	94.6	97.3	93.0	
	Replicate 2	12.1		6.0			11.7			9.1				5.1		9.1	5.8	76.3	31.8	5.9	43.8	63.3	77.3	95.6	96.6	55.9	
		13.4		5.2			13.0			8.3				2.9		6.9	6.7	14.4	9.6	92.3	60.6	43.6	84.0	91.8	91.9	87.9	
		2.6		3.0			10.5			9.0																	
		10.1		9.4			3.9			11.8																	
		5.6		6.3			5.0			3.0																	
	Distance																										
				24984.0	20771.1		419.1	8323.0	17014.0	19462.5	17611.3		22621.6	12540.5	8879.7	11922.4	11109.6	8191.3	3866.9	6821.2	3512.4	6365.9	1376.0	10115.1	785.2	8107.6	333.7
				21292.1	18310.9		706.9	20419.1	17155.1	18822.0	16848.7		21321.8	9362.7	9893.1	11903.5	10501.8	2337.9	4977.4	5658.6	4518.3	762.4	2056.0	9597.2	2434.5	10766.0	426.6
				22680.1	18098.9		1153.1	23352.9	17990.7	20023.3	18927.0		20521.5	9601.9	11073.3	12202.4	10627.8	10612.3	9697.8	2463.2	4304.0	1206.8	1609.0	9269.2	1236.2	4135.8	1129.4
				18986.2			21589.5			18779.1			19912.0	8871.2	10467.6	11865.4	11993.7	1977.7	4869.3	5967.1	4024.2	5306.2	807.3	1037.4	5817.4	1770.3	
	Replicate 1			23328.2			19669.2			15301.4			19251.3	9577.3	11021.0	12174.6	10658.7	912.9	18216.7	718.7	5116.5	694.0	1579.0	3292.9	286.3	5549.2	
				22855.7			19384.4			17871.4			15092.4	9676.5	14185.7	12112.4	10081.8	10898.5	12517.4	10765.4	15294.4	7477.5	2856.3	3872.0	1726.5		
				19260.4			415.4			21594.5			18335.0														
				17896.3			736.2			19432.6			21765.5														
Q72/Y				21601.8			1016.3			15929.7			22308.1														
		11660.0	18394.2	24576.5		21790.5	20704.9	24687.7	17134.7	14854.4		17715.6		11014.9		9726.8	11629.8	12935.7	15291.5	2930.4	2236.4	1952.7	1653.2	1089.5	1243.2	5180.1	1147.2
		18059.6	20322.0	22729.5		22281.7	20662.7	20232.6	18628.0	18324.2		23037.5		11616.8		9557.6	11125.5	1656.1	18666.9	3119.6	5502.0	4305.6	4047.2	3502.6	935.6		807.7
		22669.5	23317.5	19527.4		19261.4	21060.2	20331.1	20808.6	21381.1		18443.4		11498.6		10680.5	11464.9	15277.2	17387.9	10817.6	5912.8	39275.4	964.3	2390.9	1036.5		1056.4
		19569.8		23874.0			15085.3			22431.5				11862.7		8032.7	10547.8	12518.2	16027.4	2933.1	9668.3	1951.6	3611.1	895.3	702.0	1007.0	
	Replicate 2	16941.2		21519.4			20580.3			18095.3				11576.0		9573.9	8600.3	2674.8	13595.6	17696.7	7170.6	4808.3	3035.8	773.7	978.0	2954.4	
		17422.8		21795.2			15793.2			19567.8				11929.1		10533.4	6570.9	15240.1	15525.9	1262.0	3945.7	24665.0	1782.6	1260.2	1065.9	1785.8	
		20223.8		24301.2			15877.4			21723.5																	
		21456.1		21665.2			18954.2			20274.3																	
		16976.6		19698.4			21185.7			19874.7																	
	Velocity																										
				15.5	12.3		12.8	4.9	10.5	11.4	10.7		11.3	14.2	9.7	12.4	11.9	9.5	2.9	8.4	2.7	8.1	2.1	6.5	2.2	4.6	1.3
				13.7	11.2		6.2	10.8	10.6	11.2	9.9		10.8	10.4	10.8	11.9	12.1	6.0	3.0	6.7	3.8	1.6	2.9	7.0	5.5	5.8	2.5
				14.1	11.2		2.5	12.2	11.1	12.1	11.4		10.4	10.0	12.0	11.9	12.1	11.8	5.6	5.3	3.6	2.5	2.7	8.0	1.8	3.5	1.4
				12.5			12.6			11.1			11.6	9.5	11.3	11.4	15.2	6.7	5.8	7.0	3.0	6.9	1.7	2.1	5.0	3.6	
	Replicate 1			14.8			11.6			10.5			10.1	10.2	11.5	12.1	11.5	1.8	10.4	2.8	3.2	1.9	4.1	3.8	1.4	4.9	
				14.2			11.9			11.0			7.6	10.7	14.6	12.2	11.6	12.3	11.1	8.2	8.4	6.0	4.9	5.2	2.9		
				12.7			15.1			12.9			10.0														
				12.5			4.0			11.5			11.9														
				14.1			3.0			9.4			11.4														
		9.8	11.2	14.7		13.1	13.2	15.0	11.0	9.9		10.7		12.0		10.5	12.4	8.9	10.9	3.9	2.0	2.8	3.9	3.6	1.7	2.7	1.6
		11.6	12.8	13.9		13.7	12.4	13.0	11.5	11.2		13.5		12.6		11.5	12.3	4.1	12.2	4.9	3.6	6.7	7.9	3.7	1.7		2.4
		14.9	13.9	12.3		11.5	12.2	13.0	12.4	13.5		11.1		11.8		11.3	12.5	9.2	11.8	8.4	3.0	23.8	2.6	6.6	1.4		2.6
		11.1		14.9			10.5			13.3				13.0		11.8	11.7	8.4	11.1	3.6	7.4	1.6	5.4	1.8	1.4	3.1	
	Replicate 2	11.3		13.4			12.8			11.1				12.9		11.0	9.1	4.1	11.2	11.4	4.2	6.4	5.8	2.2	3.4	2.9	
		11.3		13.5			9.9			11.9				12.4		11.4	6.7	10.2	10.0	3.0	2.4	17.6	4.2	3.4	2.2	4.1	
		11.8		14.8			9.8			13.4																	
		13.3		14.0			10.9			13.0																	
		9.7		12.2			12.3			11.6																	

Supplemental Table 1: Raw locomotor behaviour data from male genotypes. Each value represents one individual fly that was measured. All individuals of a genotype were averaged for each replicate in the final figures.

Genotype	Age (days)	1	2	3	4	6	7	8	9	10	11	13	14	16	17	21	24	29	31	35	38	42	46	51	54	57	63
	%immobile																										
			9.3	7.0	11.2	2.3	6.4	5.9	62.3	7.4	15.3	92.6	95.4	78.5	17.2	89.5	66.4	86.9	87.2								
	Replicate 1		62.7	2.1	12.7	2.0	17.7	7.9	3.2	41.8	87.4	26.4	98.1	88.0	86.0	92.4	86.0	25.6	88.2								
			4.7	0.4	8.1	12.0	9.2	67.5	17.8	11.0	7.0	91.2	97.0	87.3	92.0	43.2	91.7	94.3	83.6								
		7.5	38.8	13.5	47.4		5.1		20.2	78.6	82.8	88.1	70.3	71.1	88.2	92.5	88.9	97.0	97.9								
	Replicate 2	10.6	2.8	12.6	27.3		9.5		8.3	81.2	87.5	89.1	87.8	50.7	93.4	97.7	86.4	87.5	93.0								
		7.3	15.0	75.2	6.0		23.7		16.0	70.7	68.9	98.0	67.7	83.5	78.0	95.8		86.5									
	Distance																										
			21233.1	1173.5	19894.1	21095.8	11740.6	10975.5	2207.5	14278.3	10189.7	1326.3	896.2	2180.2	17123.6	1455.1	5699.7	1698.7	1301.8								
	Replicate 1		4843.8	1397.2	19401.3	25181.1	8514.0	11245.4	11752.5	3782.5	786.5	20499.7	640.6	2400.4	1483.2	1238.3	1286.1	15444.6	2150.3								
DJ694/+			22422.5	1621.0	25307.8	19808.4	11220.8	2341.9	7188.4	10914.7	11944.8	1446.9	701.7	1942.1	1229.4	5826.7	1472.1	1290.0	2405.6								
		20337.2	10332.1	19059.2	10605.5		10145.5		9274.0	1133.4	1965.6	2291.9	2779.8	3320.8	1635.8	1115.2	1503.7	826.6	1707.0								
	Replicate 2	15891.7	23572.2	17138.0	16609.7		9979.2		10054.2	1230.9	1809.8	1646.9	1530.4	4389.3	1046.5	859.0	1707.6	1623.8	3037.4								
		19251.4	21186.9	3564.8	22293.3		7267.7		8062.2	1712.6	3102.0	925.6	2974.9	2031.0	13082.4	1312.8		2382.6									
	Velocity																										
			14.0	14.5	12.5	12.2	12.6	11.6	4.1	15.8	13.2	2.0	2.5	2.0	11.9	3.4	6.9	4.7	2.6								
	Replicate 1		4.5	4.6	12.4	14.7	10.1	12.5	12.5	3.1	1.5	16.3	4.0	7.0	2.0	1.7	1.9	11.5	3.4								
			14.1	1.3	15.5	12.4	12.4	5.1	8.7	12.1	13.6	1.7	2.4	1.9	2.7	4.7	2.8	1.5	5.0								
		12.9	9.3	11.9	10.7		10.3		11.5	1.7	1.7	6.7	2.0	3.8	1.9	1.6	2.6	1.4	1.4								
	Replicate 2	10.3	14.8	10.4	12.5		10.8		11.0	3.6	3.6	4.2	1.5	2.8	2.8	2.0	3.9	1.9	3.6								
		12.1	14.6	4.9	13.4		9.0		9.4	2.6	1.9	1.4	2.7	4.2	21.9	2.6		3.8									
	%immobile																										
			9.7	2.7	10.1	35.0	9.0	7.5	14.7	10.7	83.0	18.2	65.1	66.0	83.4	84.5	36.8	82.0	97.3	94.5							
	Replicate 1		12.5	1.7	12.8	6.2	5.4	8.5	20.6	5.4	6.6	93.8	89.9	83.4	71.0	85.4	9.4	98.4	42.9	66.8							
			7.7	5.0	36.0	11.7	4.9	6.1	52.6	44.8	16.6	75.0	28.8	12.6	67.8	17.3	87.8	95.1	91.4	73.8							
		6.8	4.9	8.3	5.9		5.7		29.1	8.9	89.8	87.6	87.9	52.5	17.9	24.4	96.5	92.8	94.7	62.9							
	Replicate 2	9.4	8.1	27.0	15.0		6.2		18.5	7.9	91.2	8.3	95.5	67.4	77.0	85.1	26.8	96.3	80.2	96.2							
		5.0	11.6	7.5	10.6		18.6		20.9	6.7	84.3	13.7	27.4	55.7	74.8	59.1	89.9	96.0	81.5	79.3							
	Distance																										
			16860.9	1102.6	17473.3	10132.8	11623.0	9981.7	7319.2	9267.3	1814.6	13432.0	5204.9	4563.7	2521.2	1443.3	9969.7	2569.5	882.2	1127.5							
	Replicate 1		19228.2	1436.9	18831.5	18924.2	12995.1	10233.0	6230.6	10973.3	9945.6	950.1	1274.6	2349.4	2214.9	1196.9	18547.3	266.0	6228.7	3057.0							
MHC/+			20495.8	1685.2	12862.7	15712.9	11897.3	9233.3	3188.5	3138.4	10560.2	3370.0	13976.6	12348.4	18818.9	15389.9	1339.5	645.8	759.0	2844.7							
		20936.0	21568.3	16751.2	19091.6		10078.4		6156.8	9944.3	1180.7	1826.7	1206.3	7956.8	16696.1	11700.4	928.4	998.7	945.8	3819.0							
	Replicate 2	21096.0	18587.8	12173.7	17908.9		11156.1		9546.3	9610.0	1276.3	24811.3	1092.0	3241.2	3321.2	1469.8	18405.1	595.4	2311.8	898.0							
		23832.3	23038.5	21561.9	16246.2		7911.6		9037.4	12074.7	1943.8	24264.0	12011.5	5377.3	6922.3	9618.2	1831.0	1167.1	2097.1	3090.8							
	Velocity																										
			10.9	6.1	11.0	8.3	13.0	10.4	8.6	10.5	9.8	9.5	8.2	5.9	7.5	2.5	8.5	6.0	1.6	1.6							
	Replicate 1		13.5	3.9	12.1	11.6	14.2	11.2	7.8	11.6	11.0	2.1	5.0	5.2	4.9	2.1	11.0	3.5	5.5	3.7							
			13.4	11.9	11.0	10.0	12.8	9.7	5.1	3.2	13.0	3.6	11.4	7.6	20.8	10.9	1.6	1.5	2.4	3.5							
		12.8	13.1	9.8	11.3		10.6		8.2	10.7	2.1	2.9	2.2	7.8	11.5	8.5	4.6	2.0	6.1	3.5							
	Replicate 2	12.9	12.2	8.4	11.9		11.9		11.7	10.4	1.4	15.7	10.6	2.8	6.4	4.4	14.7	1.3	4.4	4.3							
		14.0	15.3	13.0	10.1		9.4		11.5	13.2	2.7	16.2	9.6	4.0	11.3	12.3	3.8	1.7	4.1	6.7							

Supplemental Table 2: Raw locomotor behaviour data from female genotypes. Each value represents one individual fly that was measured. All individuals of a genotype were averaged for each replicate in the final figures.

Genotype	Age (days)	1	2	3	4	6	7	8	9	10	11	13	14	16	17	21	24	29	31	35	38	42	46	51	54	57	63
	%immobile																										
				20865.6	17182.3		18451.9	12978.0	16313.6	17006.3	17412.4		16519.0	9571.8	8352.0	6998.0	8323.9	5245.4	13579.8	2386.2	12784.2	3797.1	9330.2	3842.2	905.3	1188.4	1180.7
				18030.2	17326.6		1430.6	13016.5	11544.5	13833.2	17990.3		18633.9	9708.0	6450.0	9149.9	4398.4	6401.6	18362.3	7587.8	12842.2	8339.2	8987.7	1086.4	1622.5	2034.4	1452.8
				20502.8	19498.0		1715.7	11741.3	13981.3	11941.1	13867.2		16402.5	10418.3	8074.3	10514.4	6887.9	8221.4	19794.8	11158.1	17015.4	9021.6	7497.8	1682.7	1295.9	10090.9	1316.7
				19315.6			13162.3			17497.4			15796.2	7676.1	10000.4	7496.7	6454.9	2631.7	11298.8	3773.3	10389.9	1129.6	6667.1	14522.6	1642.0	1795.0	
	Replicate 1			18453.2			16324.3			11812.0			19190.3	6159.5	10129.2	7844.4	10391.2	968.7	12072.9	8402.7	16013.1	8017.7	12409.7	3150.5	6499.9	1835.6	
				17995.5			17833.2			12099.5			17370.4	9150.7	9158.5	8995.5	8387.0	7599.2	16048.2	9919.4	11018.0	11042.8	11949.4	10333.5	12249.0		
				17830.2			14311.2			13348.9			11422.6														
				18216.8			13583.2			16211.7			19535.2														
				12251.0			14745.2			11046.9			17059.4														
		17494.2	16148.3	17496.6		8672.6	15412.0	16844.3	12142.2	11274.4		18128.2		7646.2		9435.5	8963.9	8403.1	13130.2	13925.1	9999.4	1738.6	11925.6	742.7	959.5	75.6	2020.6
		17125.7	16295.9	16639.8		900.8	18338.2	15957.8	15582.8	13094.8		16712.5		9128.0		6360.3	9315.1	15870.8	15978.5	14791.8	1350.4	15830.1	3235.1	594.3	8062.1	81.8	1476.2
		18773.2	15328.5	19669.8		19417.6	17879.2	19238.4	10238.0	17314.1		15790.5		9302.9		7875.2	9517.5	4215.6	16684.1	7659.9	11216.9	21617.4	1113.8	6960.3	6455.3	86.2	1184.5
		14454.7		16411.4			13483.5			13639.5				8203.7		7415.8	7676.1	11750.9	15062.0	10091.2	9727.1	88.6	2701.0	686.3	1367.4	74.1	
	Replicate 2	14315.5		16461.9			14946.1			17113.9				7933.2		8587.8	9575.4	12485.5	10124.0	10967.4	1043.7	3375.7	10627.6	855.3	1387.6	3902.5	
		16002.3		19532.7			13198.3			15884.5				6609.6		8504.5	6770.8	10592.0	13080.4	3605.5	7296.0	7014.9	10632.7	95.1	571.9		
		15588.2		14187.0			15194.1			13705.5																	
		16527.6		17739.0			15984.6			12936.4																	
		16017.2		16179.7			11018.7			11988.2																	
	Distance																										
				20865.6	17182.3		18451.9	12978.0	16313.6	17006.3	17412.4		16519.0	9571.8	8352.0	6998.0	8323.9	5245.4	13579.8	2386.2	12784.2	3797.1	9330.2	3842.2	905.3	1188.4	1180.7
				18030.2	17326.6		1430.6	13016.5	11544.5	13833.2	17990.3		18633.9	9708.0	6450.0	9149.9	4398.4	6401.6	18362.3	7587.8	12842.2	8339.2	8987.7	1086.4	1622.5	2034.4	1452.8
				20502.8	19498.0		1715.7	11741.3	13981.3	11941.1	13867.2		16402.5	10418.3	8074.3	10514.4	6887.9	8221.4	19794.8	11158.1	17015.4	9021.6	7497.8	1682.7	1295.9	10090.9	1316.7
				19315.6			13162.3			17497.4			15796.2	7676.1	10000.4	7496.7	6454.9	2631.7	11298.8	3773.3	10389.9	1129.6	6667.1	14522.6	1642.0	1795.0	
	Replicate 1			18453.2			16324.3			11812.0			19190.3	6159.5	10129.2	7844.4	10391.2	968.7	12072.9	8402.7	16013.1	8017.7	12409.7	3150.5	6499.9	1835.6	
				17995.5			17833.2			12099.5			17370.4	9150.7	9158.5	8995.5	8387.0	7599.2	16048.2	9919.4	11018.0	11042.8	11949.4	10333.5	12249.0		
				17830.2			14311.2			13348.9			11422.6														
				18216.8			13583.2			16211.7			19535.2														
Q25/+				12251.0			14745.2			11046.9			17059.4														
		17494.2	16148.3	17496.6		8672.6	15412.0	16844.3	12142.2	11274.4		18128.2		7646.2		9435.5	8963.9	8403.1	13130.2	13925.1	9999.4	1738.6	11925.6	742.7	959.5	4541.0	2020.6
		17125.7	16295.9	16639.8		900.8	18338.2	15957.8	15582.8	13094.8		16712.5		9128.0		6360.3	9315.1	15870.8	15978.5	14791.8	1350.4	15830.1	3235.1	594.3	8062.1	3203.3	1476.2
		18773.2	15328.5	19669.8		19417.6	17879.2	19238.4	10238.0	17314.1		15790.5		9302.9		7875.2	9517.5	4215.6	16684.1	7659.9	11216.9	21617.4	1113.8	6960.3	6455.3	2839.0	1184.5
		14454.7		16411.4			13483.5			13639.5				8203.7		7415.8	7676.1	11750.9	15062.0	10091.2	9727.1	1437.0	2701.0	686.3	1367.4	4989.9	
	Replicate 2	14315.5		16461.9			14946.1			17113.9				7933.2		8587.8	9575.4	12485.5	10124.0	10967.4	1043.7	3375.7	10627.6	855.3	1387.6	3902.5	
		16002.3		19532.7			13198.3			15884.5				6609.6		8504.5	6770.8	10592.0	13080.4	3605.5	7296.0	7014.9	10632.7	983.3	571.9		
		15588.2		14187.0			15194.1			13705.5																	
		16527.6		17739.0			15984.6			12936.4																	
		16017.2		16179.7			11018.7			11988.2																	
	Velocity																										
				13.1	10.4		9.8	8.8	10.3	10.2	10.3		9.4	10.4	8.5	7.8	9.0	7.8	9.2	3.3	8.0	3.1	6.1	5.6	2.2	1.6	2.5
				11.9	10.4		3.1	9.4	7.1	8.4	11.4		10.3	10.6	7.2	9.1	6.7	8.0	11.3	6.6	8.1	6.4	6.2	2.6	3.4	2.1	1.8
				12.9	11.6		10.4	10.6	9.1	7.1	8.4		8.5	11.1	8.7	10.4	8.8	10.2	11.7	7.1	9.5	6.0	5.7	3.1	2.3	6.2	1.8
				12.2			8.8			10.0			10.0	10.0	10.5	8.6	8.5	5.5	8.5	5.0	6.9	2.3	5.2	8.9	4.2	3.6	
	Replicate 1			11.9			9.6			7.4			11.1	7.4	10.9	8.3	11.0	3.1	8.9	6.9	9.5	5.7	7.5	4.9	4.5	1.8	
				11.9			9.7			7.6			9.5	10.8	9.7	8.9	9.2	9.0	10.3	7.6	7.1	8.2	7.4	8.3	7.6		
				12.3			9.1			8.7			7.6														
				11.7			8.0			9.9			10.5														
				10.5			8.6			7.2			8.7														
		10.5	9.8	10.6		6.6	10.4	10.3	7.5	7.3		10.4		8.6		9.9	9.4	6.0	9.1	8.0	6.4	2.1	7.3	1.9	1.7	2.7	1.8
		11.9	9.8	10.2		2.9	12.1	9.5	9.2	8.3		9.6		9.5		7.6	9.8	9.3	9.9	8.5	1.7	11.5	5.8	2.5	5.9	2.6	1.6
		11.9	9.6	11.8		12.1	11.6	11.7	7.6	10.1		9.2		9.9		9.0	10.6	4.8	10.5	5.1	6.4	11.5	1.5	6.7	4.8	2.2	1.4
		9.0		10.9			8.3			8.6				8.7		8.0	8.3	6.7	9.7	5.9	6.8	2.4	3.8	1.6	3.2	3.3	
	Replicate 2	11.1		10.6			10.5			10.3				8.2		9.3	10.0	6.9	9.0	5.9	2.2	3.4	7.7	3.2	2.6	3.4	
		10.4		12.3			8.1			9.7				7.2		9.1	9.1	6.8	9.3	2.5	7.9	4.8	6.8	1.6	1.5		
		10.0		9.5			8.8			8.1																	
		10.5		10.9			9.7			8.1																	
		9.9		10.4			7.3			7.7																	

Supplemental Table 2: Raw locomotor behaviour data from female genotypes. Each value represents one individual fly that was measured. All individuals of a genotype were averaged for each replicate in the final figures.

Genotype	Age (days)	1	2	3	4	6	7	8	9	10	11	13	14	16	17	21	24	29	31	35	38	42	46	51	54	57	63
	%immobile																										
				4.1	1.7		1.9	14.2	10.9	19.1	7.4		5.3	7.4	9.8	4.2	7.6	22.7	49.3	10.7	24.8	95.1	31.7	62.3	61.8	62.8	95.3
				10.9	16.5		0.4	13.5	4.8	5.6	9.5		2.2	1.9	5.9	8.7	12.8	29.9	51.4	43.9	76.8	46.4	48.1	81.6	74.6	74.9	94.9
				3.2	32.4		0.0	9.3	3.3	3.2	8.0		3.0	2.2	5.1	11.3	17.1	13.3	13.4	13.8	53.8	94.3	83.6	88.4	54.9	85.6	67.6
				28.0			5.9			10.8			3.3	7.0	8.9	8.0	6.6	15.3	84.2	30.1	11.3	97.6	93.0	79.8	54.7	57.8	
	Replicate 1			4.7			5.1			7.1			11.0	13.4	4.2	5.2	15.3	38.6	16.8	86.5	61.2	89.6	14.7	31.8	97.7	73.3	
				4.8			3.6			12.5			5.5	43.8	2.6	2.9	18.7	40.8	25.6	34.7	55.4	96.3	41.7	88.4	94.6	73.9	
				13.9			7.5			12.0			3.5														
				16.7			6.6			48.0			4.9														
				8.5			5.3			5.9			4.4														
		13.7	14.3	7.3		10.4	11.1	6.1	4.7	7.7		2.5		9.8		3.4	5.9	84.2	93.4	79.1	56.7	83.0	94.8	89.9	92.5	94.3	51.0
		10.8	3.8	33.7		14.6	8.5	4.9	12.4	4.3		7.4		6.0		8.7	11.0	95.6	78.0	62.1	3.3	14.8	41.6	95.5	94.4	97.3	94.3
		5.1	8.1	12.0		22.0	51.2	28.0	49.0	13.4		10.1		9.6		1.2	9.5	13.1	55.9	9.6	37.8	78.3	84.0	96.5	97.1	72.5	
		14.7		11.8			6.2			3.4				37.1		12.7	11.3	16.8	47.9	57.4	75.5	74.9	51.3	98.0	93.2	90.2	
	Replicate 2	13.1		8.3			19.2			4.3				14.4		8.9	5.6	25.2	31.3	18.9	33.5	78.2	20.4	85.8	97.6	93.8	
		12.4		7.7			10.4			5.3				6.7		9.4	6.1	44.4	15.9	10.9	27.6	70.5	90.7	91.1	93.6		
		46.9		4.6			16.1			13.3																	
		8.8		8.2			11.9			3.8																	
		14.8		4.9			38.8			18.6																	
	Distance																										
				18910.2	17989.7		487.9	10084.1	15036.5	13426.1	17071.4		15885.1	8243.6	9.8	9153.0	8096.5	6240.5	5045.4	10706.0	8761.7	775.6	6224.8	5620.6	2603.0	3110.5	1087.4
				15022.6	15390.7		875.1	14253.0	17062.6	13408.7	13778.6		20543.5	9594.3	9.6	9034.7	8381.5	5216.3	5651.9	3983.9	2785.5	4433.9	3894.3	2046.6	2169.0	2447.1	1076.7
				20262.8	14764.1		984.4	17775.1	15921.4	17226.2	14080.1		20277.7	9486.5	9.9	8814.9	7401.0	7881.3	13424.7	8137.3	4175.6	1044.1	1994.7	1598.7	3205.2	1727.5	2735.0
				14093.6			16759.4			15055.4			17547.8	7328.6	9304.8	7462.1	7840.0	7745.5	1944.4	7213.3	10483.5	792.2	1101.0	2046.4	3415.8	3838.7	
	Replicate 1			19564.3			19017.5			14611.2			15811.2	6259.2	9756.6	8892.0	6455.8	3783.8	11566.9	1293.6	3465.8	1046.3	7264.7	9616.3	767.1	2525.0	
				19572.6			17964.5			16189.7			19201.1	4322.3	9793.4	9119.9	5326.6	4441.7	8681.5	7710.1	4017.1	601.0	4458.5	1604.9	946.1	2521.0	
				17678.0			14825.5			16184.1			19499.7														
				14761.3			17488.3			7438.3			18320.4														
Q72/X				17668.3			19125.9			17509.0			16245.7														
		15909.5	16325.1	16253.6		17744.9	15591.4	16419.7	13325.8	18178.1		16791.8		8438.1		10037.3	10577.7	1985.9	1099.0	2057.8	4442.5	1673.8	1111.8	1333.2	829.2	2118.8	6501.1
		17830.6	17252.0	9158.8		13422.5	17664.4	17395.8	13707.4	17118.7		16800.4		8439.8		6883.1	8534.6	898.3	2719.5	3260.0	16039.6	10833.1	4611.6	975.3	845.1	1457.0	2017.2
		19680.6	19340.9	16150.2		15566.1	7108.6	13947.6	8955.7	15884.3		14879.6		9777.9		11460.8	8633.8	11074.1	6529.7	12691.0	6593.9	23137.2	1911.6	899.8	964.4	4738.1	
		15669.8		15999.3			16284.7			19798.8				4656.9		8303.1	8422.0	11355.2	5720.6	3057.2	2413.2	2115.7	3461.5	650.4	988.9	2436.4	
	Replicate 2	17058.7		17371.9			15213.7			16073.6				6226.8		6482.9	8931.6	8871.3	9774.5	7496.8	7335.2	2245.3	10065.8	906.9	816.8	2096.0	
		16168.8		20035.7			18226.7			16416.3				9157.6		6572.4	8687.9	5596.2	14794.2	9751.3	8712.8	2922.8	972.8	909.1	1138.0		
		11099.1		17816.6			10156.8			15463.6																	
		18369.0		18715.4			16068.5			16754.0																	
		12471.4		17973.7			6119.1			13050.8																	
	Velocity																										
				11.9	10.4		15.2	9.3	9.6	9.0	10.3		8.5	9.0	9.8	8.8	8.8	8.2	3.2	6.8	5.1	3.1	4.7	7.2	3.0	1.9	1.8
				10.0	10.5		5.9	11.3	10.4	7.8	8.4		10.8	9.7	9.6	9.1	9.8	7.2	5.2	3.5	4.0	4.3	3.6	2.5	3.7	2.6	1.6
				12.7	12.1		0.1	12.1	9.6	9.8	8.5		10.4	9.8	9.9	9.0	9.0	9.4	8.7	5.5	3.5	2.4	3.2	3.3	3.3	1.6	3.3
				11.4			9.1			9.4			9.1	8.9	10.4	6.2	8.4	9.1	1.7	5.6	5.5	1.4	1.5	4.3	3.2	2.3	
	Replicate 1			13.0			10.9			8.6			8.3	8.5	10.4	8.2	7.5	5.9	7.7	4.3	3.1	1.9	4.7	7.5	1.4	1.9	
				12.5			10.2			10.4			10.3	7.5	9.9	8.8	6.3	7.1	6.5	6.4	3.6	1.5	3.7	2.0	2.3	2.1	
				12.5			8.8			10.1			10.9														
				10.4			10.5			6.9			10.1														
				11.5			11.3			10.5			9.5														
		10.5	10.8	10.2		11.2	10.4	10.2	7.7	11.0		8.7		9.3		10.5	11.4	4.1	2.4	2.8	3.9	1.7	2.0	3.4	1.5	2.3	2.7
		11.3	10.8	7.5		8.8	10.7	10.6	8.8	10.0		9.9		9.0		7.6	9.6	1.8	5.3	4.0	9.6	7.2	3.8	2.7	1.4	1.5	1.6
		11.7	11.9	10.7		11.1	6.7	11.2	9.5	10.2		8.6		11.1		11.8	9.5	7.0	5.7	8.1	4.2	34.6	4.1	1.4	1.7	1.7	
		10.8		10.4			9.7			11.5				6.7		9.6	9.6	7.6	5.9	3.3	3.3	2.4	3.1	2.0	1.7	1.9	
	Replicate 2	12.0		11.1			10.4			9.3				7.1		7.4	9.6	6.6	7.9	5.0	5.0	4.0	6.8	2.3	1.7	1.6	
		10.8		12.6			11.2			9.7				9.9		7.1	9.0	5.0	10.1	6.3	4.8	3.3	2.8	1.9	1.5		
		11.4		11.0			6.6			10.0																	
		11.3		11.8			10.2			9.7																	
		8.1		11.4			4.7			8.9																	

Supplemental Table 2: Raw locomotor behaviour data from female genotypes. Each value represents one individual fly that was measured. All individuals of a genotype were averaged for each replicate in the final figures.

Genotype	Age (days)	1	2	3	4	6	7	8	9	10	11	13	14	16	17	21	24	29	31	35	38	42	46	51	54	57	63
	%immobile																										
			7.7	2.4	9.7	14.8	7.0	25.6	15.4	18.9	32.4	90.3	8.3	81.4	42.4	93.6	23.7	96.8	86.1								
	Replicate 1		12.3	4.2	3.3	8.2	10.4	7.6	16.2	28.0	88.9	28.9	90.7	35.3	49.5	80.2	56.4	75.6	96.0								
			18.1	2.7	8.1	12.7	14.4	62.8	13.7	36.4	30.3	43.6	21.8	16.2	89.4	69.2	73.4	89.9	97.5								
		9.7	40.7	13.2	10.9		10.9		21.5	6.9	97.6	8.9	57.3	27.5	49.6	91.8	82.2	97.3	89.1								
	Replicate 2	6.5	6.6	12.3	20.8		16.8		7.3	25.7	89.0	17.4	80.4	63.1	88.1	96.9	91.6	92.5	87.3								
		12.9	63.7	8.4	9.2		6.5		7.5	12.4	95.3	6.5	89.7	61.1	74.4	59.1	94.6	95.4									
	Distance																										
			19983.7	1131.8	15924.7	17413.3	8853.4	6290.1	9252.6	9407.8	9900.3	1344.6	12252.8	2010.2	9305.7	1087.4	11116.9	775.9	1503.3								
	Replicate 1		16448.9	1303.4	21764.6	17853.1	6836.7	10457.6	5008.3	6421.2	643.1	10191.6	1039.7	9056.4	5451.2	2115.1	3285.8	2467.2	1067.0								
			17173.5	1613.7	20581.3	16292.4	7322.4	3151.3	9470.3	3702.2	5896.4	9478.6	11694.9	10851.8	1560.2	3623.0	3579.7	1272.9	771.8								
DJ694/+		19770.3	11481.9	20309.4	23095.5		9164.0		6910.1	11403.4	950.6	27675.4	5818.1	9443.0	7434.9	1088.6	1812.8	741.4	3243.4								
	Replicate 2	22582.2	18495.2	17811.4	18053.2		7897.8		8381.7	5553.1	1365.0	14968.4	1905.2	3145.0	1508.2	929.8	1098.6	1083.9	2243.1								
		15988.8	3980.8	21687.0	17601.9		11750.6		8357.7	6291.8	1203.0	20437.9	2027.5	4090.0	12186.6	2529.9	1274.0	1237.8									
	Velocity																										
			12.8	7.3	9.7	11.7	9.7	8.3	11.3	11.9	15.4	2.9	7.7	2.8	9.1	1.5	7.9	1.8	2.3								
	Replicate 1		11.9	9.1	12.8	11.2	7.8	11.5	5.6	8.7	1.6	8.1	2.5	7.6	5.5	3.9	3.1	4.0	1.4								
			12.6	6.1	12.7	10.6	8.7	7.5	11.4	3.9	8.5	9.6	8.6	7.0	1.4	5.3	5.8	2.2	1.5								
		12.5	11.2	13.1	14.8		10.4		8.8	12.3	1.8	17.9	6.2	4.9	7.7	2.5	1.9	1.4	2.0								
	Replicate 2	13.7	12.1	11.4	12.8		9.6		9.0	7.4	2.9	10.2	2.2	2.5	3.1	1.8	1.4	1.7	1.8								
		10.3	3.9	13.4	10.9		13.0		9.1	6.9	4.1	12.6	5.6	4.1	20.7	2.2	1.7	1.3									
	%immobile																										
			9.6	25.7	4.0	26.7	9.3	10.3	18.9	17.7	77.9	12.1	33.9	22.9	25.0	90.9	9.9	98.2	69.4	95.3							
	Replicate 1		4.5	8.2	13.0	12.6	14.0	8.6	13.9	8.4	25.2	14.6	50.9	21.9	28.9	62.9	45.0	64.2	85.5	94.9							
			2.2	12.8	24.6	8.7	6.0	12.1	9.5	14.5	66.4	36.0	97.5	32.7	89.7	23.9	17.1		68.2	67.6							
		8.8	13.9	13.3	9.3		2.9		5.8	10.9	10.5	7.5	96.0	51.8	68.8	58.9	85.7	84.3	96.7	78.0							
	Replicate 2	7.9	13.6	16.9	9.8		2.2		36.2	5.2	54.3	8.4	73.1	74.9	89.6	37.1	92.8	88.9	76.9	58.8							
		45.1	12.1	7.6	4.0		4.7		81.7	13.2	57.5	60.6	62.5	52.5	73.0	92.7		76.1		98.9							
	Distance																										
			16524.1	11619.7	17594.1	10603.3	9110.6	10317.9	7717.7	6148.7	1797.7	11836.1	6174.7	9277.3	5756.0	1025.2	11150.2	603.4	3050.6	1087.4							
	Replicate 1		23895.7	17622.5	16649.1	13511.6	9435.6	9254.7	4564.1	7888.9	5676.5	12339.0	5358.6	9465.0	4779.7	2913.6	7270.1	2440.8	2461.7	1076.7							
MHC/+			21097.0	14536.6	14168.7	15415.9	7827.7	7923.2	7521.5	9820.9	1499.9	9138.5	915.3	5969.7	1517.6	6741.5	10423.4		5011.0	2735.0							
		20096.2	15082.0	15356.4	16604.9		11653.1		8822.2	8327.9	9708.7	17526.2	901.3	4803.2	3706.0	3584.4	1658.6	1507.1	858.7	19125.7							
	Replicate 2	20164.1	17121.3	17504.7	14566.7		12021.4		2919.5	9208.4	4035.0	13664.5	2283.8	2449.7	1333.9	9306.7	1551.6	1898.6	33596.1	12020.8							
		11051.4	20233.0	17589.8	14686.3		10153.5		932.3	6370.6	4788.7	7182.5	4248.9	6409.5	2727.7	878.6		784.5		932.3							
	Velocity																										
			11.3	8.7	10.4	8.0	11.4	11.5	9.8	7.3	7.4	7.8	5.2	6.5	3.9	2.0	6.8	1.4	4.5	1.8							
	Replicate 1		15.1	10.8	10.7	8.7	11.9	10.3	5.0	8.7	7.5	8.6	5.9	6.7	3.5	3.1	6.7	2.2	4.5	1.6							
			13.0	9.3	10.2	9.7	9.9	9.1	8.6	11.4	3.0	8.1	1.8	4.6	1.8	4.6	7.0		4.6	3.3							
		12.6	10.3	9.9	10.3		12.4		9.4	9.3	6.0	11.0	1.5	2.6	5.8	4.0	2.0	2.9	1.9	17.1							
	Replicate 2	12.3	11.6	11.7	9.0		12.7		3.8	9.8	4.6	8.6	3.8	1.9	2.5	8.1	5.1	6.2	27.4	4.5							
		11.1	13.5	10.7	8.5		10.9		1.9	7.0	5.7	9.7	5.5	3.9	3.4	2.1		2.5		1.7							

Supplemental Table 3: Sample arena configuration table used by the extraction script to identify which genotype is in each chamber based on the configuration that is input. The number of configurations is equal to the number of genotypes tested. The number of chambers required is equal to the number of genotypes tested multiplied by the sample size of each genotype.

	Config. 1	**Config. 2**	**Config. 3**	**Config. 4**	**Config. 5**	**Config. 6**	**Config. 7**	**Config. 8**	**Config. 9**	**Config. 10**	**Config. 11**	**Config. 12**	**Config. 13**	**Config. 14**	**Config. 15**	**Config. 16**
Chamber 1	Genotype 1	Genotype 16	Genotype 15	Genotype 14	Genotype 13	Genotype 12	Genotype 11	Genotype 10	Genotype 9	Genotype 8	Genotype 7	Genotype 6	Genotype 5	Genotype 4	Genotype 3	Genotype 2
Chamber 2	Genotype 1	Genotype 16	Genotype 15	Genotype 14	Genotype 13	Genotype 12	Genotype 11	Genotype 10	Genotype 9	Genotype 8	Genotype 7	Genotype 6	Genotype 5	Genotype 4	Genotype 3	Genotype 2
Chamber 3	Genotype 1	Genotype 16	Genotype 15	Genotype 14	Genotype 13	Genotype 12	Genotype 11	Genotype 10	Genotype 9	Genotype 8	Genotype 7	Genotype 6	Genotype 5	Genotype 4	Genotype 3	Genotype 2
Chamber 4	Genotype 2	Genotype 1	Genotype 16	Genotype 15	Genotype 14	Genotype 13	Genotype 12	Genotype 11	Genotype 10	Genotype 9	Genotype 8	Genotype 7	Genotype 6	Genotype 5	Genotype 4	Genotype 3
Chamber 5	Genotype 2	Genotype 1	Genotype 16	Genotype 15	Genotype 14	Genotype 13	Genotype 12	Genotype 11	Genotype 10	Genotype 9	Genotype 8	Genotype 7	Genotype 6	Genotype 5	Genotype 4	Genotype 3
Chamber 6	Genotype 2	Genotype 1	Genotype 16	Genotype 15	Genotype 14	Genotype 13	Genotype 12	Genotype 11	Genotype 10	Genotype 9	Genotype 8	Genotype 7	Genotype 6	Genotype 5	Genotype 4	Genotype 3
Chamber 7	Genotype 3	Genotype 2	Genotype 1	Genotype 16	Genotype 15	Genotype 14	Genotype 13	Genotype 12	Genotype 11	Genotype 10	Genotype 9	Genotype 8	Genotype 7	Genotype 6	Genotype 5	Genotype 4
Chamber 8	Genotype 3	Genotype 2	Genotype 1	Genotype 16	Genotype 15	Genotype 14	Genotype 13	Genotype 12	Genotype 11	Genotype 10	Genotype 9	Genotype 8	Genotype 7	Genotype 6	Genotype 5	Genotype 4
Chamber 9	Genotype 3	Genotype 2	Genotype 1	Genotype 16	Genotype 15	Genotype 14	Genotype 13	Genotype 12	Genotype 11	Genotype 10	Genotype 9	Genotype 8	Genotype 7	Genotype 6	Genotype 5	Genotype 4
Chamber 10	Genotype 4	Genotype 3	Genotype 2	Genotype 1	Genotype 16	Genotype 15	Genotype 14	Genotype 13	Genotype 12	Genotype 11	Genotype 10	Genotype 9	Genotype 8	Genotype 7	Genotype 6	Genotype 5
Chamber 11	Genotype 4	Genotype 3	Genotype 2	Genotype 1	Genotype 16	Genotype 15	Genotype 14	Genotype 13	Genotype 12	Genotype 11	Genotype 10	Genotype 9	Genotype 8	Genotype 7	Genotype 6	Genotype 5
Chamber 12	Genotype 4	Genotype 3	Genotype 2	Genotype 1	Genotype 16	Genotype 15	Genotype 14	Genotype 13	Genotype 12	Genotype 11	Genotype 10	Genotype 9	Genotype 8	Genotype 7	Genotype 6	Genotype 5
Chamber 13	Genotype 5	Genotype 4	Genotype 3	Genotype 2	Genotype 1	Genotype 16	Genotype 15	Genotype 14	Genotype 13	Genotype 12	Genotype 11	Genotype 10	Genotype 9	Genotype 8	Genotype 7	Genotype 6
Chamber 14	Genotype 5	Genotype 4	Genotype 3	Genotype 2	Genotype 1	Genotype 16	Genotype 15	Genotype 14	Genotype 13	Genotype 12	Genotype 11	Genotype 10	Genotype 9	Genotype 8	Genotype 7	Genotype 6
Chamber 15	Genotype 5	Genotype 4	Genotype 3	Genotype 2	Genotype 1	Genotype 16	Genotype 15	Genotype 14	Genotype 13	Genotype 12	Genotype 11	Genotype 10	Genotype 9	Genotype 8	Genotype 7	Genotype 6
Chamber 16	Genotype 6	Genotype 5	Genotype 4	Genotype 3	Genotype 2	Genotype 1	Genotype 16	Genotype 15	Genotype 14	Genotype 13	Genotype 12	Genotype 11	Genotype 10	Genotype 9	Genotype 8	Genotype 7
Chamber 17	Genotype 6	Genotype 5	Genotype 4	Genotype 3	Genotype 2	Genotype 1	Genotype 16	Genotype 15	Genotype 14	Genotype 13	Genotype 12	Genotype 11	Genotype 10	Genotype 9	Genotype 8	Genotype 7
Chamber 18	Genotype 6	Genotype 5	Genotype 4	Genotype 3	Genotype 2	Genotype 1	Genotype 16	Genotype 15	Genotype 14	Genotype 13	Genotype 12	Genotype 11	Genotype 10	Genotype 9	Genotype 8	Genotype 7
Chamber 19	Genotype 7	Genotype 6	Genotype 5	Genotype 4	Genotype 3	Genotype 2	Genotype 1	Genotype 16	Genotype 15	Genotype 14	Genotype 13	Genotype 12	Genotype 11	Genotype 10	Genotype 9	Genotype 8
Chamber 20	Genotype 7	Genotype 6	Genotype 5	Genotype 4	Genotype 3	Genotype 2	Genotype 1	Genotype 16	Genotype 15	Genotype 14	Genotype 13	Genotype 12	Genotype 11	Genotype 10	Genotype 9	Genotype 8
Chamber 21	Genotype 7	Genotype 6	Genotype 5	Genotype 4	Genotype 3	Genotype 2	Genotype 1	Genotype 16	Genotype 15	Genotype 14	Genotype 13	Genotype 12	Genotype 11	Genotype 10	Genotype 9	Genotype 8
Chamber 22	Genotype 8	Genotype 7	Genotype 6	Genotype 5	Genotype 4	Genotype 3	Genotype 2	Genotype 1	Genotype 16	Genotype 15	Genotype 14	Genotype 13	Genotype 12	Genotype 11	Genotype 10	Genotype 9
Chamber 23	Genotype 8	Genotype 7	Genotype 6	Genotype 5	Genotype 4	Genotype 3	Genotype 2	Genotype 1	Genotype 16	Genotype 15	Genotype 14	Genotype 13	Genotype 12	Genotype 11	Genotype 10	Genotype 9
Chamber 24	Genotype 8	Genotype 7	Genotype 6	Genotype 5	Genotype 4	Genotype 3	Genotype 2	Genotype 1	Genotype 16	Genotype 15	Genotype 14	Genotype 13	Genotype 12	Genotype 11	Genotype 10	Genotype 9
Chamber 25	Genotype 9	Genotype 8	Genotype 7	Genotype 6	Genotype 5	Genotype 4	Genotype 3	Genotype 2	Genotype 1	Genotype 16	Genotype 15	Genotype 14	Genotype 13	Genotype 12	Genotype 11	Genotype 10
Chamber 26	Genotype 9	Genotype 8	Genotype 7	Genotype 6	Genotype 5	Genotype 4	Genotype 3	Genotype 2	Genotype 1	Genotype 16	Genotype 15	Genotype 14	Genotype 13	Genotype 12	Genotype 11	Genotype 10
Chamber 27	Genotype 9	Genotype 8	Genotype 7	Genotype 6	Genotype 5	Genotype 4	Genotype 3	Genotype 2	Genotype 1	Genotype 16	Genotype 15	Genotype 14	Genotype 13	Genotype 12	Genotype 11	Genotype 10
Chamber 28	Genotype 10	Genotype 9	Genotype 8	Genotype 7	Genotype 6	Genotype 5	Genotype 4	Genotype 3	Genotype 2	Genotype 1	Genotype 16	Genotype 15	Genotype 14	Genotype 13	Genotype 12	Genotype 11
Chamber 29	Genotype 10	Genotype 9	Genotype 8	Genotype 7	Genotype 6	Genotype 5	Genotype 4	Genotype 3	Genotype 2	Genotype 1	Genotype 16	Genotype 15	Genotype 14	Genotype 13	Genotype 12	Genotype 11
Chamber 30	Genotype 10	Genotype 9	Genotype 8	Genotype 7	Genotype 6	Genotype 5	Genotype 4	Genotype 3	Genotype 2	Genotype 1	Genotype 16	Genotype 15	Genotype 14	Genotype 13	Genotype 12	Genotype 11
Chamber 31	Genotype 11	Genotype 10	Genotype 9	Genotype 8	Genotype 7	Genotype 6	Genotype 5	Genotype 4	Genotype 3	Genotype 2	Genotype 1	Genotype 16	Genotype 15	Genotype 14	Genotype 13	Genotype 12
Chamber 32	Genotype 11	Genotype 10	Genotype 9	Genotype 8	Genotype 7	Genotype 6	Genotype 5	Genotype 4	Genotype 3	Genotype 2	Genotype 1	Genotype 16	Genotype 15	Genotype 14	Genotype 13	Genotype 12
Chamber 33	Genotype 11	Genotype 10	Genotype 9	Genotype 8	Genotype 7	Genotype 6	Genotype 5	Genotype 4	Genotype 3	Genotype 2	Genotype 1	Genotype 16	Genotype 15	Genotype 14	Genotype 13	Genotype 12
Chamber 34	Genotype 12	Genotype 11	Genotype 10	Genotype 9	Genotype 8	Genotype 7	Genotype 6	Genotype 5	Genotype 4	Genotype 3	Genotype 2	Genotype 1	Genotype 16	Genotype 15	Genotype 14	Genotype 13
Chamber 35	Genotype 12	Genotype 11	Genotype 10	Genotype 9	Genotype 8	Genotype 7	Genotype 6	Genotype 5	Genotype 4	Genotype 3	Genotype 2	Genotype 1	Genotype 16	Genotype 15	Genotype 14	Genotype 13
Chamber 36	Genotype 12	Genotype 11	Genotype 10	Genotype 9	Genotype 8	Genotype 7	Genotype 6	Genotype 5	Genotype 4	Genotype 3	Genotype 2	Genotype 1	Genotype 16	Genotype 15	Genotype 14	Genotype 13
Chamber 37	Genotype 13	Genotype 12	Genotype 11	Genotype 10	Genotype 9	Genotype 8	Genotype 7	Genotype 6	Genotype 5	Genotype 4	Genotype 3	Genotype 2	Genotype 1	Genotype 16	Genotype 15	Genotype 14
Chamber 38	Genotype 13	Genotype 12	Genotype 11	Genotype 10	Genotype 9	Genotype 8	Genotype 7	Genotype 6	Genotype 5	Genotype 4	Genotype 3	Genotype 2	Genotype 1	Genotype 16	Genotype 15	Genotype 14
Chamber 39	Genotype 13	Genotype 12	Genotype 11	Genotype 10	Genotype 9	Genotype 8	Genotype 7	Genotype 6	Genotype 5	Genotype 4	Genotype 3	Genotype 2	Genotype 1	Genotype 16	Genotype 15	Genotype 14
Chamber 40	Genotype 14	Genotype 13	Genotype 12	Genotype 11	Genotype 10	Genotype 9	Genotype 8	Genotype 7	Genotype 6	Genotype 5	Genotype 4	Genotype 3	Genotype 2	Genotype 1	Genotype 16	Genotype 15
Chamber 41	Genotype 14	Genotype 13	Genotype 12	Genotype 11	Genotype 10	Genotype 9	Genotype 8	Genotype 7	Genotype 6	Genotype 5	Genotype 4	Genotype 3	Genotype 2	Genotype 1	Genotype 16	Genotype 15
Chamber 42	Genotype 14	Genotype 13	Genotype 12	Genotype 11	Genotype 10	Genotype 9	Genotype 8	Genotype 7	Genotype 6	Genotype 5	Genotype 4	Genotype 3	Genotype 2	Genotype 1	Genotype 16	Genotype 15
Chamber 43	Genotype 15	Genotype 14	Genotype 13	Genotype 12	Genotype 11	Genotype 10	Genotype 9	Genotype 8	Genotype 7	Genotype 6	Genotype 5	Genotype 4	Genotype 3	Genotype 2	Genotype 1	Genotype 16
Chamber 44	Genotype 15	Genotype 14	Genotype 13	Genotype 12	Genotype 11	Genotype 10	Genotype 9	Genotype 8	Genotype 7	Genotype 6	Genotype 5	Genotype 4	Genotype 3	Genotype 2	Genotype 1	Genotype 16
Chamber 45	Genotype 15	Genotype 14	Genotype 13	Genotype 12	Genotype 11	Genotype 10	Genotype 9	Genotype 8	Genotype 7	Genotype 6	Genotype 5	Genotype 4	Genotype 3	Genotype 2	Genotype 1	Genotype 16
Chamber 46	Genotype 16	Genotype 15	Genotype 14	Genotype 13	Genotype 12	Genotype 11	Genotype 10	Genotype 9	Genotype 8	Genotype 7	Genotype 6	Genotype 5	Genotype 4	Genotype 3	Genotype 2	Genotype 1
Chamber 47	Genotype 16	Genotype 15	Genotype 14	Genotype 13	Genotype 12	Genotype 11	Genotype 10	Genotype 9	Genotype 8	Genotype 7	Genotype 6	Genotype 5	Genotype 4	Genotype 3	Genotype 2	Genotype 1
Chamber 48	Genotype 16	Genotype 15	Genotype 14	Genotype 13	Genotype 12	Genotype 11	Genotype 10	Genotype 9	Genotype 8	Genotype 7	Genotype 6	Genotype 5	Genotype 4	Genotype 3	Genotype 2	Genotype 1

Supplemental Table 4: One-way ANOVA analysis of change in locomotion with age in males.

Q25/+ replicate 1					
ANOVA			**Percent Immobility**	**Distance**	**Velocity**
	F(DFn,DFd)		F(21,107)=21.50	F(21,107)=16.53	F(21,107)=9.487
	p value		<0.0001	<0.0001	<0.0001
Dunnett's multiple comparison test (α=0.05)	**3 vs. 4**	**95% CI of diff.**	-35.69 to 27.50	-5265 to 9976	-2.963 to 7.640
		Significant?	No	No	No
	3 vs. 7	**95% CI of diff.**	-16.16 to 28.53	7533 to 18310	-0.8569 to 6.641
		Significant?	No	Yes	No
	3 vs. 8	**95% CI of diff.**	-36.86 to 26.34	-2360 to 12881	-1.091 to 9.512
		Significant?	No	No	No
	3 vs. 9	**95% CI of diff.**	-24.05 to 39.15	-5989 to 9252	-2.907 to 7.696
		Significant?	No	No	No
	3 vs. 10	**95% CI of diff.**	-19.95 to 24.74	-4060 to 6717	-1.778 to 5.720
		Significant?	No	No	No
	3 vs. 11	**95% CI of diff.**	-23.86 to 39.33	-6548 to 8693	-2.944 to 7.659
		Significant?	No	No	No
	3 vs. 14	**95% CI of diff.**	-14.79 to 29.90	-5315 to 5462	-1.492 to 6.005
		Significant?	No	No	No
	3 vs. 16	**95% CI of diff.**	-18.26 to 31.70	3330 to 15379	-2.075 to 6.308
		Significant?	No	Yes	No
	3 vs. 17	**95% CI of diff.**	-18.44 to 31.52	3475 to 15524	-1.792 to 6.590
		Significant?	No	Yes	No
	3 vs. 21	**95% CI of diff.**	-17.36 to 32.60	2925 to 14974	-1.999 to 6.383
		Significant?	No	Yes	No
	3 vs. 24	**95% CI of diff.**	-24.35 to 25.62	4480 to 16529	-1.607 to 6.775
		Significant?	No	Yes	No
	3 vs. 29	**95% CI of diff.**	-67.81 to -17.85	10015 to 22064	2.923 to 11.31
		Significant?	Yes	Yes	Yes
	3 vs. 31	**95% CI of diff.**	-42.25 to 7.709	1508 to 13557	0.1645 to 8.547
		Significant?	No	Yes	Yes
	3 vs. 35	**95% CI of diff.**	-67.41 to -17.45	7775 to 19824	3.231 to 11.61
		Significant?	Yes	Yes	Yes
	3 vs. 38	**95% CI of diff.**	-74.31 to -24.35	8842 to 20891	4.969 to 13.35
		Significant?	Yes	Yes	Yes
	3 vs. 42	**95% CI of diff.**	-64.86 to -14.90	7224 to 19273	3.037 to 11.42
		Significant?	Yes	Yes	Yes
	3 vs. 46	**95% CI of diff.**	-78.56 to -28.60	10166 to 22215	5.972 to 14.35
		Significant?	Yes	Yes	Yes
	3 vs. 51	**95% CI of diff.**	-82.93 to -32.97	9552 to 21601	4.130 to 12.51
		Significant?	Yes	Yes	Yes
	3 vs. 54	**95% CI of diff.**	-100.6 to -50.62	12045 to 24094	4.658 to 13.04
		Significant?	Yes	Yes	Yes
	3 vs. 57	**95% CI of diff.**	-93.53 to -43.57	11476 to 23525	6.546 to 14.93
		Significant?	Yes	Yes	Yes
	3 vs. 63	**95% CI of diff.**	-109.8 to -46.61	10744 to 25985	4.368 to 14.97
		Significant?	Yes	Yes	Yes

	Q25/+ replicate 2				
ANOVA			Percent Immobility	Distance	Velocity
	F(DFn,DFd)		F(21,104)=11.18	F(21,104)=7.679	F(21,104)=8.936
	p value		<0.0001	<0.0001	<0.0001
Dunnett's multiple comparison test (α=0.05)	1 vs. 2	95% CI of diff.	-40.67 to 42.91	-10471 to 10592	-4.554 to 5.567
		Significant?	No	No	No
	1 vs. 3	95% CI of diff.	-25.95 to 33.15	-6257 to 8636	-2.387 to 4.769
		Significant?	No	No	No
	1 vs. 6	95% CI of diff.	-45.92 to 37.66	-6788 to 14274	-2.925 to 7.196
		Significant?	No	No	No
	1 vs. 7	95% CI of diff.	-25.99 to 33.11	-5623 to 9271	-1.825 to 5.331
		Significant?	No	No	No
	1 vs. 8	95% CI of diff.	-34.60 to 48.97	-9924 to 11138	-3.851 to 6.270
		Significant?	No	No	No
	1 vs. 9	95% CI of diff.	-34.93 to 48.64	-9464 to 11599	-3.319 to 6.802
		Significant?	No	No	No
	1 vs. 10	95% CI of diff.	-32.60 to 26.50	-2602 to 12291	-0.1873 to 6.969
		Significant?	No	No	No
	1 vs. 13	95% CI of diff.	-47.54 to 36.04	-7535 to 13527	-3.430 to 6.691
		Significant?	No	No	No
	1 vs. 16	95% CI of diff.	-27.48 to 38.59	2431 to 19082	-1.769 to 6.231
		Significant?	No	Yes	No
	1 vs. 21	95% CI of diff.	-30.24 to 35.83	2384 to 19035	-1.668 to 6.333
		Significant?	No	Yes	No
	1 vs. 24	95% CI of diff.	-39.75 to 26.33	2338 to 18989	-1.801 to 6.200
		Significant?	No	Yes	No
	1 vs. 29	95% CI of diff.	-47.27 to 18.81	147.2 to 16798	1.672 to 9.673
		Significant?	No	Yes	Yes
	1 vs. 31	95% CI of diff.	-42.22 to 23.86	-2644 to 14008	-1.240 to 6.761
		Significant?	No	No	No
	1 vs. 35	95% CI of diff.	-46.97 to 19.10	460.3 to 17111	1.814 to 9.815
		Significant?	No	Yes	Yes
	1 vs. 38	95% CI of diff.	-90.39 to -24.32	7481 to 24132	4.156 to 12.16
		Significant?	Yes	Yes	Yes
	1 vs. 42	95% CI of diff.	-79.63 to -13.56	2693 to 19344	-0.7204 to 7.280
		Significant?	Yes	Yes	No
	1 vs. 46	95% CI of diff.	-98.11 to -32.04	8088 to 24740	3.418 to 11.42
		Significant?	Yes	Yes	Yes
	1 vs. 51	95% CI of diff.	-108.0 to -41.92	9783 to 26434	5.515 to 13.52
		Significant?	Yes	Yes	Yes
	1 vs. 54	95% CI of diff.	-101.1 to -34.98	8977 to 25628	6.024 to 14.03
		Significant?	Yes	Yes	Yes
	1 vs. 57	95% CI of diff.	-81.55 to -15.47	-380.0 to 16271	3.625 to 11.63
		Significant?	Yes	No	Yes
	1 vs. 63	95% CI of diff.	-119.7 to -36.16	8214 to 29277	4.674 to 14.79
		Significant?	Yes	Yes	Yes

Q72/Y replicate 1					
ANOVA			Percent Immobility	Distance	Velocity
	F(DFn,DFd)		F(21,106)=19.65	F(21,106)=16.27	F(21,106)=13.82
	p value		<0.0001	<0.0001	<0.0001
Dunnett's multiple comparison test (α=0.05)	3 vs. 4	95% CI of diff.	-33.59 to 32.65	-5485 to 10227	-2.352 to 6.840
		Significant?	No	No	No
	3 vs. 7	95% CI of diff.	-19.95 to 26.88	8644 to 19755	1.697 to 8.197
		Significant?	No	Yes	Yes
	3 vs. 8	95% CI of diff.	-41.14 to 25.10	-3789 to 11923	-0.1224 to 9.070
		Significant?	No	No	No
	3 vs. 9	95% CI of diff.	-34.03 to 32.21	-3811 to 11901	-1.522 to 7.670
		Significant?	No	No	No
	3 vs. 10	95% CI of diff.	-24.63 to 22.21	-2703 to 8407	-0.6916 to 5.808
		Significant?	No	No	No
	3 vs. 11	95% CI of diff.	-32.71 to 33.53	-4220 to 11492	-1.450 to 7.742
		Significant?	No	No	No
	3 vs. 14	95% CI of diff.	-22.57 to 24.27	-4249 to 6861	-0.03104 to 6.469
		Significant?	No	No	No
	3 vs. 16	95% CI of diff.	-26.93 to 25.43	5283 to 17704	-0.6692 to 6.598
		Significant?	No	Yes	No
	3 vs. 17	95% CI of diff.	-26.85 to 25.51	4301 to 16722	-1.510 to 5.757
		Significant?	No	Yes	No
	3 vs. 21	95% CI of diff.	-22.76 to 29.60	3191 to 15612	-1.799 to 5.467
		Significant?	No	Yes	No
	3 vs. 24	95% CI of diff.	-29.95 to 22.42	4392 to 16814	-2.248 to 5.018
		Significant?	No	Yes	No
	3 vs. 29	95% CI of diff.	-61.72 to -9.356	9399 to 21821	2.144 to 9.410
		Significant?	Yes	Yes	Yes
	3 vs. 31	95% CI of diff.	-58.27 to -5.901	6197 to 18618	3.676 to 10.94
		Significant?	Yes	Yes	Yes
	3 vs. 35	95% CI of diff.	-81.07 to -28.71	9822 to 22243	3.742 to 11.01
		Significant?	Yes	Yes	Yes
	3 vs. 38	95% CI of diff.	-70.57 to -18.20	9093 to 21514	6.030 to 13.30
		Significant?	Yes	Yes	Yes
	3 vs. 42	95% CI of diff.	-92.67 to -40.30	11585 to 24007	5.654 to 12.92
		Significant?	Yes	Yes	Yes
	3 vs. 46	95% CI of diff.	-107.4 to -55.00	13507 to 25928	7.080 to 14.35
		Significant?	Yes	Yes	Yes
	3 vs. 51	95% CI of diff.	-71.79 to -19.43	9024 to 21445	4.733 to 12.00
		Significant?	Yes	Yes	Yes
	3 vs. 54	95% CI of diff.	-101.5 to -49.09	13173 to 25595	7.022 to 14.29
		Significant?	Yes	Yes	Yes
	3 vs. 57	95% CI of diff.	-67.27 to -11.85	8793 to 21939	5.458 to 13.15
		Significant?	Yes	Yes	Yes
	3 vs. 63	95% CI of diff.	-125.5 to -59.23	12946 to 28658	7.477 to 16.67
		Significant?	Yes	Yes	Yes

Q72/Y replicate 2					
ANOVA			Percent Immobility	Distance	Velocity
	F(DFn,DFd)		F(21,102)=33.11	F(21,102)=16.09	F(21,102)=13.83
	p value		<0.0001	<0.0001	<0.0001
Dunnett's multiple comparison test (α=0.05)	1 vs. 2	95% CI of diff.	-22.65 to 31.69	-11070 to 6376	-5.966 to 3.984
		Significant?	No	No	No
	1 vs. 3	95% CI of diff.	-13.99 to 24.43	-10025 to 2312	-5.611 to 1.424
		Significant?	No	No	No
	1 vs. 6	95% CI of diff.	-22.57 to 31.77	-11503 to 5943	-6.102 to 3.848
		Significant?	No	No	No
	1 vs. 7	95% CI of diff.	-18.19 to 20.24	-6715 to 5621	-3.433 to 3.603
		Significant?	No	No	No
	1 vs. 8	95% CI of diff.	-23.01 to 31.33	-12143 to 5304	-6.984 to 2.965
		Significant?	No	No	No
	1 vs. 9	95% CI of diff.	-25.28 to 29.07	-9249 to 8197	-4.940 to 5.010
		Significant?	No	No	No
	1 vs. 10	95% CI of diff.	-17.45 to 20.97	-7451 to 4885	-3.966 to 3.069
		Significant?	No	No	No
	1 vs. 13	95% CI of diff.	-21.75 to 32.60	-10124 to 7322	-5.073 to 4.877
		Significant?	No	No	No
	1 vs. 16	95% CI of diff.	-15.98 to 26.98	-148.2 to 13644	-4.732 to 3.134
		Significant?	No	No	No
	1 vs. 21	95% CI of diff.	-22.36 to 20.61	1751 to 15543	-3.541 to 4.325
		Significant?	No	Yes	No
	1 vs. 24	95% CI of diff.	-15.97 to 26.99	1445 to 15237	-3.063 to 4.803
		Significant?	No	Yes	No
	1 vs. 29	95% CI of diff.	-47.61 to -4.650	1384 to 15177	0.2338 to 8.100
		Significant?	Yes	Yes	Yes
	1 vs. 31	95% CI of diff.	-28.40 to 14.56	-4648 to 9145	-3.490 to 4.376
		Significant?	No	No	No
	1 vs. 35	95% CI of diff.	-67.54 to -24.58	4975 to 18767	1.856 to 9.722
		Significant?	Yes	Yes	Yes
	1 vs. 38	95% CI of diff.	-62.09 to -19.13	5695 to 19488	3.936 to 11.80
		Significant?	Yes	Yes	Yes
	1 vs. 42	95% CI of diff.	-73.96 to -31.00	-1392 to 12401	-2.126 to 5.740
		Significant?	Yes	No	No
	1 vs. 46	95% CI of diff.	-92.47 to -49.51	8919 to 22712	2.752 to 10.62
		Significant?	Yes	Yes	Yes
	1 vs. 51	95% CI of diff.	-98.85 to -55.89	9783 to 23575	4.147 to 12.01
		Significant?	Yes	Yes	Yes
	1 vs. 54	95% CI of diff.	-105.4 to -62.41	10441 to 24234	5.728 to 13.59
		Significant?	Yes	Yes	Yes
	1 vs. 57	95% CI of diff.	-90.67 to -41.68	7736 to 23462	3.932 to 12.90
		Significant?	Yes	Yes	Yes
	1 vs. 63	95% CI of diff.	-110.6 to -56.28	8604 to 26050	4.474 to 14.42
		Significant?	Yes	Yes	Yes

DJ694/+ replicate 1					
ANOVA			Percent Immobility	Distance	Velocity
	F(DFn,DFd)		F(16,34)=4.874	F(16,34)=4.229	F(16,34)=1.818
	p value		<0.0001	0.0002	0.0705
Dunnett's multiple comparison test (α=0.05)	3 vs. 7	95% CI of diff.	-40.67 to 85.49	1229 to 28310	-7.203 to 15.30
		Significant?	No	Yes	No
	3 vs. 10	95% CI of diff.	-48.16 to 78.00	-18909 to 8173	-13.87 to 8.637
		Significant?	No	No	No
	3 vs. 14	95% CI of diff.	-42.95 to 83.21	-19403 to 7679	-13.51 to 8.993
		Significant?	No	No	No
	3 vs. 16	95% CI of diff.	-48.61 to 77.55	-7866 to 19215	-12.08 to 10.42
		Significant?	No	No	No
	3 vs. 17	95% CI of diff.	-64.60 to 61.56	-5562 to 21520	-10.09 to 12.41
		Significant?	No	No	No
	3 vs. 21	95% CI of diff.	-65.29 to 60.88	-4424 to 22658	-8.817 to 13.69
		Significant?	No	No	No
	3 vs. 24	95% CI of diff.	-57.58 to 68.59	-7033 to 20049	-10.70 to 11.80
		Significant?	No	No	No
	3 vs. 29	95% CI of diff.	-74.10 to 52.06	-5015 to 22067	-9.784 to 12.72
		Significant?	No	No	No
	3 vs. 31	95% CI of diff.	-107.6 to 18.57	-5132 to 21950	-7.041 to 15.46
		Significant?	No	No	No
	3 vs. 35	95% CI of diff.	-134.3 to -8.175	1880 to 28961	-3.310 to 19.19
		Significant?	Yes	Yes	No
	3 vs. 38	95% CI of diff.	-122.1 to 4.035	451.5 to 27533	-4.039 to 18.46
		Significant?	No	Yes	No
	3 vs. 42	95% CI of diff.	-102.6 to 23.59	-3986 to 23095	-5.940 to 16.56
		Significant?	No	No	No
	3 vs. 46	95% CI of diff.	-112.6 to 13.60	-214.3 to 26867	-3.645 to 18.86
		Significant?	No	No	No
	3 vs. 51	95% CI of diff.	-118.9 to 7.285	-193.5 to 26888	-4.250 to 18.25
		Significant?	No	No	No
	3 vs. 54	95% CI of diff.	-106.4 to 19.73	-3519 to 23563	-6.286 to 16.22
		Significant?	No	No	No
	3 vs. 57	95% CI of diff.	-123.8 to 2.356	673.2 to 27755	-4.064 to 18.44
		Significant?	No	Yes	No

DJ694/+ replicate 2					
ANOVA			Percent Immobility	Distance	Velocity
	F(DFn,DFd)		F(15,30)=17.51	F(15,30)=8.646	F(15,30)=4.644
	p value		<0.0001	<0.0001	0.0002
Dunnett's multiple comparison test (α=0.05)	1 vs. 3	95% CI of diff.	-44.34 to 23.52	-9191 to 9451	-9.463 to 7.140
		Significant?	No	No	No
	1 vs. 7	95% CI of diff.	-59.23 to 8.628	-4082 to 14561	-5.618 to 10.98
		Significant?	No	No	No
	1 vs. 10	95% CI of diff.	-52.40 to 15.46	-7331 to 11312	-8.758 to 7.845
		Significant?	No	No	No
	1 vs. 16	95% CI of diff.	-38.27 to 29.59	41.53 to 18684	-6.578 to 10.02
		Significant?	No	Yes	No
	1 vs. 21	95% CI of diff.	-40.30 to 27.56	42.16 to 18684	-7.178 to 9.425
		Significant?	No	Yes	No
	1 vs. 24	95% CI of diff.	-102.3 to -34.46	7813 to 26456	0.8415 to 17.44
		Significant?	Yes	Yes	Yes
	1 vs. 29	95% CI of diff.	-105.2 to -37.37	6880 to 25522	1.042 to 17.65
		Significant?	Yes	Yes	Yes
	1 vs. 31	95% CI of diff.	-117.2 to -49.38	7551 to 26193	-0.6505 to 15.95
		Significant?	Yes	Yes	No
	1 vs. 35	95% CI of diff.	-100.8 to -32.90	6744 to 25386	1.360 to 17.96
		Significant?	Yes	Yes	Yes
	1 vs. 38	95% CI of diff.	-93.92 to -26.06	5925 to 24568	-0.1623 to 16.44
		Significant?	Yes	Yes	No
	1 vs. 42	95% CI of diff.	-112.0 to -44.16	3917 to 22560	-5.456 to 11.15
		Significant?	Yes	Yes	No
	1 vs. 46	95% CI of diff.	-120.8 to -52.96	8077 to 26719	1.397 to 18.00
		Significant?	Yes	Yes	Yes
	1 vs. 51	95% CI of diff.	-117.1 to -41.25	6466 to 27309	-0.7849 to 17.78
		Significant?	Yes	Yes	No
	1 vs. 54	95% CI of diff.	-115.8 to -47.96	7561 to 26204	1.113 to 17.72
		Significant?	Yes	Yes	Yes
	1 vs. 57	95% CI of diff.	-125.0 to -49.08	5700 to 26543	-0.01104 to 18.55
		Significant?	Yes	Yes	No

MHC/+ replicate 1					
ANOVA			Percent Immobility	Distance	Velocity
	F(DFn,DFd)		F(17,36)=4.009	F(17,36)=3.138	F(17,36)=2.739
	p value		0.0002	0.0019	0.0054
Dunnett's multiple comparison test (α=0.05)	3 vs. 7	95% CI of diff.	-54.90 to 68.55	5308 to 29599	-3.094 to 13.77
		Significant?	No	Yes	No
	3 vs. 10	95% CI of diff.	-71.37 to 52.07	-9673 to 14618	-7.153 to 9.707
		Significant?	No	No	No
	3 vs. 14	95% CI of diff.	-69.39 to 54.06	-8207 to 16084	-5.796 to 11.06
		Significant?	No	No	No
	3 vs. 16	95% CI of diff.	-58.15 to 65.29	-5456 to 18835	-9.155 to 7.705
		Significant?	No	No	No
	3 vs. 17	95% CI of diff.	-59.11 to 64.33	-3100 to 21191	-6.286 to 10.57
		Significant?	No	No	No
	3 vs. 21	95% CI of diff.	-81.06 to 42.38	1137 to 25428	-2.964 to 13.89
		Significant?	No	Yes	No
	3 vs. 24	95% CI of diff.	-72.05 to 51.39	-1077 to 23214	-4.240 to 12.62
		Significant?	No	No	No
	3 vs. 29	95% CI of diff.	-87.16 to 36.28	-724.0 to 23567	-7.086 to 9.773
		Significant?	No	No	No
	3 vs. 31	95% CI of diff.	-114.1 to 9.358	798.8 to 25090	-0.8969 to 15.96
		Significant?	No	Yes	No
	3 vs. 35	95% CI of diff.	-113.0 to 10.44	-102.6 to 24188	-4.017 to 12.84
		Significant?	No	No	No
	3 vs. 38	95% CI of diff.	-105.7 to 17.70	295.6 to 24587	-2.033 to 14.83
		Significant?	No	Yes	No
	3 vs. 42	95% CI of diff.	-125.8 to -2.370	-1136 to 23156	-6.883 to 9.976
		Significant?	Yes	No	No
	3 vs. 46	95% CI of diff.	-114.1 to 9.301	706.1 to 24997	-0.9674 to 15.89
		Significant?	No	Yes	No
	3 vs. 51	95% CI of diff.	-96.41 to 27.04	-3236 to 21055	-2.853 to 14.01
		Significant?	No	No	No
	3 vs. 54	95% CI of diff.	-143.6 to -20.17	5556 to 29847	0.5132 to 17.37
		Significant?	Yes	Yes	Yes
	3 vs. 57	95% CI of diff.	-129.0 to -5.518	4093 to 28384	1.024 to 17.88
		Significant?	Yes	Yes	Yes
	3 vs. 63	95% CI of diff.	-130.1 to -6.665	4373 to 28664	1.235 to 18.09
		Significant?	Yes	Yes	Yes

MHC/+ replicate 2					
ANOVA			Percent Immobility	Distance	Velocity
	F(DFn,DFd)		F(16,34)=7.196	F(16,34)=5.601	F(16,34)=4.114
	p value		<0.0001	<0.0001	0.0003
Dunnett's multiple comparison test (α=0.05)	1 vs. 3	95% CI of diff.	-53.00 to 50.77	-11546 to 13325	-8.025 to 7.409
		Significant?	No	No	No
	1 vs. 7	95% CI of diff.	-59.08 to 44.68	-7310 to 17561	-4.874 to 10.56
		Significant?	No	No	No
	1 vs. 10	95% CI of diff.	-55.32 to 48.45	-8230 to 16641	-5.594 to 9.840
		Significant?	No	No	No
	1 vs. 16	95% CI of diff.	-54.99 to 48.77	-196.0 to 24675	-5.133 to 10.30
		Significant?	No	No	No
	1 vs. 21	95% CI of diff.	-67.64 to 36.12	1272 to 26143	-4.939 to 10.50
		Significant?	No	Yes	No
	1 vs. 24	95% CI of diff.	-52.65 to 51.12	-1024 to 23847	-5.944 to 9.490
		Significant?	No	No	No
	1 vs. 29	95% CI of diff.	-133.2 to -29.45	8052 to 32923	3.439 to 18.87
		Significant?	Yes	Yes	Yes
	1 vs. 31	95% CI of diff.	-81.32 to 22.44	-7448 to 17423	-6.094 to 9.341
		Significant?	No	No	No
	1 vs. 35	95% CI of diff.	-115.1 to -11.31	4749 to 29620	-1.971 to 13.46
		Significant?	Yes	Yes	No
	1 vs. 38	95% CI of diff.	-103.3 to 0.4314	3994 to 28865	0.6628 to 16.10
		Significant?	No	Yes	Yes
	1 vs. 42	95% CI of diff.	-101.4 to 2.399	539.5 to 25410	-4.215 to 11.22
		Significant?	No	Yes	No
	1 vs. 46	95% CI of diff.	-101.0 to 2.775	1923 to 26794	-2.858 to 12.58
		Significant?	No	Yes	No
	1 vs. 51	95% CI of diff.	-115.9 to -12.11	2465 to 27335	-2.191 to 13.24
		Significant?	Yes	Yes	No
	1 vs. 54	95% CI of diff.	-139.8 to -36.06	8599 to 33470	3.818 to 19.25
		Significant?	Yes	Yes	Yes
	1 vs. 57	95% CI of diff.	-130.3 to -26.51	7734 to 32605	0.6677 to 16.10
		Significant?	Yes	Yes	Yes
	1 vs. 63	95% CI of diff.	-124.3 to -20.50	6917 to 31788	0.6488 to 16.08
		Significant?	Yes	Yes	Yes

Supplemental Table 5: One-way ANOVA analysis of change in locomotion with age in females.

Q25/+ replicate 1					
ANOVA			Percent Immobility	Distance	Velocity
	F(DFn,DFd)		F(21,106)=11.20	F(21,106)=13.70	F(21,106)=13.83
	p value		<0.0001	<0.0001	<0.0001
Dunnett's multiple comparison test (α=0.05)	3 vs. 4	95% CI of diff.	-30.49 to 35.08	-6353 to 6673	-1.893 to 4.401
		Significant?	No	No	No
	3 vs. 7	95% CI of diff.	-20.59 to 25.79	1161 to 10372	1.247 to 5.698
		Significant?	No	Yes	Yes
	3 vs. 8	95% CI of diff.	-34.28 to 31.30	-929.7 to 12097	-0.7147 to 5.579
		Significant?	No	No	No
	3 vs. 9	95% CI of diff.	-31.54 to 34.04	10923 to 23950	0.05678 to 6.351
		Significant?	No	Yes	Yes
	3 vs. 10	95% CI of diff.	-23.63 to 22.74	-309.8 to 8901	1.303 to 5.754
		Significant?	No	No	Yes
	3 vs. 11	95% CI of diff.	-31.64 to 33.93	-4774 to 8252	-1.168 to 5.126
		Significant?	No	No	No
	3 vs. 14	95% CI of diff.	-18.76 to 27.61	-3325 to 5886	0.2878 to 4.738
		Significant?	No	No	Yes
	3 vs. 16	95% CI of diff.	-23.35 to 28.50	4232 to 14530	-0.4982 to 4.478
		Significant?	No	Yes	No
	3 vs. 17	95% CI of diff.	-22.37 to 29.47	4319 to 14617	0.2830 to 5.259
		Significant?	No	Yes	Yes
	3 vs. 21	95% CI of diff.	-24.39 to 27.46	4513 to 14811	0.6726 to 5.649
		Significant?	No	Yes	Yes
	3 vs. 24	95% CI of diff.	-33.76 to 18.09	5539 to 15837	0.6594 to 5.635
		Significant?	No	Yes	Yes
	3 vs. 29	95% CI of diff.	-53.52 to -1.676	7835 to 18133	2.278 to 7.254
		Significant?	Yes	Yes	Yes
	3 vs. 31	95% CI of diff.	-29.65 to 22.19	-2180 to 8118	-0.4465 to 4.529
		Significant?	No	No	No
	3 vs. 35	95% CI of diff.	-55.27 to -3.421	5808 to 16107	3.480 to 8.456
		Significant?	Yes	Yes	Yes
	3 vs. 38	95% CI of diff.	-29.75 to 22.09	-330.9 to 9967	1.357 to 6.333
		Significant?	No	No	Yes
	3 vs. 42	95% CI of diff.	-55.03 to -3.187	6122 to 16420	4.268 to 9.244
		Significant?	Yes	Yes	Yes
	3 vs. 46	95% CI of diff.	-39.88 to 11.97	3539 to 13837	3.195 to 8.171
		Significant?	No	Yes	Yes
	3 vs. 51	95% CI of diff.	-76.42 to -24.57	7243 to 17541	3.988 to 8.964
		Significant?	Yes	Yes	Yes
	3 vs. 54	95% CI of diff.	-80.12 to -28.28	8977 to 19275	5.522 to 10.50
		Significant?	Yes	Yes	Yes
	3 vs. 57	95% CI of diff.	-89.72 to -34.85	9324 to 20222	6.335 to 11.60
		Significant?	Yes	Yes	Yes
	3 vs. 63	95% CI of diff.	-112.8 to -47.26	10332 to 23358	6.859 to 13.15
		Significant?	Yes	Yes	Yes

Q25/+ replicate 2					
ANOVA			Percent Immobility	Distance	Velocity
	F(DFn,DFd)		F(21,103)=11.58	F(21,103)=13.17	F(21,103)=13.16
	p value		<0.0001	<0.0001	<0.0001
Dunnett's multiple comparison test (α=0.05)	1 vs. 2	95% CI of diff.	-34.33 to 42.46	-6392 to 7055	-2.736 to 4.420
		Significant?	No	No	No
	1 vs. 3	95% CI of diff.	-22.79 to 31.51	-5645 to 3864	-2.754 to 2.307
		Significant?	No	No	No
	1 vs. 6	95% CI of diff.	-70.33 to 6.459	-131.7 to 13315	-0.1870 to 6.969
		Significant?	No	No	No
	1 vs. 7	95% CI of diff.	-24.26 to 30.04	-3549 to 5959	-1.584 to 3.476
		Significant?	No	No	No
	1 vs. 8	95% CI of diff.	-30.00 to 46.79	-7815 to 5632	-3.494 to 3.663
		Significant?	No	No	No
	1 vs. 9	95% CI of diff.	-39.36 to 37.43	7835 to 21282	-1.121 to 6.035
		Significant?	No	Yes	No
	1 vs. 10	95% CI of diff.	-24.62 to 29.68	-2605 to 6904	-0.6333 to 4.427
		Significant?	No	No	No
	1 vs. 13	95% CI of diff.	-33.18 to 43.61	-7345 to 6102	-2.727 to 4.429
		Significant?	No	No	No
	1 vs. 16	95% CI of diff.	-24.87 to 35.84	2803 to 13434	-0.9260 to 4.732
		Significant?	No	Yes	No
	1 vs. 21	95% CI of diff.	-27.41 to 33.30	2910 to 13541	-1.062 to 4.596
		Significant?	No	Yes	No
	1 vs. 24	95% CI of diff.	-27.60 to 33.11	2304 to 12934	-1.789 to 3.869
		Significant?	No	Yes	No
	1 vs. 29	95% CI of diff.	-38.46 to 22.24	387.0 to 11018	1.014 to 6.672
		Significant?	No	Yes	Yes
	1 vs. 31	95% CI of diff.	-36.25 to 24.46	-3070 to 7561	-1.834 to 3.823
		Significant?	No	No	No
	1 vs. 35	95% CI of diff.	-39.46 to 21.25	766.5 to 11397	1.770 to 7.427
		Significant?	No	Yes	Yes
	1 vs. 38	95% CI of diff.	-63.48 to -2.769	4168 to 14799	2.505 to 8.162
		Significant?	Yes	Yes	Yes
	1 vs. 42	95% CI of diff.	-68.60 to -7.895	2438 to 13069	1.813 to 7.470
		Significant?	Yes	Yes	Yes
	1 vs. 46	95% CI of diff.	-67.61 to -6.899	4234 to 14865	2.262 to 7.920
		Significant?	Yes	Yes	Yes
	1 vs. 51	95% CI of diff.	-104.9 to -44.23	9136 to 19767	4.834 to 10.49
		Significant?	Yes	Yes	Yes
	1 vs. 54	95% CI of diff.	-88.78 to -28.07	7806 to 18437	4.492 to 10.15
		Significant?	Yes	Yes	Yes
	1 vs. 57	95% CI of diff.	-99.63 to -35.38	6735 to 17986	4.758 to 10.75
		Significant?	Yes	Yes	Yes
	1 vs. 63	95% CI of diff.	-119.3 to -42.50	7972 to 21418	5.399 to 12.56
		Significant?	Yes	Yes	Yes

Q72/X replicate 1					
ANOVA			Percent Immobility	Distance	Velocity
	F(DFn,DFd)		F(21,107)=18.60	F(21,107)=15.83	F(21,107)=20.69
	p value		<0.0001	<0.0001	<0.0001
Dunnett's multiple comparison test (α=0.05)	3 vs. 4	95% CI of diff.	-37.83 to 25.14	-5317 to 8228	-2.533 to 4.089
		Significant?	No	No	No
	3 vs. 7	95% CI of diff.	-15.78 to 28.75	767.4 to 10345	0.2971 to 4.980
		Significant?	No	Yes	Yes
	3 vs. 8	95% CI of diff.	-33.28 to 29.69	-3306 to 10239	-2.413 to 4.210
		Significant?	No	No	No
	3 vs. 9	95% CI of diff.	-27.31 to 35.66	-5275 to 8269	-1.412 to 5.210
		Significant?	No	No	No
	3 vs. 10	95% CI of diff.	-25.56 to 18.97	-1846 to 7732	0.2676 to 4.950
		Significant?	No	No	Yes
	3 vs. 11	95% CI of diff.	-29.26 to 33.71	-4245 to 9299	-0.6199 to 6.002
		Significant?	No	No	No
	3 vs. 14	95% CI of diff.	-16.53 to 28.00	-5433 to 4144	-0.3329 to 4.350
		Significant?	No	No	No
	3 vs. 16	95% CI of diff.	-27.00 to 22.79	4611 to 15319	0.2225 to 5.458
		Significant?	No	Yes	Yes
	3 vs. 17	95% CI of diff.	-20.46 to 29.32	7336 to 18044	-0.8526 to 4.383
		Significant?	No	Yes	No
	3 vs. 21	95% CI of diff.	-21.10 to 28.69	3404 to 14112	0.7769 to 6.012
		Significant?	No	Yes	Yes
	3 vs. 24	95% CI of diff.	-27.35 to 22.43	4900 to 15607	0.8500 to 6.085
		Significant?	No	Yes	Yes
	3 vs. 29	95% CI of diff.	-41.14 to 8.646	6265 to 16973	1.316 to 6.551
		Significant?	No	Yes	Yes
	3 vs. 31	95% CI of diff.	-54.49 to -4.703	4431 to 15139	3.660 to 8.896
		Significant?	Yes	Yes	Yes
	3 vs. 35	95% CI of diff.	-51.00 to -1.215	5642 to 16350	3.796 to 9.031
		Significant?	Yes	Yes	Yes
	3 vs. 38	95% CI of diff.	-61.59 to -11.80	6535 to 17243	5.006 to 10.24
		Significant?	Yes	Yes	Yes
	3 vs. 42	95% CI of diff.	-100.9 to -51.16	10701 to 21409	6.715 to 11.95
		Significant?	Yes	Yes	Yes
	3 vs. 46	95% CI of diff.	-66.51 to -16.73	7993 to 18701	5.575 to 10.81
		Significant?	Yes	Yes	Yes
	3 vs. 51	95% CI of diff.	-86.44 to -36.66	8394 to 19102	4.685 to 9.920
		Significant?	Yes	Yes	Yes
	3 vs. 54	95% CI of diff.	-87.39 to -37.61	9965 to 20673	6.311 to 11.55
		Significant?	Yes	Yes	Yes
	3 vs. 57	95% CI of diff.	-85.73 to -35.95	9455 to 20163	7.075 to 12.31
		Significant?	Yes	Yes	Yes
	3 vs. 63	95% CI of diff.	-106.9 to -43.93	9098 to 22643	6.194 to 12.82
		Significant?	Yes	Yes	Yes

Q72/X replicate 2					
ANOVA			Percent Immobility	Distance	Velocity
	F(DFn,DFd)		F(21,102)=15.74	F(21,102)=13.73	F(21,102)=5.692
	p value		<0.0001	<0.0001	<0.0001
Dunnett's multiple comparison test (α=0.05)	1 vs. 2	95% CI of diff.	-29.28 to 42.98	-8646 to 5424	-6.657 to 6.096
		Significant?	No	No	No
	1 vs. 3	95% CI of diff.	-20.91 to 30.19	-5554 to 4395	-4.389 to 4.629
		Significant?	No	No	No
	1 vs. 6	95% CI of diff.	-36.21 to 36.05	-6584 to 7486	-5.865 to 6.888
		Significant?	No	No	No
	1 vs. 7	95% CI of diff.	-29.23 to 21.86	-2550 to 7399	-2.591 to 6.427
		Significant?	No	No	No
	1 vs. 8	95% CI of diff.	-33.56 to 38.70	-6928 to 7143	-6.173 to 6.580
		Significant?	No	No	No
	1 vs. 9	95% CI of diff.	-42.59 to 29.67	-3003 to 11067	-4.197 to 8.557
		Significant?	No	No	No
	1 vs. 10	95% CI of diff.	-18.20 to 32.89	-5472 to 4477	-3.679 to 5.339
		Significant?	No	No	No
	1 vs. 13	95% CI of diff.	-27.18 to 45.08	-7164 to 6906	-4.603 to 8.150
		Significant?	No	No	No
	1 vs. 16	95% CI of diff.	-26.93 to 30.20	2684 to 13808	-3.025 to 7.057
		Significant?	No	Yes	No
	1 vs. 21	95% CI of diff.	-20.36 to 36.76	2177 to 13300	-3.182 to 6.901
		Significant?	No	Yes	No
	1 vs. 24	95% CI of diff.	-21.21 to 35.92	1502 to 12626	-3.946 to 6.136
		Significant?	No	Yes	No
	1 vs. 29	95% CI of diff.	-59.52 to -2.396	3837 to 14960	0.5007 to 10.58
		Significant?	Yes	Yes	Yes
	1 vs. 31	95% CI of diff.	-66.70 to -9.580	3694 to 14817	-0.3580 to 9.724
		Significant?	Yes	Yes	No
	1 vs. 35	95% CI of diff.	-52.64 to 4.480	4081 to 15205	0.9040 to 10.99
		Significant?	No	Yes	Yes
	1 vs. 38	95% CI of diff.	-52.04 to 5.083	2877 to 14001	0.6901 to 10.77
		Significant?	No	Yes	Yes
	1 vs. 42	95% CI of diff.	-79.57 to -22.45	3312 to 14436	-3.051 to 7.031
		Significant?	Yes	Yes	No
	1 vs. 46	95% CI of diff.	-76.81 to -19.68	6778 to 17901	2.058 to 12.14
		Significant?	Yes	Yes	Yes
	1 vs. 51	95% CI of diff.	-105.8 to -48.65	9521 to 20645	3.550 to 13.63
		Significant?	Yes	Yes	Yes
	1 vs. 54	95% CI of diff.	-107.7 to -50.60	9536 to 20660	4.245 to 14.33
		Significant?	Yes	Yes	Yes
	1 vs. 57	95% CI of diff.	-104.2 to -43.78	7573 to 19345	3.705 to 14.38
		Significant?	Yes	Yes	Yes
	1 vs. 63	95% CI of diff.	-99.45 to -14.72	3520 to 20019	1.244 to 16.20
		Significant?	Yes	Yes	Yes

	DJ694/+ replicate 1		Percent Immobility	Distance	Velocity
ANOVA		F(DFn,DFd)	F(16,34)=5.492	F(16,34)=8.481	F(16,34)=3.345
		p value	<0.0001	<0.0001	0.0015
Dunnett's multiple comparison test (α=0.05)	3 vs. 7	95% CI of diff.	-42.94 to 62.05	8344 to 24694	-2.187 to 12.07
		Significant?	No	Yes	No
	3 vs. 10	95% CI of diff.	-46.85 to 58.14	-9730 to 6620	-6.412 to 7.842
		Significant?	No	No	No
	3 vs. 14	95% CI of diff.	-51.70 to 53.29	-7493 to 8857	-5.830 to 8.424
		Significant?	No	No	No
	3 vs. 16	95% CI of diff.	-50.39 to 54.60	2023 to 18373	-3.410 to 10.84
		Significant?	No	Yes	No
	3 vs. 17	95% CI of diff.	-71.82 to 33.18	3061 to 19411	-3.763 to 10.49
		Significant?	No	Yes	No
	3 vs. 21	95% CI of diff.	-54.95 to 50.05	1783 to 18133	-4.119 to 10.13
		Significant?	No	Yes	No
	3 vs. 24	95% CI of diff.	-67.57 to 37.43	3183 to 19533	-2.858 to 11.40
		Significant?	No	Yes	No
	3 vs. 29	95% CI of diff.	-90.33 to 14.67	4214 to 20564	-3.185 to 11.07
		Significant?	No	Yes	No
	3 vs. 31	95% CI of diff.	-94.07 to 10.92	2689 to 19039	-1.538 to 12.72
		Significant?	No	Yes	No
	3 vs. 35	95% CI of diff.	-80.06 to 24.94	1364 to 17715	-0.9234 to 13.33
		Significant?	No	Yes	No
	3 vs. 38	95% CI of diff.	-84.09 to 20.90	2388 to 18738	-0.4877 to 13.77
		Significant?	No	Yes	No
	3 vs. 42	95% CI of diff.	-100.2 to 4.788	4255 to 20605	-0.02897 to 14.22
		Significant?	No	Yes	No
	3 vs. 46	95% CI of diff.	-120.8 to -15.84	7418 to 23769	1.752 to 16.01
		Significant?	Yes	Yes	Yes
	3 vs. 51	95% CI of diff.	-90.99 to 14.00	3699 to 20050	-0.2787 to 13.98
		Significant?	No	Yes	No
	3 vs. 54	95% CI of diff.	-127.3 to -22.27	8188 to 24538	2.644 to 16.90
		Significant?	Yes	Yes	Yes
	3 vs. 57	95% CI of diff.	-133.0 to -28.03	8580 to 24930	3.581 to 17.83
		Significant?	Yes	Yes	Yes

	DJ694/+ replicate 2				
ANOVA			Percent Immobility	Distance	Velocity
	F(DFn,DFd)		F(15,31)=22.03	F(15,31)=14.02	F(15,31)=6.386
	p value		<0.0001	<0.0001	<0.0001
Dunnett's multiple comparison test (α=0.05)	1 vs. 3	95% CI of diff.	-59.45 to 4.843	-219.5 to 16475	-4.478 to 10.67
		Significant?	No	No	No
	1 vs. 7	95% CI of diff.	-33.77 to 30.53	-8836 to 7859	-8.050 to 7.102
		Significant?	No	No	No
	1 vs. 10	95% CI of diff.	-36.12 to 28.18	-8484 to 8211	-8.264 to 6.887
		Significant?	No	No	No
	1 vs. 16	95% CI of diff.	-33.84 to 30.46	1496 to 18190	-6.406 to 8.745
		Significant?	No	Yes	No
	1 vs. 21	95% CI of diff.	-34.54 to 29.76	3217 to 19911	-4.364 to 10.79
		Significant?	No	Yes	No
	1 vs. 24	95% CI of diff.	-37.48 to 26.82	3350 to 20045	-4.277 to 10.88
		Significant?	No	Yes	No
	1 vs. 29	95% CI of diff.	-116.4 to -52.15	9927 to 26622	1.613 to 16.76
		Significant?	Yes	Yes	Yes
	1 vs. 31	95% CI of diff.	-33.41 to 30.88	-9927 to 6767	-8.969 to 6.183
		Significant?	No	No	No
	1 vs. 35	95% CI of diff.	-98.25 to -33.96	7849 to 24544	-0.06367 to 15.09
		Significant?	Yes	Yes	No
	1 vs. 38	95% CI of diff.	-73.03 to -8.732	5540 to 22235	0.7232 to 15.87
		Significant?	Yes	Yes	Yes
	1 vs. 42	95% CI of diff.	-93.20 to -28.90	4057 to 20751	-5.925 to 9.227
		Significant?	Yes	Yes	No
	1 vs. 46	95% CI of diff.	-105.1 to -40.78	9584 to 26278	2.428 to 17.58
		Significant?	Yes	Yes	Yes
	1 vs. 51	95% CI of diff.	-111.9 to -47.65	9705 to 26399	2.935 to 18.09
		Significant?	Yes	Yes	Yes
	1 vs. 54	95% CI of diff.	-117.5 to -53.21	10079 to 26773	3.113 to 18.26
		Significant?	Yes	Yes	Yes
	1 vs. 57	95% CI of diff.	-114.5 to -42.59	7371 to 26037	1.796 to 18.74
		Significant?	Yes	Yes	Yes

MHC/+ replicate 1					
ANOVA			**Percent Immobility**	**Distance**	**Velocity**
		F(DFn,DFd)	F(17,35)=6.366	F(17,35)=17.76	F(17,35)=15.44
		p value	<0.0001	<0.0001	<0.0001
Dunnett's multiple comparison test (α=0.05)	**3 vs. 7**	**95% CI of diff.**	-55.34 to 35.03	560.2 to 11265	).003162 to 7.082
		Significant?	No	Yes	Yes
	3 vs. 10	**95% CI of diff.**	-53.61 to 36.76	-984.2 to 9721	-0.8411 to 6.238
		Significant?	No	No	No
	3 vs. 14	**95% CI of diff.**	-55.76 to 34.61	1976 to 12681	0.7832 to 7.862
		Significant?	No	Yes	Yes
	3 vs. 16	**95% CI of diff.**	-49.55 to 40.83	6362 to 17067	-1.515 to 5.564
		Significant?	No	Yes	No
	3 vs. 17	**95% CI of diff.**	-50.11 to 40.27	5988 to 16693	-0.6916 to 6.387
		Significant?	No	Yes	No
	3 vs. 21	**95% CI of diff.**	-53.84 to 36.53	8552 to 19257	1.817 to 8.896
		Significant?	No	Yes	Yes
	3 vs. 24	**95% CI of diff.**	-53.28 to 37.09	7200 to 17905	0.4462 to 7.525
		Significant?	No	Yes	Yes
	3 vs. 29	**95% CI of diff.**	-96.27 to -5.899	12162 to 22867	3.642 to 10.72
		Significant?	Yes	Yes	Yes
	3 vs. 31	**95% CI of diff.**	-60.64 to 29.73	4049 to 14754	1.412 to 8.491
		Significant?	No	Yes	Yes
	3 vs. 35	**95% CI of diff.**	-100.5 to -10.13	11004 to 21709	5.310 to 12.39
		Significant?	Yes	Yes	Yes
	3 vs. 38	**95% CI of diff.**	-65.59 to 24.79	6916 to 17621	3.657 to 10.74
		Significant?	No	Yes	Yes
	3 vs. 42	**95% CI of diff.**	-87.61 to 2.762	11135 to 21840	6.528 to 13.61
		Significant?	No	Yes	Yes
	3 vs. 46	**95% CI of diff.**	-98.95 to -8.572	11593 to 22298	6.385 to 13.46
		Significant?	Yes	Yes	Yes
	3 vs. 51	**95% CI of diff.**	-63.79 to 26.59	5539 to 16244	2.746 to 9.825
		Significant?	No	Yes	Yes
	3 vs. 54	**95% CI of diff.**	-126.3 to -25.28	12999 to 24968	7.340 to 15.25
		Significant?	Yes	Yes	Yes
	3 vs. 57	**95% CI of diff.**	-114.2 to -23.78	11645 to 22350	5.050 to 12.13
		Significant?	Yes	Yes	Yes
	3 vs. 63	**95% CI of diff.**	-125.7 to -35.32	13520 to 24225	7.337 to 14.42
		Significant?	Yes	Yes	Yes

	MHC/+ replicate 2				
ANOVA			Percent Immobility	Distance	Velocity
	F(DFn,DFd)		F(16,32)=8.236	F(16,32)=3.518	F(16,32)=2.209
	p value		<0.0001	0.0012	0.0276
Dunnett's multiple comparison test (α=0.05)	1 vs. 3	95% CI of diff.	-38.41 to 53.21	-13809 to 13059	-10.09 to 10.55
		Significant?	No	No	No
	1 vs. 7	95% CI of diff.	-37.81 to 53.80	-13147 to 13721	-9.073 to 11.56
		Significant?	No	No	No
	1 vs. 10	95% CI of diff.	-32.88 to 58.74	-11616 to 15252	-7.583 to 13.05
		Significant?	No	No	No
	1 vs. 16	95% CI of diff.	-28.50 to 63.12	-7606 to 19262	-10.33 to 10.30
		Significant?	No	No	No
	1 vs. 21	95% CI of diff.	-66.42 to 25.19	-554.7 to 26313	-3.356 to 17.28
		Significant?	No	No	No
	1 vs. 24	95% CI of diff.	-34.94 to 56.67	-4299 to 22569	-7.027 to 13.61
		Significant?	No	No	No
	1 vs. 29	95% CI of diff.	-65.98 to 25.63	-2508 to 24360	-3.765 to 16.87
		Significant?	No	No	No
	1 vs. 31	95% CI of diff.	-50.71 to 40.91	-9121 to 17747	-8.060 to 12.57
		Significant?	No	No	No
	1 vs. 35	95% CI of diff.	-102.4 to -10.78	1192 to 28060	-1.905 to 18.73
		Significant?	Yes	Yes	No
	1 vs. 38	95% CI of diff.	-84.93 to 6.684	-884.2 to 25984	-1.113 to 19.52
		Significant?	No	No	No
	1 vs. 42	95% CI of diff.	-102.3 to -10.72	1081 to 27949	-2.199 to 18.43
		Significant?	Yes	Yes	No
	1 vs. 46	95% CI of diff.	-88.09 to 3.524	-919.9 to 25948	-3.059 to 17.57
		Significant?	No	No	No
	1 vs. 51	95% CI of diff.	-119.9 to -17.43	479.2 to 30518	-3.066 to 20.00
		Significant?	Yes	Yes	No
	1 vs. 54	95% CI of diff.	-108.3 to -16.69	2273 to 29141	-2.177 to 18.46
		Significant?	Yes	Yes	No
	1 vs. 57	95% CI of diff.	-117.4 to -14.97	-15143 to 14896	-14.19 to 8.875
		Significant?	Yes	No	No
	1 vs. 63	95% CI of diff.	-103.8 to -12.16	-7023 to 19845	-6.068 to 14.57
		Significant?	Yes	No	No

DATA EXTRACTION SCRIPT

```
%Loading in data and setting up initial parameters
% Replace ArenaConfigsMHCMale by the name of your configuration file
load(“ArenaConfigsMHCMale")
% Change frametonalyze value to accommodate length of analyzed video file, 5400: 3 min at 30 frames/s
frametoanalyze=5400;
trkdatasize=size(trk.data);
Numberofarena=trkdatasize(1,1);
% Remove or add ‘number’ to the required number of configurations
Arenaconfig=menu('Choose Configuration','1','2','3','4','5','6','7','8','9','10','11','12','13','14','15','16');
% Replace ArenaConfigsMHCMale by the name of your configuration file
Configuration=ArenaConfigsMHCMale(1:48,Arenaconfig+1);
Conf=table2array(Configuration);
prompt={'Enter Age'};
title="Input";
dims=[1 40];
AgeAsText=inputdlg(prompt,title,dims);
Age=str2double(AgeAsText);
prompt={'Starting Arena'};
title="Input";
dims=[1 40];
ArenaAsText=inputdlg(prompt,title,dims);
Arena=str2double(ArenaAsText);
flydata=zeros(frametoanalyze+2,Numberofarena*4);
%Extracting data of interest and forming an array
for i=1:Numberofarena
    %Setting up starting points for calculations
    framenumber=frametoanalyze;
    distance=0;
    timeimmobile=0;
    velocity=0;
    VelocityFrames=5399;
    %Calculating data of interest
        for j=2:frametoanalyze
            if or(isnan(trk.data(i,j,2)),isnan(trk.data(i,j-1,2)))
            else
                distance=distance+sqrt((trk.data(i,j-1,2)-trk.data(i,j,2))^2+(trk.data(i,j-1,1)-trk.data(i,j,1))^2);
```

```
            end
            if or(isnan(feat.data(i,j,1)),isnan(trk.data(i,j,2)))
                VelocityFrames=VelocityFrames-1;
            else
                if feat.data(i,j,1)<1
                    timeimmobile=timeimmobile+1;
                else
                velocity=velocity+feat.data(i,j,1);
                end
            end
        end
        %Calculating numbers of frames to analyze
    for j=1:frametoanalyze
        if or(isnan(trk.data(i,j,1)),isnan(trk.data(i,j,2)))
            framenumber=framenumber-1;
        end
        %Extracting x, y, velocity, and distance and placing them in to
        %their respective columns. Adds data of interest at the bottom.
        flydata(:,(4*(i-1)+1))=[trk.data(i,1:frametoanalyze,1),framenumber,0]';
        flydata(:,(4*(i-1)+2))=[trk.data(i,1:frametoanalyze,2),distance,VelocityFrames]';
        flydata(:,(4*(i-1)+3))=[feat.data(i,1:frametoanalyze,1),timeimmobile,velocity/(VelocityFrames-timeimmobile)]';
        flydata(:,(4*(i-1)+4))=[feat.data(i,1:frametoanalyze,9),0,0]';
    end
end
%Processing Data and compiling in to a table
flyprocesseddata=cell(Numberofarena,8);
for i=1:Numberofarena
flyprocesseddata(i,1)={Conf(((i-1)+Arena),1)};
flyprocesseddata(i,2)={"Male"};
flyprocesseddata(i,3)={Age};
flyprocesseddata(i,4)={Arena+(i-1)};
flyprocesseddata(i,5)={flydata(5401,(4*(i-1)+1))};
flyprocesseddata(i,6)={((flydata(5401,(4*(i-1)+3)))/VelocityFrames)*100};
flyprocesseddata(i,7)={flydata(5401,(4*(i-1)+2))};
flyprocesseddata(i,8)={flydata(5402,(4*(i-1)+3))};
end
%Sets up table with variable names
FlyFinal=cell2table(flyprocesseddata,...
    'VariableNames',{'Genotype','Gender','Age','Arena','Frames','PercentImmobile','Distance','Velocity'});
```

```
%Writing to file
if i==1
    %If analyzing data from a single arena, arena number added to name of file
    % Replace MHC Male by the desired name for the saved file
    FileName=strcat("MHC Male",cell2mat(AgeAsText),cell2mat(ArenaAsText),".xls");
else
    % Replace MHC Male by the desired name for the saved file
    FileName=strcat("MHC Male",cell2mat(AgeAsText),".xls");
end
%Prompting save path
[file,path]=uiputfile(FileName);
CSVFileName=strcat(path,'Flydata',FileName);
FileName=strcat(path,file);
writetable(FlyFinal,FileName);
xlswrite(CSVFileName,flydata)
```

END OF DATA EXTRACTION SCRIPT

DATA COMPILATION SCRIPT

```
dname = uigetdir();
Filelist=struct2table(dir(dname));
FileList=Filelist(Filelist.isdir==false,'name');
FileList2=Filelist(endsWith(Filelist.name,'.xls'),'name');
DirSize=size(FileList2);
FileName=strcat(dname,"/",cell2mat(FileList2{1,1}));
Compilation=readtable(FileName);
for i=2:DirSize(1,1)
    FileName=strcat(dname,"/",cell2mat(FileList2{i,1}));
    Compilation=[Compilation;readtable(FileName)];
end
FileName=strcat(dname,"/Compilation.xls");
writetable(Compilation,FileName)
```

END OF DATA COMPILATION SCRIPT