AUTOMATED QUANTIFICATION OF CLIMBING PERFORMANCE IN *D. MELANOGASTER*

The Impact of Age, Mitonuclear Genotype and Diet

Chen Ye | Zemplen Pataki | Denise Yoon
Tyler Devlin | Julia Dewey | Brian Franklin | Matthew McAteer | Cynthia Hale-Phillips | Adam Spierer | Lei Zhu | David Rand

BACKGROUND

Aging

Senescence is a cyclical process: as an organism's physiological and systematic balance declines, so too do other factors. Some are easily quantified, such as life span, but others, such as health span, are more abstract. We use many assays to assess and standardize these more fluid qualities.

Climbing Assays

One such assay applicable to *Drosophila* is a climbing assay. Flies instinctively climb in response to stressful stimulus—and the resultant speed metrics may serve as an indicator of overall health. Speed has been a previous metric of health span, but the manual nature of the data acquisition limited its complexity.

One previous metric has been a climbing index, in which Tinkerhess et al. 2012 (JoVE) divided the climbing area into quartiles and calculated a weighted average based on the quantity of flies within each zone after a set timespan. This 'binning' of data is less effective over extended time-frames, cannot track individual flies, and has limited resolution.

Development of automated tracking techniques would grant higher fidelity and dimensionality of data, enabling applications which cannot be realistically performed using manual methods—such as continuous measurements over long periods of time, or high volume assays.

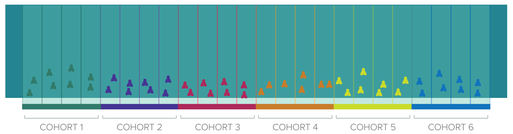
INTRODUCTION

We performed automated climbing assays on *D. melanogaster* cohorts which varied in age, nuclear and mitochondrial genotype, and diet. In conjunction with biochemical, mitochondrial, and starvation assays, we determined whether factors associated with aging were co-related or behaved independently.

Another goal of this experiment was to develop and demonstrate protocols and techniques for automating climbing assays.

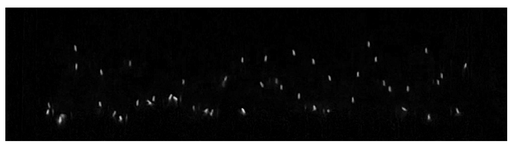
METHODS

1. 6 cohorts of 8 flies are loaded separately into a climbing apparatus comprised of 24 serological pipettes, each with 2 flies per tube.



2. Apparatus is jolted to bring flies to the bottom of the device and the flies are recorded for 25 seconds. This is repeated 3 times per load

3. Video files are transferred to Brown's supercomputer, OSCAR, and preprocessed in Fiji, an image analysis program, to remove backgrounds, leaving only silhouettes of flies.



4. Flies are tracked in Fiji plugin TrackMate using Lapacian of Gaussian blob detection and a Linear Assignment Problem tracker.

trackmate

$$g(x,y,t) = \frac{1}{2\pi t^2} e^{-\frac{x^2+y^2}{2t^2}}$$

$$\nabla^2 L = L_{xx} + L_{yy}$$

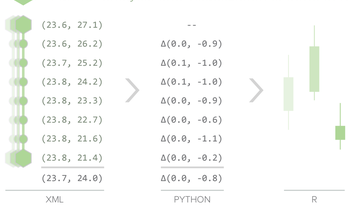
$$\sum_{i \in A} \sum_{i \in A} C(i,j) x_{ij}$$

$$\sum_{j \in T} x_{ij} = 1 \text{ for } i \in A$$

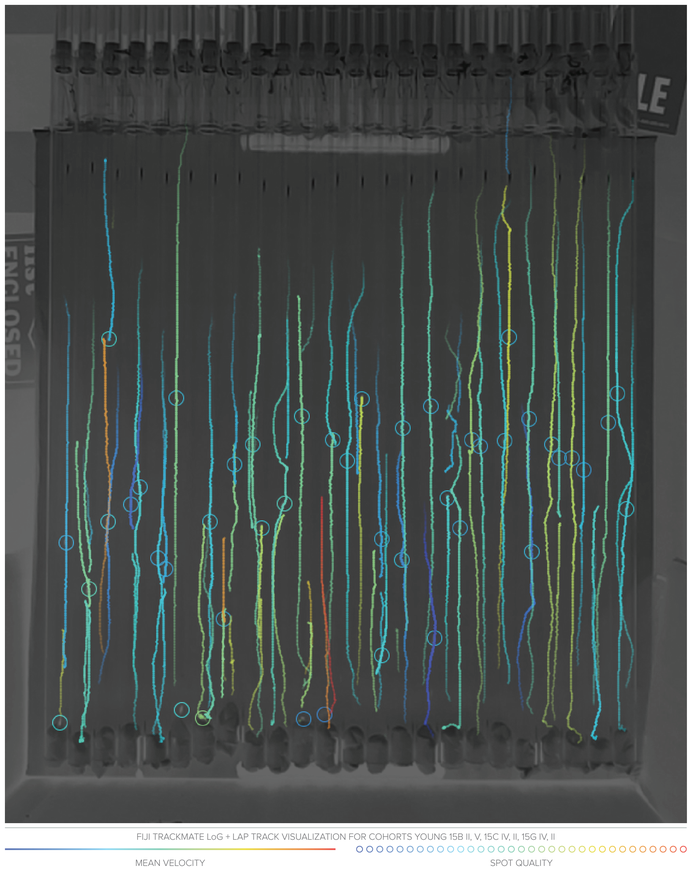
$$\sum_{i \in A} x_{ij} = 1 \text{ for } j \in T$$

$$x_{ij} \geq 0 \text{ for } i,j \in A,T$$

5. Data is exported to IPython and post-processed. Further analysis and visualization is done in R.



RESULTS



A tracked video capture may be visualized in TrackMate. In this capture, tracks are drawn for the preceding and proceeding 1.6 seconds of video. Track colour indicates mean track velocity and spot colour indicates spot recognition quality.

ANOVA for automatically tracked data

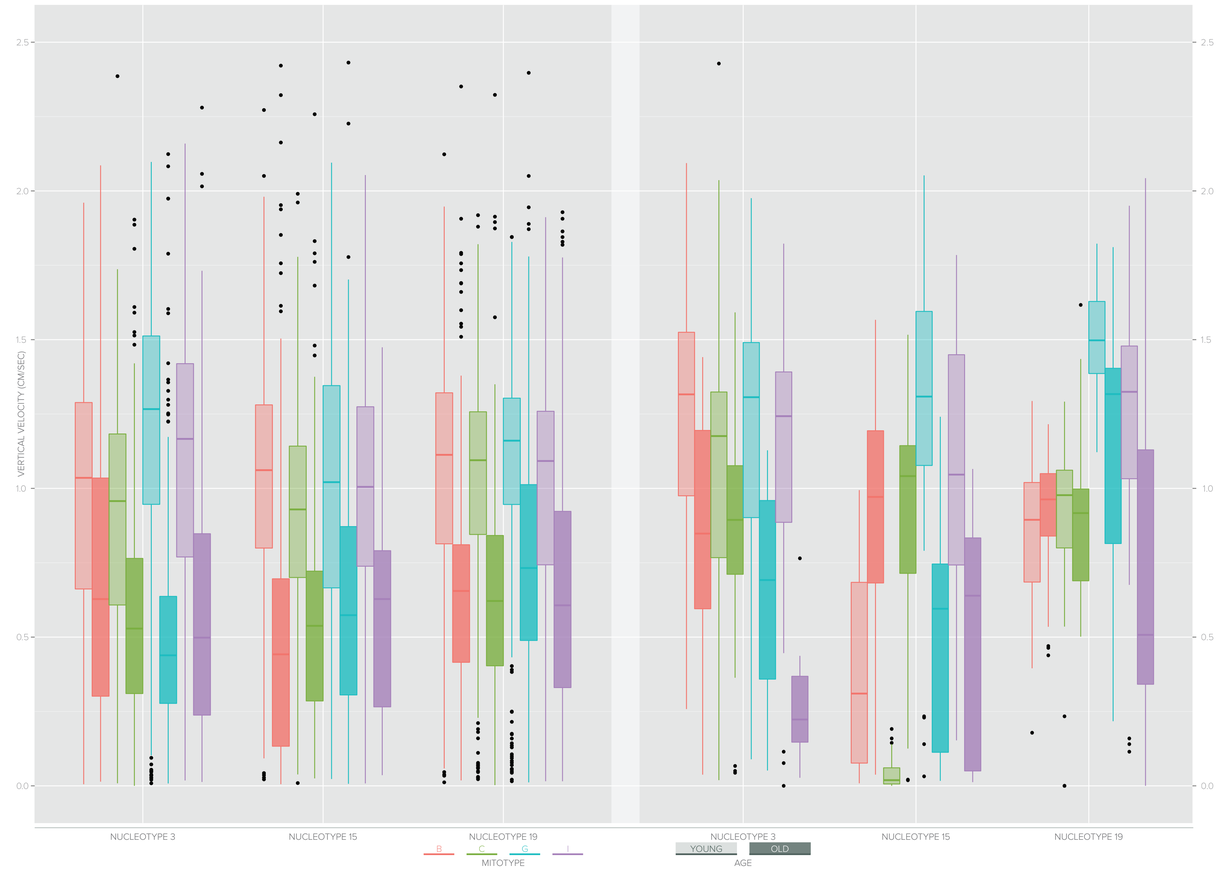
	DOF	SUM OF SQUARES	MEAN SQUARE	F-VALUE	P-VALUE(>F)	SIGNIFICANCE
AGE	1	349.1	349.1	2125.961	$< 2.000\times10^{-16}$	•••
NUCLEAR	2	9.7	4.8	29.415	1.850×10^{-13}	•••
MITO	3	13.9	4.6	28.243	$< 2.000\times10^{-16}$	•••
DIET	2	3.5	1.7	10.564	2.610×10^{-5}	•••
AGE:NUCLEAR	2	2.4	1.2	7.294	6.840×10^{-4}	•••
AGE:MITO	3	3.2	1.1	6.438	2.380×10^{-4}	•••
NUCLEAR:MITO	6	4.3	0.7	4.322	2.320×10^{-4}	•••
AGE:DIET	2	0.6	0.3	1.946	1.429×10^{-1}	
NUCLEAR:DIET	4	3.2	0.8	4.933	5.690×10^{-4}	•••
MITO:DIET	6	4.5	0.8	4.615	1.090×10^{-4}	•••
AGE:NUCLEAR:MITO	6	17.4	2.9	17.692	$< 2.000\times10^{-16}$	•••
AGE:NUCLEAR:DIET	4	0.1	0	0.153	9.618×10^{-1}	
AGE:MITO:DIET	6	5.2	0.9	5.278	1.930×10^{-5}	•••
NUCLEAR:MITO:DIET	12	15	1.2	7.609	3.310×10^{-14}	•••
AGE:NUCLEAR:MITO:DIET	12	6.9	0.6	3.502	3.400×10^{-5}	•••
RESIDUALS	9026	1482.3	0.2			

ANOVA for manually tracked data

	DOF	SUM OF SQUARES	MEAN SQUARE	F-VALUE	P-VALUE(>F)	SIGNIFICANCE
AGE	1	4.009	4.009	33.018	3.080×10^{-8}	•••
NUCLEAR	2	4.934	2.467	20.316	8.230×10^{-9}	•••
MITO	3	1.750	0.583	4.803	2.931×10^{-3}	••
DIET	2	0.484	0.242	1.994	1.387×10^{-1}	
AGE:NUCLEAR	2	3.885	1.942	15.995	3.330×10^{-7}	•••
AGE:MITO	3	12.884	4.281	35.256	$< 2.000\times10^{-16}$	•••
NUCLEAR:MITO	6	6.169	1.028	8.467	2.910×10^{-8}	•••
AGE:DIET	2	0.369	0.185	1.521	2.208×10^{-1}	
NUCLEAR:DIET	4	1.077	0.269	2.218	6.812×10^{-2}	
MITO:DIET	6	0.468	0.078	0.643	6.960×10^{-1}	
AGE:NUCLEAR:MITO	6	3.35	0.558	4.598	2.040×10^{-4}	•••
AGE:NUCLEAR:DIET	4	0.66	0.165	1.358	2.497×10^{-1}	
AGE:MITO:DIET	6	0.485	0.081	0.666	6.774×10^{-1}	
NUCLEAR:MITO:DIET	12	4.134	0.345	2.837	1.229×10^{-3}	••
AGE:NUCLEAR:MITO:DIET	12	3.626	0.302	2.488	4.511×10^{-3}	••
RESIDUALS	216	26.23	0.121			

SIGNIFICANCE CODES
0 ••• 0.001 •• 0.01 • 0.05 · 0.1 1

Averaged Climbing Velocity by Cohort



AUTOMATICALLY TRACKED DATA

A median velocity is calculated for each distinct track, and then tracks of the same fly type are averaged together. We use the median to reduce noise.

MANUALLY TRACKED DATA

For manually tracked data, fly elevation is measured every 3 seconds for 9 seconds. Velocity is then computed per interval, and averaged together by type.

CONCLUSIONS

It should be noted that we are continuing to refine our data-processing protocol, and automated data results are extremely preliminary.

Having said that, a relationship between age and velocity is immediately and strikingly apparent within our Velocity × Type charts. This effect strongly appears in ANOVA data as well. ANOVA additionally reveals relationships between nucleotype, mitotype, age, and velocity.

This correlates with our findings using other assays, as well as manual tracking of the data, indicating that the climbing assay protocol we developed is accurate and precise enough to successfully capture fly velocity trends. Additionally, this indicates that climbing assays overall correlate well with other measures of healhspan, such as starvation assays.

In general, our data indicates that climbing velocity depended strongly on fly age, and to a lesser extent, nucleotype and mitotype. Strikingly, however, the effect of age on velocity appears to be modulated by genotype—most flies slow down as they age, but some nucleotypes and mitotypes hold steady, and others even speed up as they age.

This is evidence to support our hypothesis that the impacts of aging effectors are co-related.

FUTURE DIRECTIONS

The code, protocols, and hardware we've developed for automated fly tracking have exciting applications in many other experiments. There is much room for further automation and streamlining of our techniques, and we are working to further refine these methods, with the potential of developing a set of standards applicable outside our own lab.

Inside our lab, we intend to focus on the impact of fly exercise on health span, and our tracking techniques will be used to continuously assay flies for time to fatigue as they are exercised for long periods of time. The unmonitored mass processing of data will be another proving ground for our protocols, and will be used to improve code stability and efficiency.

We would also like to further analyze existing and future data we've gathered, applying metrics such as number of drops, particle size, ratio of vertical to horizontal movement, and potentially many others.

CITATIONS

Jaqaman, K., Loerke, D., Mettlen, M., Kuwata, H., Grinstein, S., Schmid, S. L., & Danuser, G. (2008). Robust single-particle tracking in live-cell time-lapse sequences. *Nature Methods, 5*(8), 695–702. doi:10.1038/nmeth.1237

Piazza, N., Gosangi, B., Devilla, S., Arking, R., & Wessells, R. (2009). Exercise-training in young Drosophila melanogaster reduces age-related decline in mobility and cardiac performance. *PloS One, 4*(6), e5886. doi:10.1371/journal.pone.0005886

Schindelin, J., Arganda-Carreras, I., Frise, E., Kaynig, V., Longair, M., Pietzsch, T., ... Cardona, A. (2012). Fiji: an open-source platform for biological-image analysis. *Nature Methods, 9*(7), 676–82. doi:10.1038/nmeth.2019

Tinkerhess, M. J., Ginzberg, S., Piazza, N., & Wessells, R. J. (2012). Endurance training protocol and longitudinal performance assays for Drosophila melanogaster. *Journal of Visualized Experiments : JoVE,* (61), e3786. doi:10.3791/3786

ACKNOWLEDGMENTS

We would like to thank Jim Mossman, Lei Zhu, Leann Barnes, John Santiago, David Rand and Adam Spierer for their help.

Part of this research was conducted using computational and visualization resources and services at the Center for Computation and Visualization, Brown University.

This Research was funded by a grant from the Howard Hughes Medical Institute.

CONTACT

Resources for this project may be found at

yeesus.com/Excelsior

Chen Ye: cye@brown.edu | 507-301-6165