AUTOMATED QUANTIFICATION OF CLIMBING PERFORMANCE IN *D. MELANOGASTER*

The Impact of Age, Mitonuclear Genotype and Diet

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BACKGROUND

Aging

Senescence is a multifaceted process which both influences and is influenced by a multitude of factors within an organism. Some are obvious, such as life span, but others, such as health, are more abstract. We use many assays to quantify and standardize measuring these more fluid qualities.

Climbing Assays

One such assay applicable to drosophila is a climbing assay—flies instinctively climb in response to stress—and the resultant speed metrics may serve as an indicator of overall health.

However, processing climbing data has predominantly been a manual task, which is tedious and limits the complexity of the data gathered.

For example, in [need citation, need title author year], researchers divided the climbing area into zones and calculated an index based on the quantity of flies within each zone after a set timespan. This 'binning' of motion data is less effective over extended periods of time, cannot track individual flies, and has limited resolution.

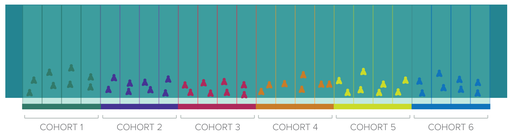
Development of automated tracking techniques would grant higher fidelity and dimensionality of data, enabling applications which cannot be realistically performed using manual methods, such as continuous measurements over long periods of time, or high volume assays.

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 behaved independently of each other, or were co-related. A secondary goal of this experiment was to develop and demonstrate protocols and techniques for automated climbing assay analysis.

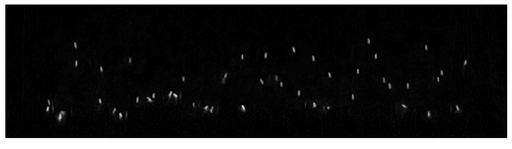
METHODS

1. 6 cohorts of 8 flies each are loaded into climbing apparati comprised of 24 serological pipettes, such that there are 2 flies per tube.



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

3. Videos are transferred to OSCAR and preprocessed in Fiji, an image analysis program, to remove backgrounds & enhance fly sihouettes



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

trackmate

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

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

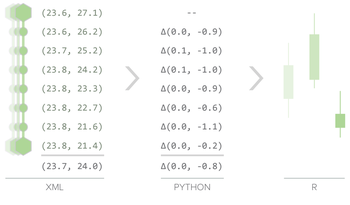
$$\sum_{i \in A} \sum_{i \in A} C(i,j) x_{ij}$$

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

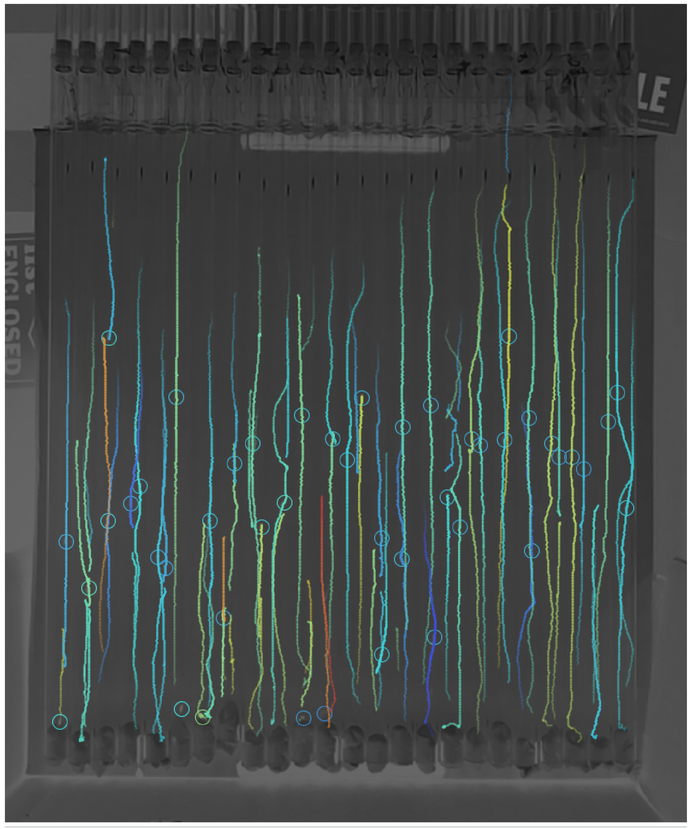
$$\sum_{i \in A} x_{ij} = 1 \text{ for } j \in T$$

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

5. Data is exported to IPython and postprocessed. 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 pre-ceeding and proceeding 1.6 seconds of video. Track colour indicates mean track velocity and spot colour indicates spot recognition quality.

	Degrees of Freedom		Sum of Squares		Mean Square		F-value		p-value(>F)		Significance	
	Auto	Manual	Auto	Manual	Auto	Manual	Auto	Manual	Auto	Manual	Auto	Manual
Nuclear	2		19	2296	9.5	1148	10.404	18.026	3.06E-05	5.76E-08	***	***
Mito	3		14	3511	4.7	1170	5.16	18.376	0.001456	1.18E-10	**	***
Diet	2		4	402	1.8	201	1.946	3.158	0.142838	0.0445		*
Age	1		793	34310	793.2	34310	872.258	538.658	< 2e-16	< 2E-16	***	***
Nuclear:Mito	6		5	2366	0.8	394	0.852	6.191	0.529292	5.18E-06		***
Nuclear:Diet	4		13	847	3.1	212	3.463	3.322	0.007812	0.0115	**	*
Mito:Diet	6		12	479	1.9	80	2.137	1.253	0.046077	0.2807	*	
Nuclear:Age	2		10	2349	5	1175	5.518	18.441	0.004027	4.03E-08	**	***
Mito:Age	3		6	3069	2	1023	2.19	16.062	0.087065	1.84E-09	.	***
Diet:Age	2		0	407	0	204	0.044	3.198	0.956822	0.0428		*
Nuclear:Mito:Diet	12		28	2856	2.4	238	2.596	3.737	0.001891	3.79E-05	**	***
Nuclear:Mito:Age	6		23	2481	3.8	414	4.183	6.492	0.000331	2.59E-06	***	***
Nuclear:Diet:Age	4		3	862	0.9	215	0.936	3.383	0.441718	0.0104		*
Mito:Diet:Age	6		16	472	2.7	79	2.96	1.234	0.006898	0.2899	**	
Nuclear:Mito:Diet:Age	12		17	2864	1.4	239	1.556	3.747	0.096959	3.63E-05	.	***

Velocity x Fly Type

Auto v. Manual

CONCLUSIONS

ANOVA tests on the gathered automated data revealed clear trends, with mean vertical climbing velocity of flies being predicted to a very high significance by Age, Nucleotype, Mitotypes C, G, and I, and Diets IV and V. This distinction of velocity by cohort is evident visually as well, when Velocity × Type charts are examined.

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

Overall, our data indicates that climbing velocity depended strongly on fly age, and to a secondary extent by factors that modify the effect of aging—most strongly, nuclear genotype, and to a lesser extent, diet and mitochondrial genotype. Strikingly however, ANOVA revealed that the specific impacts of certain mitotypes were linked to genotype, and certain diets favored certain nucleotypes.

This is evidence to support our hypothesis that the impacts of aging affectors 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 continously assay flies 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 exisiting 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

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