Correlation between Time and Climbing Success in *Drosophila*: Comparison in Normal and Stressed Conditions

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Abstract. The Drosophila Climbing Assay (DCA) is often used when Drosophila is positioned as a model organism to study the effects of disease or environmental conditions. The purpose of this study was to analyze the consistency of the relationship between the two variables of climbing ability in fly imago, both under normal conditions and under stress conditions. The predictor variable of this study was the duration of climbing, while the criterion variable was climbing success. A total of 72 experimental units were prepared and divided into 3 conditions, i.e., 24 fly bottles cultured on normal medium, 24 fly bottles cultured for one generation on lead-containing medium, and 24 others cultured for two generations on lead-containing medium. The results of the Spearman's Rho correlation test indicated that the duration of climbing duration was always significantly negatively correlated with climbing success in all three conditions: natural conditions ($r_s=-0.438$, $p=0.032$), stress over a generation ($r_s=-0.864$, $p<0.001$), as well as stress for two generations ($r_s=-0.436$, $p=0.033$). Furthermore, the results of the parallelism test report that the three lines were not parallel ($p<0.05$). The slope of the regression line on the normal condition data was clearly different from the stress condition data for two generations. Through the scatter plot, the two regression lines intersect. Both variables are consistently proven to have a significant relationship and can show different levels of influence when implemented under certain stress conditions.

1 Introduction

The fruit fly (*Drosophila melanogaster*) is a popular model organism used in the field of biology [1]. This small, cosmopolitan insect is able to adapt well to laboratory conditions, making it easier for researchers to involve it in various laboratory expressions [2]. Apart from that, fruit flies also have complex physiological responses to various artificial conditions so

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that they can be positioned as research subjects to study various environmental conditions [3–12]. Various assays involving fruit flies have also been developed and researchers can study the effect of certain conditions or treatments on the organism's response [13–14]. One assay that is usually included in research using fruit flies is the Drosophila Climbing Assay (DCA) [15–16].

DCA is part of the behavioral assay. This assay aims to measure the ability of fruit flies to climb a vertical plane using certain parameters, such as speed [17–19], height [20–21], and the number of flies that successfully climb to a certain point [6–18–19–21–23]. This assay is capable of providing important data but is known as a simple and cheap assay [24]. The assay's ability to gauge locomotor activity and the flies' responsiveness to various stimuli complements Drosophila's innate genetic variability. In line with the advantages of this assay, DCA has ultimately become a procedure that is often used to evaluate various compounds that are predicted to be toxic [20–25–29]. In addition, by taking advantage of the short generation time of fruit flies [2], researchers can practically design fast research to reveal the effect of toxicity of various compounds on changes in fruit fly behavior.

Discussing of toxicity studies, the superior characteristics of fruit flies also position these insects as ideal model organisms for studying the complexities of toxicology [30–31]. In general, toxicity studies are aimed at evaluating the potential danger of certain compounds to human health [32–33]. In line with this, fruit flies have genetic similarities and molecular pathways with humans [34]. Various health conditions and disease-related metabolism can also be studied through fruit flies [35]. In addition, because fruit flies are able to produce large numbers of offspring quickly [36], it will make it easier for researchers to screen for various potentially toxic compounds more efficiently. By involving DCA, researchers can reveal the toxicity of various substances from a behavioral change perspective.

One group of toxic compounds that is often studied by researchers is the heavy metal group. Even though it is often studied [17–20–21–23–37–41], research related to this group of compounds is still continuing today in order to address the impact of heavy metal pollution that continues to threaten human and environmental life. The presence of heavy metals such as lead, mercury, cadmium, and others in the environment still raises global concerns considering the level of exposure which is currently still high in various locations [42–45]. Apart from that, there are still many aspects that have not been fully revealed from the various studies that have been reported. Studies that examine the impact of exposure in the long term and across generations are also rarely conducted. Therefore, research related to the impact of heavy metals remains an important research focus in order to comprehensively reveal its impact, support better policy development, and maintain ecosystem balance and human welfare.

In this study, an in-depth exploration of the untapped potential of the DCA as a tool capable of revealing the toxicity of certain compounds is proposed. An approach will be adopted by measuring two key parameters, namely climbing duration [46] and number of flies that successfully climb [6–18–19–21–23]. These two parameters will be observed in three groups of flies that receive different conditions, including control conditions, acute exposure, and chronic exposure The main objective is to perform a Spearman-based correlation analysis on the relationship between these two variables. In addition, we will use statistical methods to form regression lines in each condition and test the parallelism between these regression lines. The results of the parallelism test are expected to provide an overview of whether the regression lines in toxic exposure conditions have a significant intersection with the regression lines in control conditions. This can be interpreted as the potency of DCA data to detect changes due to exposure to toxic compounds. The findings from this study will make an important contribution to a deeper understanding of the potential and informative power of DCA as a tool that can reveal the toxicity effects of a compound more accurately and efficiently within the framework of toxicological research.

2 Method

2.1 Research design

This study involved a total of 72 experimental units that were prepared and divided into three different conditions. In the first condition, 24 fly bottles were cultured in normal medium. In the second condition, 24 fly bottles were cultured for one generation in a medium containing heavy metal. In the third condition, 24 fly bottles were cultured for two generations in a medium containing heavy metal. The design of this study aims to observe the effects of heavy metal exposure on Drosophila flies under various conditions. This grouping compares the impact of lead exposure under different exposure conditions, namely exposure to high concentrations but only for one generation and exposure to low concentrations but for two generations. In addition, this arrangement allows for analysis of changes in fly behavioral responses in response to heavy metal exposure. Thus, this study design provides a solid experimental basis for identifying the potential impact of heavy metal exposure on Drosophila flies.

2.2 Research variable

The variables measured in this study involve the Drosophila Climbing Assay under three different conditions: normal conditions without heavy metal exposure, conditions exposed to heavy metals for one generation, and conditions exposed to heavy metals for two generations. This study evaluates two main variables of the climbing assay: climbing duration and climbing success. The predictor variable in this study is the movement duration (climbing duration) of Drosophila flies. This movement duration reflects how long the flies are able to climb within a certain period of time. This serves as an indicator of the fly's motor response and physical activity in response to various treatment conditions. The criterion variable is success in climbing (climbing success). These variables measure whether the flies are able to reach the top of the climbing assay apparatus. This serves as an indicator of the fly's overall fitness and ability to cope with environmental challenges.

2.3 Data collection procedures

The procedure for measuring climbing duration was carried out based on a method adapted from Fauzi et al. (2020). First, a special climbing tube for *D. melanogaster* was prepared. Then, 5 adult *D. melanogaster* from each treatment to be tested were placed in the climbing tube. Each climbing tube had been marked at a height of 8 cm from the bottom of the tube. Before testing, adult *D. melanogaster* were allowed to stay in the climbing tube for a few minutes to reduce stress levels. After the condition of adult *D. melanogaster* stabilized, the test tube was tapped and shaken gently to ensure that all flies were at the bottom of the tube. Observations were made on the behavior of adult *D. melanogaster* in their efforts to climb to reach the set climbing limit, which is a height of 8 cm. Observations were made by giving flies the opportunity to climb for a certain period. Recording was done for 60 seconds, and during that time, recording was made of the duration of movement for each individual fly. If the fly failed to climb or had not reached the predetermined height limit until the recording time ran out, then the duration of movement for that fly was recorded as 60 seconds. Next, the climbing time of the five flies in each tube was calculated and recorded. This approach allows for observing variations in fly ability in responding to different environmental conditions, especially in the context of heavy metal exposure.

The procedure for collecting data on the climbing success variable also refers to the methodology adopted from Fauzi et al. (2020). First, a special climbing tube for *D. melanogaster* was prepared. Then, 10 adult *D. melanogaster* from each treatment to be tested were placed in the climbing tube. Each climbing tube had been marked at a height of 8 cm from the bottom of the tube. Before testing, adult *D. melanogaster* were allowed to stay in the climbing tube for a few minutes to reduce any stress that may occur. After the condition of adult *D. melanogaster* was deemed stable, the test tube was tapped and shaken gently so that all flies were at the bottom of the bottle. Observations were made on the behavior of adult *D. melanogaster* who were given the opportunity to climb to reach the set climbing limit, which is a height of 8 cm, with an observation duration of 10 seconds. During this time, data collection was carried out to record the number of flies that successfully reached the predetermined height limit (8 cm). This approach allows researchers to observe the proportion of flies that can overcome physical obstacles and reach vertical movement goals in the context of different treatment conditions, including heavy metal exposure.

2.4 Data analysis

The data generated from measuring movement duration and climbing success in each condition was processed using a series of statistical analysis techniques. First, Spearman's rho correlation analysis was performed to evaluate the relationship between movement duration and climbing success in each treatment condition. If there is a significant correlation, a simple linear regression test is run for each condition. Next, to assess the parallelism of the regression lines in the three different conditions, a regression line parallelism test is performed. This test provides insight into whether the regression lines from the three conditions run parallel or intersect. The results of this test provide important information about whether fly responses to heavy metal exposure are uniformly reflected in movement duration and climbing success. Finally, visualization of the alignment of the three regression lines is done through a scatter plot graph. This graph provides a clear visual representation of the relationship pattern between movement duration and climbing success variables in three different conditions. All analyses in this study were conducted using RStudio software.

3 Results and Discussion

The analysis using Spearman's Rho correlation test revealed compelling findings. It revealed a consistent and statistically significant negative correlation between climbing duration and climbing success across all three conditions under investigation. In the natural conditions, the duration of climbing was found to be negatively correlated with climbing success (r_s=-0.438, p=0.032). Similarly, the same trend was observed in the conditions of stress exposure for one generation (r_s=-0.864, p<0.001) and stress exposure for two generations (r_s=-0.436, p=0.033). These results suggest a robust association between the time taken to climb and the success achieved, emphasizing the potential influence of varying stressors on Drosophila's climbing behavior. The correlations, as summarized in Table 1, provide a foundational understanding for the subsequent analyses and discussions in this study.

These findings suggest that as the duration of climbing increases, the likelihood of achieving successful climbs decreases. The correlation pattern revealed in this research could be attributed to the physiological and behavioral adjustments that naturally undergo when facing environmental challenges. This correlation results can be interpreted as an adaptive response of Drosophila to stressors. Longer climbing duration might indicate a higher level of physical strain or fatigue, which subsequently leads to reduced climbing success. This finding is in line with other research which reports the evolutionary adaptive response of fruit flies in the form of changes in sleep patterns to starvation stress [47]. Other studies also report that stressful conditions due to inbreeding also give rise to adaptive responses in these insects [48]. Regarding physical strain on climbing success, muscle fatigue resistance is an important factor in climbing [49]. In humans, climbing involves intermittent and continuous contractions of the arm muscles. In conditions of difficulty climbing and the climbing duration is too long, the muscles use anaerobic pathways and cause fatigue which ultimately reduces climbing success [50].

Table 1. Summary of Spearman's Rank Correlation test results.

Statistic	Zero generation	One generation	Two generation
Coefficient (r_s)	-0.4382	-0.8634	-0.4357
p-value	0.03223	< 0.001	0.03334
Covariance	-19.5217	-42.5109	-20.6087
Sample size (*n*)	24	24	24

The emergence of the impact of consuming media containing heavy metals on fly locomotors in this study also strengthens the impact of this substance on other variables, such as egg laying and feeding behavior [51] reproductive fitness [52], as well as reducing fly survival [53]. Stressors, such as exposure to heavy metals, can disrupt the insects' physiological processes, affecting their overall mobility and motor performance [52–54]. Furthermore, the consistent correlation across varying exposure conditions implies that the observed relationship is not limited to a specific stressor intensity or duration.

These findings may have revealed new information regarding potential stress response biomarkers of fruit flies. The negative correlation resulting from the results of this research analysis indicates that the level of climbing success is a sensitive indicator of the organism's overall health and resilience. The consistent direction of the correlation between the three conditions examined in this study can be attributed to the link between the physiological responses of fruit flies and their behavioral adaptations when exposed to toxic substances. Apart from that, this correlation is also in line with the concept that stress-induced alterations in energy allocation [55–56] and physiological trade-offs could manifest as observable changes in behavior [57–58]. The stressors, potentially including heavy metal exposure, could disrupt neural pathways [59], muscle function [60], or even energy metabolism, thereby influencing the insects' motor skills and, consequently, their climbing performance.

Table 2. Summary of Simple Linear Regression test results.

Conditions	**Coefficients**					**ANOVA**	
		Unstandardized	**SE**	***t***	***p***	***F***	***p***
1	Intercept	9.948	0.227	43.884	< 0.001	7.528	0.012
	Duration	-0.111	0.040	-2.744	0.012		
2	Intercept	9.239	0.536	17/243	< 0.001	44.586	< 0.001
	Duration	-0.181	0.027	-6.677	< 0.001		
3	Intercept	9.306	0.269	34.605	< 0.001	5.220	0.032
	Duration	-0.054	0.024	-2.285	0.032		

Furthermore, the results of the linear regression analyses, as presented in Table 2, provide valuable insights into the relationship between climbing duration and climbing success within each exposure condition. In the natural condition, the regression equation $\hat{y}=9.948 - 0.111X$ indicates that for every unit increase in climbing duration (X), the predicted climbing success ($\hat{y}$) decreases by 0.111. This suggests that longer climbing durations are associated with reduced chances of achieving successful climbs in the absence of stressors. Similarly, under stress exposure for one generation, the regression equation ŷ=9.239 - 0.181X illustrates a more pronounced negative relationship. In this case, an increment in climbing duration corresponds to a larger reduction in predicted climbing success, reflecting the intensified impact of stress on Drosophila's climbing behavior. In line with these two regression lines, the regression equation for the group of flies exposed to low concentrations after two generations is ŷ=9.306 - 0.054X.

The three regression equations, just like the correlation results, have the same pattern. These three regression equations will produce a decreasing line as an indication of the direction of the relationship between the duration of climbing and the number of flies that successfully climb. Based on the similarities between the two analysis results, the two measured DCA parameters show consistent findings. Both the long duration and the failure to climb indicate the unhealthy condition experienced by the fly. Healthy flies will be able to climb quickly [61]. However, if the body condition is not perfect, the fly's ability to climb will be disrupted or lost, rather, the regression equation for the condition of stress exposure for two generations portrays a relatively milder negative relationship between climbing duration and climbing success. This result could mean that the prolonged stress exposure might lead to an attenuated reduction in climbing success as climbing duration increases.

To ensure that there are differences in the slopes of the three regressions, a parallelism test was carried out. The assessment of parallelism among the three regression lines, yielded significant results with a p-value<0.05. This outcome underscores that not all the lines are parallel. The significance of this result suggests that the impact of stressors, particularly exposure to heavy metals over one or two generations, introduces distinct variations in the relationship between climbing duration and climbing success compared to the natural condition.

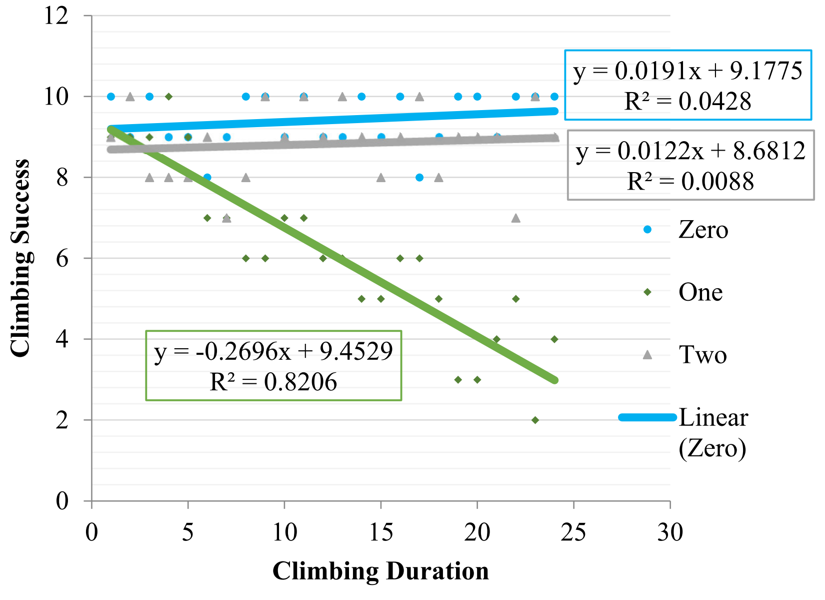

Fig. 1. Scatterplot that visualizes the regression line of the three conditions.

To clarify the non-parallelism of the three regression lines, Figure 1 presents a scatter plot of the three conditions examined in this study. Based on the figure, it is clear that the regression line on the exposure data for one generation intersects with the other two regression lines. The collision of these regression lines indicates that the influence of exposure to heavy metals over two generations changed the predicted relationship between climbing duration and climbing success rate of flies and resulted in a deviation from the pattern observed under natural conditions. This deviation could be caused by a stronger decline in the health condition of flies exposed to stress over a longer exposure duration.

In biology and toxicology research, conditions of shorter exposure but with higher doses are called acute exposure [62]. On the other hand, conditions of low exposure but over a long period of time are called chronic exposure [62]. Acute exposure is usually able to produce radiation effects quickly, while chronic exposure allows the effects to accumulate over time [63–65]. These two types of exposure can impact different health conditions due to differences in dose, duration, and mechanisms of action for both [66–67].

Acute exposure can cause severe effects on biological tissue [68–69]. With high doses, the substance that enters the body is also high so that it can directly damage cellular components, causing cell death or genetic mutations [70]. This condition will lead to tissue damage and even death [71]. On the other hand, chronic exposure usually has milder impacts in the early stages of exposure [64–72]. The health impacts will increase the longer the exposure is received [64–73].

In addition, the body's repair mechanisms when exposed to acute and chronic exposure are also different. Acute exposure may overwhelm cellular repair processes, resulting in immediate harm [65–74]. In contrast, in chronic exposure, cells still have the opportunity to adapt and repair the damage they receive, thereby potentially reducing tissue damage [72–75]. In typical DCA data, acute exposure can lead to severe damage to nerve tissue [76–77]. High concentrations of heavy metals can damage neural circuits and muscle function. This exposure will cause a rapid decline in climbing ability because the flies have difficulty coordinating their movements effectively. On the other hand, when exposed to chronicity, the neural circuits and muscles still can adapt. This adaptation may involve compensatory mechanisms that help mitigate the initial damage and maintain climbing ability to a certain degree.

4 Conclusion

Overall, this study explores the behavioral responses of *D. melanogaster* to varying environmental exposures, focusing on the relationship between climbing duration and climbing success in three different conditions. The results of the Spearman correlation analysis show that in all tested conditions, there is a significant negative correlation between climbing duration and climbing success. These findings indicate the presence of complex adaptive responses in climbing behavior, especially when *Drosophila* faces exposure to environmental stressors. Furthermore, linear regression analysis presents interesting results. In natural conditions and under conditions of acute and chronic exposure, regression shows a negative relationship between climbing duration and climbing success. The significance of the regression line parallelism test reveals variations in this relationship among the three conditions. Moreover, scatterplots depict that the regression line of the condition exposed to heavy metals for two generations intersects with the regression line of natural conditions. These findings indicate more complex changes in climbing behavior when Drosophila experiences longer-term exposure to heavy metals.

In conclusion, this study permeates understanding of *Drosophila* behavioral adaptation to environmental changes and underlines the importance of a holistic approach in analyzing behavioral responses. These results have relevant implications in the fields of toxicology and ecology, showing that the climbing assay is a sensitive evaluation tool for environmental impacts on organism behavior. Therefore, this study not only provides new insights into understanding organism interactions with environmental stressors but also provides a foothold for further research into exploring the complexity of adaptation at the behavioral and physiological levels.

References

1. F. P. Fischer, R. A. Karge, Y. G. Weber, H. Koch, S. Wolking, & A. Voigt, Drosophila melanogaster as a versatile model organism to study genetic epilepsies: An overview. *Frontiers in Molecular Neuroscience*, **16** (2023). https://doi.org/10.3389/fnmol.2023.1116000.
2. M. Yamaguchi & H. Yoshida, Drosophila as a model organism. *Drosophila Models for Human Diseases, Advances in Experimental Medicine and Biology* (Singapore: Springer Nature Singapore Pte Ltd, 2018), pp. 1–10. https://doi.org/10.1007/978-981-13-0529-0_1.
3. A. Raj, P. Shah, & N. Agrawal, Sedentary behavior and altered metabolic activity by AgNPs ingestion in Drosophila melanogaster. *Scientific Reports*, **7** (2017). https://doi.org/10.1038/s41598-017-15645-6.
4. S.-H. Lee, H.-Y. Lee, E.-J. Lee, D. Khang, & K.-J. Min, Effects of carbon nanofiber on physiology of Drosophila. *International Journal of Nanomedicine*, **10** (2015) 3687–3697. https://doi.org/10.2147/IJN.S82637.
5. P. K. Mishra, A. Ekielski, S. Mukherjee, S. Sahu, S. Chowdhury, M. Mishra, S. Talegaonkar, L. Siddiqui, & H. Mishra, Wood-based cellulose nanofibrils: Haemocompatibility and impact on the development and behaviour of drosophila melanogaster. *Biomolecules*, **9** (2019). https://doi.org/10.3390/biom9080363.
6. S. Yan, N. Li, Y. Guo, Y. Chen, C. Ji, M. Yin, J. Shen, & J. Zhang, Chronic exposure to the star polycation (SPc) nanocarrier in the larval stage adversely impairs life history traits in Drosophila melanogaster. *Journal of Nanobiotechnology*, **20** (2022). https://doi.org/10.1186/s12951-022-01705-1.
7. K. Sood, J. Kaur, H. Singh, S. Kumar Arya, & M. Khatri, Comparative toxicity evaluation of graphene oxide (GO) and zinc oxide (ZnO) nanoparticles on Drosophila melanogaster. *Toxicology Reports*, **6** (2019) 768–781. https://doi.org/10.1016/j.toxrep.2019.07.009.
8. N. Songvorawit, P. Phengphuang, & T. Khongkhieo, Fluorescent silica nanoparticles as an internal marker in fruit flies and their effects on survivorship and fertility. *Scientific Reports*, **12** (2022). https://doi.org/10.1038/s41598-022-24301-7.
9. G. Fedele, E. W. Green, E. Rosato, & C. P. Kyriacou, An electromagnetic field disrupts negative geotaxis in Drosophila via a CRY-dependent pathway. *Nature Communications*, **5** (2014). https://doi.org/10.1038/ncomms5391.
10. J.-E. Bae, S. Bang, S. Min, S.-H. Lee, S.-H. Kwon, Y. Lee, Y.-H. Lee, J. Chung, & K.-S. Chae, Positive geotactic behaviors induced by geomagnetic field in Drosophila. *Molecular Brain*, **9** (2016). https://doi.org/10.1186/s13041-016-0235-1.
11. A. R. McNeil, S. N. Jolley, A. A. Akinleye, M. Nurilov, Z. Rouzyi, A. J. Milunovich, M. C. Chambers, & A. F. Simon, Conditions affecting social space in Drosophila melanogaster. *Journal of Visualized Experiments*, **2015** (2015). https://doi.org/10.3791/53242.
12. Y. Xia, W. Xu, S. Meng, N. K. H. Lim, W. Wang, & F.-D. Huang, An Efficient and Reliable Assay for Investigating the Effects of Hypoxia/Anoxia on Drosophila. *Neuroscience Bulletin*, **34** (2018) 397–402. https://doi.org/10.1007/s12264-017-0173-7.

13. B. Ugur, K. Chen, & H. J. Bellen, Drosophila tools and assays for the study of human diseases. *Disease Models & Mechanisms*, **9** (2016) 235–244. https://doi.org/10.1242/dmm.023762.
14. S. A. Deshpande, G. B. Carvalho, A. Amador, A. M. Phillips, S. Hoxha, K. J. Lizotte, & W. W. Ja, Quantifying Drosophila food intake: comparative analysis of current methodology. *Nature Methods*, **11** (2014) 535–540. https://doi.org/10.1038/nmeth.2899.
15. S. Manjila & G. Hasan, Flight and climbing assay for assessing motor functions in Drosophila. *Bio-Protocol*, **8** (2018) 1–9. https://doi.org/10.21769/bioprotoc.2742.
16. W. Cao, L. Song, J. Cheng, N. Yi, L. Cai, F.-D. Huang, & M. Ho, An automated rapid iterative negative geotaxis assay for analyzing adult climbing behavior in a Drosophila model of neurodegeneration. *Journal of Visualized Experiments*, **2017** (2017). https://doi.org/10.3791/56507.
17. A. E. Peppriell, J. T. Gunderson, I. N. Krout, D. Vorojeikina, & M. D. Rand, Latent effects of early-life methylmercury exposure on motor function in Drosophila. *Neurotoxicology and Teratology*, **88** (2021). https://doi.org/10.1016/j.ntt.2021.107037.
18. S. Ohiomokhare, F. Olaolorun, A. Ladagu, F. Olopade, M.-J. R. Howes, E. Okello, J. Olopade, & P. L. Chazot, The pathopharmacological interplay between vanadium and iron in parkinson's disease models. *International Journal of Molecular Sciences*, **21** (2020) 1–15. https://doi.org/10.3390/ijms21186719.
19. Q. Wu, X. Du, X. Feng, H. Cheng, Y. Chen, C. Lu, M. Wu, & H. Tong, Chlordane exposure causes developmental delay and metabolic disorders in Drosophila melanogaster. *Ecotoxicology and Environmental Safety*, **225** (2021). https://doi.org/10.1016/j.ecoenv.2021.112739.
20. T. D. Algarve, C. E. Assmann, T. Aigaki, & I. B. M. da Cruz, Parental and preimaginal exposure to methylmercury disrupts locomotor activity and circadian rhythm of adult Drosophila melanogaster. *Drug and Chemical Toxicology*, **43** (2020) 255–265. https://doi.org/10.1080/01480545.2018.1485689.
21. Y. Zhang, M. B. Wolosker, Y. Zhao, H. Ren, & B. Lemos, Exposure to microplastics cause gut damage, locomotor dysfunction, epigenetic silencing, and aggravate cadmium (Cd) toxicity in Drosophila. *Science of the Total Environment*, **744** (2020). https://doi.org/10.1016/j.scitotenv.2020.140979.
22. A. O. Abolaji, J. P. Kamdem, T. H. Lugokenski, E. O. Farombi, D. O. Souza, T. L. da Silva Loreto, & J. B. T. Rocha, Ovotoxicants 4-vinylcyclohexene 1,2-monoepoxide and 4-vinylcyclohexene diepoxide disrupt redox status and modify different electrophile sensitive target enzymes and genes in Drosophila melanogaster. *Redox Biology*, **5** (2015) 328–339. https://doi.org/10.1016/j.redox.2015.06.001.
23. O. Ibraheem, T. A. Oyewole, A. Adedara, A. O. Abolaji, O. M. Ogundipe, J. Akinyelu, C. T. Eze, S. Albogami, S. S. Alotaibi, O. S. Adeyemi, G. E.-S. Batiha, M. Alorabi, & M. De Waard, Ackee (Blighia sapida K.D. Koenig) Leaves and Arils Methanolic Extracts Ameliorate CdCl2-Induced Oxidative Stress Biomarkers in Drosophila melanogaster. *Oxidative Medicine and Cellular Longevity*, **2022** (2022). https://doi.org/10.1155/2022/3235031.
24. J. Harbottle, P. Strangward, C. Alnuamaani, S. Lawes, S. Patel, & A. Prokop, Making research fly in schools: Drosophila as a powerful modern tool for teaching biology. *School Science Review*, **97** (2016) 18–22.

25. A. K. Lima, H. Dhillon, & A. R. Dillman, ShK-Domain-Containing Protein from a Parasitic Nematode Modulates Drosophila melanogaster Immunity. *Pathogens*, **11** (2022). https://doi.org/10.3390/pathogens11101094.
26. M. R. Poetini, E. A. S. Musachio, S. M. Araujo, F. P. Almeida, M. M. M. Dahleh, V. C. Bortolotto, D. E. Janner, F. C. Pinheiro, B. P. Ramborger, R. Roehrs, D. La Rosa Novo, M. F. Mesko, G. P. Guerra, & M. Prigol, Iron overload during the embryonic period develops hyperactive like behavior and dysregulation of biogenic amines in Drosophila melanogaster. *Developmental Biology*, **475** (2021) 80–90. https://doi.org/10.1016/j.ydbio.2021.03.006.
27. M. P. Singh, R. Himalian, S. Shabir, A. A. Obaid, A. S. Alamri, C. M. Galanakis, S. K. Singh, & E. Vamanu, Protection of Phytoextracts against Rotenone-Induced Organismal Toxicities in Drosophila melanogaster via the Attenuation of ROS Generation. *Applied Sciences (Switzerland)*, **12** (2022). https://doi.org/10.3390/app12199822.
28. Z. Chen, F. Wang, D. Wen, & R. Mu, Exposure to bisphenol A induced oxidative stress, cell death and impaired epithelial homeostasis in the adult Drosophila melanogaster midgut. *Ecotoxicology and Environmental Safety*, **248** (2022). https://doi.org/10.1016/j.ecoenv.2022.114285.
29. T. O. Johnson, A. O. Abolaji, S. Omale, I. Y. Longdet, R. J. Kutshik, B. O. Oyetayo, A. E. Adegboyega, & A. Sagay, Benzo[a]pyrene and Benzo[a]pyrene-7,8-dihydrodiol-9,10-epoxide induced locomotor and reproductive senescence and altered biochemical parameters of oxidative damage in Canton-S Drosophila melanogaster. *Toxicology Reports*, **8** (2021) 571–580. https://doi.org/10.1016/j.toxrep.2021.03.001.
30. M. D. Rand, J. M. Tennessen, T. F. C. Mackay, & R. R. H. Anholt, Perspectives on the Drosophila melanogaster model for advances in toxicological science. *Current Protocols*, **3** (2023). https://doi.org/10.1002/cpz1.870.
31. L. J. Sudmeier, S. P. Howard, & B. Ganetzky, A Drosophila model to investigate the neurotoxic side effects of radiation exposure. *DMM Disease Models and Mechanisms*, **8** (2015) 669–677. https://doi.org/10.1242/dmm.019786.
32. J. Patocka, R. Wu, E. Nepovimova, M. Valis, W. Wu, & K. Kuca, Chemistry and toxicology of major bioactive substances in inocybe mushrooms. *International Journal of Molecular Sciences*, **22** (2021) 2218. https://doi.org/10.3390/ijms22042218.
33. G. J. Myatt, E. Ahlberg, Y. Akahori, D. Allen, A. Amberg, L. T. Anger, A. Aptula, S. Auerbach, L. Beilke, P. Bellion, R. Benigni, J. Bercu, E. D. Booth, D. Bower, A. Brigo, N. Burden, Z. Cammerer, M. T. D. Cronin, K. P. Cross, L. Custer, M. Dettwiler, K. Dobo, K. A. Ford, M. C. Fortin, S. E. Gad-McDonald, N. Gellatly, V. Gervais, K. P. Glover, S. Glowienke, J. Van Gompel, S. Gutsell, B. Hardy, J. S. Harvey, J. Hillegass, M. Honma, J.-H. Hsieh, C.-W. Hsu, K. Hughes, C. Johnson, R. Jolly, D. Jones, R. Kemper, M. O. Kenyon, M. T. Kim, N. L. Kruhlak, S. A. Kulkarni, K. Kümmerer, P. Leavitt, B. Majer, S. Masten, S. Miller, J. Moser, M. Mumtaz, W. Muster, L. Neilson, T. I. Oprea, G. Patlewicz, A. Paulino, E. Lo Piparo, M. Powley, D. P. Quigley, M. V. Reddy, A.-N. Richarz, P. Ruiz, B. Schilter, R. Serafimova, W. Simpson, L. Stavitskaya, R. Stidl, D. Suarez-Rodriguez, D. T. Szabo, A. Teasdale, A. Trejo-Martin, J.-P. Valentin, A. Vuorinen, B. A. Wall, P. Watts, A. T. White, J. Wichard, K. L. Witt, A. Woolley, D. Woolley, C. Zwickl, & C. Hasselgren, In silico toxicology protocols. *Regulatory Toxicology and Pharmacology*, **96** (2018) 1–17. https://doi.org/10.1016/j.yrtph.2018.04.014.
34. K. G. Hales, C. A. Korey, A. M. Larracuente, & D. M. Roberts, Genetics on the fly: A primer on the drosophila model system. *Genetics*, **201** (2015) 815–842. https://doi.org/10.1534/genetics.115.183392.

35. F. Liguori, U. B. Pandey, & F. A. Digilio, Editorial: Drosophila as a model to study neurodegenerative diseases. *Frontiers in neuroscience*, **17** (2023) 1275253. https://doi.org/10.3389/fnins.2023.1275253.
36. D. P. Snustad & M. J. Simmons, *Principles of genetics*, 7th ed (Wiley, 2015).
37. D. Fatmawati, D. Khoiroh, S. Zubaidah, H. Susanto, M. Agustin, & A. Fauzi, Wing morphological changes of Drosophila melanogaster exposed with Lead in nine generations. *AIP Conference Proceedings* (AIP Publishing, 2022).
38. D. Khoiroh, L. Hindun, D. Fatmawati, S. Zubaidah, H. Susanto, & A. Fauzi, Drosophila melanogaster behavior study: Does plumbum affect pupation and climbing ability of imago? *AIP Conference Proceedings* (AIP Publishing, 2023), p. 020099. https://doi.org/10.1063/5.0111891.
39. O. Ibraheem, D. Bankole, A. Adedara, A. O. Abolaji, T. H. Fatoki, J. M. Ajayi, & C. T. Eze, Methanolic leaves and arils extracts of ackee (Blighia sapida) plant ameliorate mercuric chloride-induced oxidative stress in drosophila melanogaster. *Biointerface Research in Applied Chemistry*, **11** (2021) 7528–7542. https://doi.org/10.33263/BRIAC111.75287542.
40. A. E. Peppriell, J. T. Gunderson, D. Vorojeikina, & M. D. Rand, Methylmercury myotoxicity targets formation of the myotendinous junction. *Toxicology*, **443** (2020). https://doi.org/10.1016/j.tox.2020.152561.
41. J. T. Gunderson, A. E. Peppriell, I. N. Krout, D. Vorojeikina, & M. D. Rand, Neuroligin-1 Is a Mediator of Methylmercury Neuromuscular Toxicity. *Toxicological Sciences*, **184** (2021) 236–251. https://doi.org/10.1093/toxsci/kfab114.
42. Z. Alasmary, T. Todd, G. M. Hettiarachchi, T. Stefanovska, V. Pidlisnyuk, K. Roozeboom, L. Erickson, L. Davis, & O. Zhukov, Effect of soil treatments and amendments on the nematode community under miscanthus growing in a lead contaminated military site. *Agronomy*, **10** (2020) 1727. https://doi.org/10.3390/agronomy10111727.
43. J. J. Clark & A. C. Knudsen, Extent, characterization, and sources of soil lead contamination in small-urban residential neighborhoods. *Journal of Environmental Quality*, **42** (2013) 1498–1506. https://doi.org/10.2134/jeq2013.03.0100.
44. E. Obeng-Gyasi, Sources of lead exposure in various countries. *Reviews on Environmental Health*, **34** (2019) 25–34. https://doi.org/10.1515/reveh-2018-0037.
45. M. M. Onakpa, A. A. Njan, & O. C. Kalu, A review of heavy metal contamination of food crops in Nigeria. *Annals of global health*, **84** (2018) 488–494. https://doi.org/10.29024/aogh.2314.
46. A. Fauzi, S. Zubaidah, & H. Susanto, The study of larva and adult behavior of Drosophila melanogaster: Do strains affect behavior? In A. Taufiq, H. Susanto, H. Nur, M. Aziz, C.-R. Chang, H. Lee, M. Diantoro, N. Mufti, N.A.N.N. Malek, I.C. Wang, D.T. Iskandar, G. Elbers, S. Sunaryono, S. Zubaidah, S. Sumari, A. Aulanni'am, A.B. Nandiyanto, I. Wibowo, & A.Y. Handaya,eds., *AIP Conference Proceedings* (Malang: AIP Publishing, 2020), pp. 0400141–0400147. https://doi.org/10.1063/5.0002429.
47. M. E. Slocumb, J. M. Regalado, M. Yoshizawa, G. G. Neely, P. Masek, A. G. Gibbs, & A. C. Keene, Enhanced sleep is an evolutionarily adaptive response to starvation stress in Drosophila. *PLOS ONE*, **10** (2015) e0131275. https://doi.org/10.1371/journal.pone.0131275.
48. M. F. Schou, J. Bechsgaard, J. Muñoz, & T. N. Kristensen, Genome-wide regulatory deterioration impedes adaptive responses to stress in inbred populations of Drosophila melanogaster *. *Evolution*, **72** (2018) 1614–1628. https://doi.org/10.1111/evo.13497.

49. Climbro Team, Performance factors in sport climbing. (2020). https://climbro.com/2020/04/performance-factors-in-sport-climbing/.
50. A. W. Sheel, Physiology of sport rock climbing. *British Journal of Sports Medicine*, **38** (2004) 355–359. https://doi.org/10.1136/bjsm.2003.008169.
51. S. Bahadorani & A. J. Hilliker, Biological and behavioral effects of heavy metals in Drosophila melanogaster adults and larvae. *Journal of Insect Behavior*, **22** (2009) 399–411. https://doi.org/10.1007/s10905-009-9181-4.
52. K. P. Nanda & H. Firdaus, Dietary cadmium induced declined locomotory and reproductive fitness with altered homeostasis of essential elements in Drosophila melanogaster. *Comparative Biochemistry and Physiology Part C: Toxicology & Pharmacology*, **255** (2022) 109289. https://doi.org/10.1016/j.cbpc.2022.109289.
53. F. A. Al-Momani & A. M. Massadeh, Effect of different heavy-metal concentrations on Drosophila melanogaster larval growth and development. *Biological Trace Element Research*, **108** (2005) 271–278. https://doi.org/10.1385/BTER:108:1-3:271.
54. L. Bonilla-Ramirez, M. Jimenez-Del-Rio, & C. Velez-Pardo, Acute and chronic metal exposure impairs locomotion activity in Drosophila melanogaster: a model to study Parkinsonism. *BioMetals*, **24** (2011) 1045–1057. https://doi.org/10.1007/s10534-011-9463-0.
55. W. M. De Coen & C. R. Janssen, The use of biomarkers in Daphnia magna toxicity testing. IV. Cellular Energy Allocation: a new methodology to assess the energy budget of toxicant-stressed Daphnia populations. *Journal of Aquatic Ecosystem Stress and Recovery*, **6** (1997) 43–55. https://doi.org/10.1023/A:1008228517955.
56. Y. Ma, M. C. Dias, & H. Freitas, Drought and salinity stress responses and microbe-induced tolerance in plants. *Frontiers in Plant Science*, **11** (2020). https://doi.org/10.3389/fpls.2020.591911.
57. T. C. Salzman, A. L. McLaughlin, D. F. Westneat, & P. H. Crowley, Energetic trade-offs and feedbacks between behavior and metabolism influence correlations between pace-of-life attributes. *Behavioral Ecology and Sociobiology*, **72** (2018) 54. https://doi.org/10.1007/s00265-018-2460-3.
58. C. K. R. Willis, Trade-offs influencing the physiological ecology of hibernation in temperate-zone bats. *Integrative and Comparative Biology*, **57** (2017) 1214–1224. https://doi.org/10.1093/icb/icx087.
59. N. Singh & B. Sharma, On the mechanisms of heavy metal-induced neurotoxicity: Amelioration by plant products. *Proceedings of the National Academy of Sciences, India Section B: Biological Sciences* (2021), pp. 743–751. https://doi.org/10.1007/s40011-021-01272-9.
60. M. Wu, Y. Shu, & Y. Wang, Exposure to mixture of heavy metals and muscle strength in children and adolescents: A population-based study. *Environmental Science and Pollution Research*, **29** (2022) 60269–60277. https://doi.org/10.1007/s11356-022-19916-2.
61. A. N. Spierer, D. Yoon, C.-T. Zhu, & D. M. Rand, FreeClimber: Automated quantification of climbing performance in Drosophila. *Journal of Experimental Biology*, (2020). https://doi.org/10.1242/jeb.229377.
62. G. Heinemeyer, M. Jantunen, & P. Hakkinen, *The practice of consumer exposure assessment* (Springer International Publishing, 2020).
63. K. Rehman, F. Fatima, I. Waheed, & M. S. H. Akash, Prevalence of exposure of heavy metals and their impact on health consequences. *Journal of Cellular Biochemistry*, **119** (2018) 157–184. https://doi.org/10.1002/jcb.26234.

64. N. Liu, Y. Peng, X. Zhong, Z. Ma, S. He, Y. Li, W. Zhang, Z. Gong, & Z. Yao, Effects of exposure to low-dose ionizing radiation on changing platelets: A prospective cohort study. *Environmental Health and Preventive Medicine*, **26** (2021) 14. https://doi.org/10.1186/s12199-021-00939-z.
65. T. A. Choi, S. V. Costes, & R. J. Abergel, Understanding the health impacts and risks of exposure to radiation. In J. Ahn, C. Carson, M. Jensen, K. Juraku, S. Nagasaki, & S. Tanaka,eds., *Reflections on the Fukushima Daiichi Nuclear Accident* (Cham: Springer International Publishing, 2015), pp. 259–281. https://doi.org/10.1007/978-3-319-12090-4_13.
66. R. Dhakal, M. Yosofvand, M. Yavari, R. Abdulrahman, R. Schurr, N. Moustaid-Moussa, & H. Moussa, Review of biological effects of acute and chronic radiation exposure on Caenorhabditis elegans. *Cells*, **10** (2021) 1966. https://doi.org/10.3390/cells10081966.
67. R. Yahyapour, P. Amini, S. Rezapour, M. Cheki, A. Rezaeyan, B. Farhood, D. Shabeeb, A. E. Musa, H. Fallah, & M. Najafi, Radiation-induced inflammation and autoimmune diseases. *Military Medical Research*, **5** (2018) 9. https://doi.org/10.1186/s40779-018-0156-7.
68. B. A. Hollingsworth, D. R. Cassatt, A. L. DiCarlo, C. I. Rios, M. M. Satyamitra, T. A. Winters, & L. P. Taliaferro, Acute radiation syndrome and the microbiome: Impact and review. *Frontiers in Pharmacology*, **12** (2021). https://doi.org/10.3389/fphar.2021.643283.
69. H. Fukunaga & K. M. Prise, Non-uniform radiation-induced biological responses at the tissue level involved in the health risk of environmental radiation: a radiobiological hypothesis. *Environmental Health*, **17** (2018) 93. https://doi.org/10.1186/s12940-018-0444-4.
70. C. Jia, Q. Wang, X. Yao, & J. Yang, The role of DNA damage induced by low/high dose ionizing radiation in cell carcinogenesis. *Exploratory Research and Hypothesis in Medicine*, (2021). https://doi.org/10.14218/ERHM.2021.00020.
71. J. Sia, R. Szmyd, E. Hau, & H. E. Gee, Molecular mechanisms of radiation-induced cancer cell death: A primer. *Frontiers in Cell and Developmental Biology*, **8** (2020). https://doi.org/10.3389/fcell.2020.00041.
72. G. L. Russo, I. Tedesco, M. Russo, A. Cioppa, M. G. Andreassi, & E. Picano, Cellular adaptive response to chronic radiation exposure in interventional cardiologists. *European Heart Journal*, **33** (2012) 408–414. https://doi.org/10.1093/eurheartj/ehr263.
73. K. Kamiya, K. Ozasa, S. Akiba, O. Niwa, K. Kodama, N. Takamura, E. K. Zaharieva, Y. Kimura, & R. Wakeford, Long-term effects of radiation exposure on health. *The Lancet*, **386** (2015) 469–478. https://doi.org/10.1016/S0140-6736(15)61167-9.
74. K. Tanigawa, Case review of severe acute radiation syndrome from whole body exposure: concepts of radiation-induced multi-organ dysfunction and failure. *Journal of Radiation Research*, **62** (2021) i15–i20. https://doi.org/10.1093/jrr/rraa121.
75. D. Wodarz, R. Sorace, & N. L. Komarova, Dynamics of cellular responses to radiation. *PLoS Computational Biology*, **10** (2014) e1003513. https://doi.org/10.1371/journal.pcbi.1003513.
76. D. R. Ortega, D. F. G. Esquivel, T. B. Ayala, B. Pineda, S. Gómez Manzo, J. M. Quino, P. C. Mora, & V. P. de la Cruz, Cognitive impairment induced by lead exposure during lifespan: Mechanisms of lead neurotoxicity. *Toxics*, **9** (2021) 23. https://doi.org/10.3390/toxics9020023.

77. J.-W. Lee, H. Choi, U.-K. Hwang, J.-C. Kang, Y. J. Kang, K. Il Kim, & J.-H. Kim, Toxic effects of lead exposure on bioaccumulation, oxidative stress, neurotoxicity, and immune responses in fish: A review. *Environmental Toxicology and Pharmacology*, **68** (2019) 101–108. https://doi.org/10.1016/j.etap.2019.03.010.