Effects of Martian magnetic and gravitational fields across multiple generations of the nematode *C. elegans*

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Abstract

Life on Earth evolved under a specific set of environmental conditions, including consistent gravitational and magnetic fields. However, planned human missions to Mars in the coming decades will expose terrestrial organisms to radically different conditions, with Martian gravity being approximately 38% of Earth's and a significantly reduced magnetic field. Understanding the combined effects of these factors is crucial, as they may impact biological systems that evolved under different conditions. In this study, we investigated the effects of simulated Martian gravity and hypomagnetic fields on the nematode *Caenorhabditis elegans* across six generations. We used an integrated experimental setup consisting of clinostats to mimic the reduced Martian gravity, and Merritt coil magnetic cages to model the decreased Martian magnetic fields. We assessed behavioral, morphological, and physiological responses of *C. elegans*. High-throughput automated assays revealed significant reductions in motor output and morphological dimensions for animals in the Mars treatment compared to matched "earth" controls. We assessed neurological function by means of chemotaxis assays and found a progressive decline in performance for worms raised under the Martian paradigm compared to Earth controls. Our results show that worms grown under Martian-like conditions exhibit progressive physiological alterations across generations, suggesting that the unique environment of Mars might pose challenges to biological function and adaptation. These findings contribute to understanding how living organisms may respond to the combined effects of reduced gravity and hypomagnetic fields, providing insights relevant for future human exploration and potential colonization of Mars.

Introduction

The Earth is the only known planet where life has been confirmed to exist. Various environmental factors, such as water availability, temperature range, magnetic field, and gravitational forces contributed to creating an environment that supported abiogenesis and the subsequent evolution of a wide range of life forms (Groombridge and Jenkins, 2002). While life may have independently evolved under diverse conditions outside our planet, Earth-based life is constrained to a narrow range of physical variables shaped by its evolution on our planet's surface (Wharton, 2007). In addition to a stable supply of solar radiation and a relatively rich mineral composition, life on Earth evolved in the presence of our planet's gravitational and magnetic force fields.

Gravity and its role in biological systems

The Earth's gravity is a fundamental factor in the evolution and mechanics of life (Morey-Holton, 2003). It exerts a constant force directed toward the Earth's center, defining the weight of objects and influencing various chemical, biological, and ecological processes (Guillen et al., 2008). Gravity has shaped the development of life from early aquatic organisms to terrestrial vertebrates, requiring adaptations for fluid dynamics, structural support, and locomotion (Morey-Holton, 2003). Its influence on biological processes is well-documented across numerous studies (Klaus et al., 1997; Hammond et al., 2010; Boyle and Hughes-Fulford, 2021).

Research on gravity's effects has employed various methods to simulate microgravity on Earth (see Ferranti et al., 2020). These include the drop towers (Dittrich, 2014), parabolic flights (Pletser, 2016), neutral buoyancy tanks (Neufeld and Charles, 2015), and clinostats (Klauss, 2001). These techniques help create environments that mimic microgravity for extended periods, allowing researchers to study its

impact on cellular and physiological processes (Dedolph and Dipert, 1971; Hasemstein et al., 2015). With the advent of space exploration and the deployment of the International Space Station (ISS), microgravity environments have been generated by placing objects in continual free fall, offering a unique platform for gravity-related studies (Penley et al., 2002). However, it's important to note that the ISS does not provide an environment with significantly reduced gravitational force. The gravity experienced on the ISS is about 90% of what is felt on Earth's surface; the sensation of microgravity is due to the station and its occupants being in constant free fall around the planet, akin to a continuous drop tower experiment.

Pioneering work during the shuttle program's Space Life Sciences-1 (SLS-1) mission demonstrated the profound effects of microgravity on biological systems; for example, jellyfish raised in space showed sensory organ and swimming impairments compared to their Earth-based counterparts (Spangenberg et al., 1994). This and other studies have since established that microgravity alters cellular metabolism and gene expression in diverse organisms (Häder, 2005).

Hypomagnetic fields and their effects on biological systems

In addition to gravity, Earth's magnetic field has also played a crucial role in shaping the evolution of life. The geomagnetic field protects the planet from cosmic radiation and solar wind, providing a stable environment for various life forms (Kirschvink et al., 2010). Many organisms, from bacteria to vertebrates, have evolved mechanisms to detect and respond to Earth's magnetic field, a phenomenon known as magnetoreception (Johnsen and Lohmann, 2008). For example, magnetotactic bacteria use Earth's magnetic field for navigation, and migratory birds rely on it for orientation during long flights (Gould, 1980). Thus, Earth's magnetic field has been a critical factor in the survival and behavior of many species.

In addition, recent studies have demonstrated that hypomagnetic fields impact various biological systems. For example, experiments have shown that hypomagnetic conditions can alter growth rates, reproduction, and cellular metabolism in microorganisms (Fu et al., 2016). Animal studies have linked hypomagnetic fields to changes in behavior, circadian rhythms, and neurological function (Zhang et al., 2004). At the molecular level, hypomagnetic fields may affect the spin states of radical pairs involved in biochemical reactions, potentially disrupting processes like DNA repair and cellular signaling (Ritz et al., 2000).

Effects of Martian physics on terrestrial organisms

For decades work has been underway to send terrestrial organisms to Mars. This includes plans to send humans to Mars in the next few decades (Moore, 2010). Compared to Earth, on Mars terrestrial organisms will face different gravitational and magnetic fields. Mars gravity is about 38% that or the Earth, and it lacks a global magnetic field similar to Earth's, resulting in a "hypomagnetic" environment where organisms are exposed to lower and more variable magnetic fields (Acuna et al., 1999). Understanding how this reduced gravity and magnetic field environment might impact biological systems is crucial, as life on Earth has never been exposed to such conditions for extended periods. Hypomagnetic fields could disrupt processes dependent on magnetoreception and alter fundamental cellular activities, such as gene expression, cellular signaling, and reactive oxygen species (ROS) production (Zhang et al., 2020).

The use of *C. elegans* in space exploration

To understand the potential impacts of Martian-like conditions, including hypomagnetic fields and reduced gravity, researchers often turn to model organisms. *Caenorhabditis elegans* (*C. elegans*), a nematode that reaches adulthood within three days, is particularly suited for such studies due to its short lifespan, which allows for multigenerational studies (Byerly et al., 1976). Despite being a simple organism, *C. elegans* shares many genetic and biological pathways with more complex organisms, including humans, making it an excellent model for studying fundamental biological processes affected by space conditions (Markaki and Tavernarakis, 2020).

Previous studies have used *C. elegans* to examine the effects of microgravity on muscle function, gene expression, and neuronal health, providing insights relevant to human physiology (Adachi et al., 2008; Selch et al., 2008). However, research combining both hypomagnetic and microgravity conditions remains limited. Given that both factors are likely to influence biological systems on Mars, a comprehensive understanding requires studies that simultaneously model reduced gravity and magnetic field environments.

Toward an integrated approach for future Martian missions

While previous studies have explored the effects of either reduced gravity or hypomagnetic fields on biological systems, few have examined the combined impact of these two conditions. As human exploration of Mars becomes a reality, it is crucial to understand how organisms adapt to environments that differ significantly in both gravitational and magnetic fields from those on Earth. Our study aims to address this gap by exposing *C. elegans* to modeled Martian magnetic and gravitational fields, providing a more holistic understanding of the challenges that life might face on Mars.

In this study we raised *C. elegans* nematodes under modeled Martian magnetic and gravitational fields for six generations. We used a combination of approaches including high throughput automated behavioral assays to compare motor and neural function in animals raised under Martian versus Terrestrial magnetic and gravity conditions.

Methods

Animal strains

In this study we used (N2) wild-type *C. elegans* nematodes obtained from the Caenorhabditis Genetic Center. Animals were raised on OP50 *E.coli* bacteria at 20±2°C following established protocols (Brenner, 1974).

Animal husbandry

To study animals across six generations, approximately 3-4 day 1 adult worms were allowed to lay eggs in plates containing nematode growth media (NGM) agar seeded with a lawn of OP50 *E.coli* for one hour. Plates with freshly laid eggs were placed inside a PVC receptacle at the center of our experimental setup (see below). After 3.5 days plates were removed from the experimental setup and day-1 adults were once again allowed to lay eggs for one hour in new NGM plates before these were replaced in the setup once more to continue the transgenerational experiment. Following the 1-hour egg laying protocol, adult day-1 worms were used for the behavioral and physiological experiments. For each condition (Mars or Earth) four individual NGM plates with worms were propagated independently for six generations.

Integrated Magnetic and Gravitational Field Cages

To manipulate the effective magnetic and gravitational field experienced by developing nematodes we constructed two identical experimental setups (Figure 1). Each setup consisted of a clinostat used to manipulate the experienced gravitational field, plus a 20 cm^3 four-coil Merritt magnetic cage used to manipulate the magnetic field experienced by the animals. The animals sat at the center of this cage surrounded by a grounded Faraday cage comprised of copper fabric as a means to cancel out the electric field generated by the Magnetic coil system.

In each of the two identical systems, the Clinostat inclination and the Merritt cage orientation could be controlled independently. Both Merritt Cages were powered by a single DP50V5A constant voltage constant current programmable control power supply which delivered the same current to each of the two Merrit cages.

The effective magnetic field at the center of each magnetic cage was measured using a DC MilliGaus meter (AlphaLab Inc). The magnetic cages were aligned so that the magnetic vector they generated summed with the earth's magnetic vector in the lab in order to produce a net magnetic field ranging between 5±5 milliGaus (Martian conditions), or 650±10 milligaus (Earth conditions). Magnetic

conditions within the cages were measured before at the start and end of each generation of animals placed in the setup. The electric field generated by the cages was removed by the use of grounded faraday cages inside the Merritt system.

To alter the effective gravitational pulled experienced by the nematodes we calculated the angle at which each clinostat needed to be positioned to model each net gravitational pull. Martian gravity is approximately 0.38g (38% that of the Earth's). We used an equation based on Newtonian mechanics: $\sin(\propto) = \frac{desired\ grgavity}{Earth's gravity}$. Therefore, for Martian gravity sin(∝)= 0.38g/1g= 0.38. Solving for ∝, ∝= arcsin(0.38)= 22.3°. For Earth set up, one clinostat was setup at 90° to the ground.

Filming

Swimming. Plates containing Day 1 adult *C. elegans* were flooded with 2 ml of liquid NGM solution. Animals were then placed in a dissecting microscope and filmed for 30 seconds at 30 fps using a Pointgray USBC video camera. TIFF sequences were saved for later analysis.

Crawling. Day 1 adults were transferred to NGM plates with no bacteria. A Basler acA4024-29um infrared camera was used to generate 17 sec 30 fps films of nematodes crawling. ***Swimming frequency measurement***

Image sequences were manually analyzed using ImageJ. Eight to twelve animals per plate were measured from each plate and a total of twenty-four to thirty worms were analyzed for each generation. The maximal number of uninterrupted swimming cycles produced by each animal was divided by the time elapsed during the interval to obtain the swimming frequency.

Tierpsy kinematic analysis

The automated behavioral analysis software Tierpsy Tracker (Javer et al., 2018) was used to detect and analyze the movement of crawling worms. Each culture plate was analyzed individually.

Chemotaxis assay

Chemotaxis was assessed using standard procedures (Queirós et al., 2021). Day one *C. elegans* were placed at the center of agar plates with Diacetyl as an attractant source at one side of the 10 cm chemotaxis plate. After allowing the worms to migrate for 30 minutes at 22 ± 1 °C, the number of worms on each side (attractant and neutral) was quantified. The chemotaxis index was calculated to evaluate the response. All assays were performed in sextuplicate, following established protocols (Margie et al., 2013)

Result

Morphological differences

To compare kinematics and morphological differences between our treatment animals we used an unbiased high-throughput open-source platform for analyzing worm behavioral data (Figure 2, Javer et al., 2018). We found that animals in all treatments experienced morphological changes over the duration of the experiments (Figure 3). However, animals raised under Martian conditions displayed consistent reduction in their width compared to animals raised under Earth conditions (Table 1). These results indicate that worms grown under Mars conditions exhibit a significant reduction in midbody width, head contour width, and tail width compared to their Earth counterparts especially with prolonged exposure.

Worm Curvature

To compare the curvature between worms grown under Earth and Mars conditions, we analyzed neck, midbody, and tail curvatures across generations (Figure 4). Mars-grown worms exhibited consistent differences in neck curvature compared to their Earth counterparts, with significant variation observed in generation 6 (Table 2). Similarly, midbody curvature showed a significant difference between Mars- and Earth-grown worms in generation 4. However, no significant differences were found in tail curvature across all generations. These results suggest that Martian conditions selectively impact specific body regions, with neck and midbody curvatures showing distinct changes over generations.

Postural Alterations

To analyze postural changes, we measured the Eigenworms of C. *elegans* to assess shape variation in *the groups* grown under Earth and Mars conditions (Figure 5). The first seven Eigenworms (α1 to α7) represent the principal modes of shape variation, with α1 capturing primary bending motions and subsequent Eigenworms capturing finer aspects of posture. Our analysis showed a slight increase in the first Eigenworm projection over generations for both conditions, with significant differences in the 6th generation between Earth- and Mars-grown worms (Table 3). The fourth Eigenworm projection remained stable for Earth-grown worms, while a decreasing trend was observed for Mars-grown worms, suggesting adaptive morphological responses to Mars conditions. Significant differences were observed between the 6th generation of both groups for the fourth Eigenworm projection. These results indicate that shape variation in Mars-grown worms changes over time, likely due to environmental stress.

Swimming Frequency

We compared the swimming frequencies of day-1 *C. elegans* grown under Earth and Martian conditions after 2, 4, and 6 generation. We found that worms raised under Martian conditions displayed a significantly lower swimming frequency compared with Earth controls (Figure 6). While the difference in swimming frequency was present throughout the experiment, for each treatment swimming frequency did not change over time (ANOVA but did not increase overtime (Table 4).

Chemotaxis

Chemotaxis behavior in *C. elegans* was assessed across generations grown under Earth and Mars conditions. A significant reduction in the chemotaxis index was observed in Mars-grown worms compared to Earth-grown counterparts, especially in later generations (Figure 7). While no significant difference was found at generation 2, Mars-grown worms exhibited progressively lower chemotaxis indices at generations 4 and 6. These results suggest that worms under Martian conditions experience a decline in chemotactic ability over time (Table 5). This reduced sensory function highlights the potential long-term impacts of Mars-like conditions on neural responses in *C. elegans.*

Discussion

Human missions to Mars have been under consideration for a while now as NASA is hoping to land their first humans in the 2030s (Drake et al., 2010). While this is a remarkable technological feat, it comes with important considerations for the safety of the human component of the mission. One of the many areas of concern is the unknown biological response of astronauts to the relatively different environmental condition of Mars when compared to Earth (McKay & Stoker, 1989). Mars has a significantly weaker global magnetic field compared to Earth, with its surface field strength less than 50 nT (Albee & Palluconi, 1990). This suggests that no dynamo action is currently occurring in the interior of Mars (Albee & Palluconi, 1990). However, this was not the case in the past as the remanent magnetization of meteorites indicates that Mars may have had a surface magnetic field in earlier years, comparable in magnitude to Earth's magnetic field (Curtis & Ness, 1988). This indicates that Mars has undergone evolution in terms of its magnetic properties. Unlike Earth's strong, global magnetosphere, Mars only has localized magnetic fields in its crust, which create small-scale magnetic anomalies (Albee et al., 1998; Connerney et al., 1999). One of the importance of the stronger Earth magnetic field is the protection of the Earth from the solar wind. The solar wind is a continuous stream of charged particles, primarily electrons and protons, that is emitted from the Sun's outer layers and travels through space affecting the radial magnetic field component of any planet (Acuña et al., 2001; Connerney et al., 1999). The solar wind interacts with Mars by forming a magnetic pileup boundary (MPB), where the solar wind plasma and interplanetary magnetic field drape around the planet's ionosphere, creating a partial "magnetic barrier." This boundary provides some shielding but is less effective compared to a global magnetosphere (Cloutier et al., 1999). Due to the weak magnetic protection, Mars has experienced significant atmospheric

stripping. The solar wind continuously interacts directly with the Martian atmosphere, leading to loss of atmospheric particles over geological time scales (Crider et al., 2002).

Research involving animals inhabiting in Mars-like condition may provide an insight to expected physiological reactions of living organisms. In this paper, we present the observed physiological differences between *C. elegans* grown in Mars-like and Earth-like conditions. This study investigated the effects of Mars-like conditions on the morphology and behavior of *C. elegans* across multiple generations. The findings reveal significant alterations in both kinematics and chemotactic responses, suggesting that prolonged exposure to Martian-like environments can impact the physiological and behavioral traits of these model organisms.

The analysis of curvature, particularly the neck and midbody curvatures, showed consistent morphological differences between worms raised under Earth and Mars conditions. Specifically, Mars-grown worms exhibited reduced neck curvature, particularly by generation 6, indicating a potential adaptive response to the altered gravitational and environmental conditions. This aligns with the previous findings that suggest environmental stressors can lead to morphological changes in C. *elegans* (Androwski, et al., 2017; Wang and Amar, 2024), likely as a survival mechanism in unfavorable conditions. The lack of significant differences in tail curvature suggests that not all aspects of morphology are uniformly affected, pointing to a more subtle interaction between environmental stress and developmental plasticity.

In terms of behavior, the chemotaxis index significantly declined in worms grown under Mars conditions, especially from generation 4 onward. The persistent decrease in chemotactic ability indicates a deterioration in sensory processing or behavioral responsiveness, which may stem from neurophysiological decline to prolonged exposure to Martian conditions. The significant differences observed in generations 4 and 6 highlight the potential cumulative effects of environmental stressors on sensory behaviors over generations. These findings are critical, as they suggest that physiological declination may not only be immediate but can persist or even worsen over time, raising concerns about the long-term viability of organisms subjected to extraterrestrial environments. They are also in line with the findings of Kim and Jin who found that stressors may contribute to physiological and behavioral changes (Kim and Jin, 2015; Giles and Rankin, 2009).

Additionally, the use of Eigenworms to analyze shape variation provided insights into the principal modes of morphological change in *C. elegans*. The increasing trend in the first Eigenworm projection coefficients across generations under both Earth and Mars conditions suggests that while the primary mode of shape variation remains stable, subtle changes may reflect adaptive processes to different environmental pressures. In contrast, the decreasing trend in the fourth Eigenworm projection for Mars-grown worms could indicate a selection against certain morphological traits in the face of harsh environmental conditions.

Overall, the results of this study shows the complex interplay between environmental conditions, morphological adaptations, and behavioral responses in *C. elegans*. The observed reductions in chemotaxis and significant morphological changes point to potential long-term consequences of living in Martian-like conditions. These findings raise important questions about the implications for higher organisms, including humans, during extended space missions or colonization efforts on Mars. Future research should explore the underlying mechanisms driving these changes and assess the broader implications for life in extraterrestrial environments.

References

Acuña, M. H., Connerney, J. E. P., Wasilewski, P., Lin, R. P., Mitchell, D., Anderson, K. A., ... & Ness, N. F. (2001). Magnetic field of Mars: Summary of results from the aerobraking and mapping orbits. *Journal of Geophysical Research: Planets*, *106*(E10), 23403-23417.

Albee, A. L., & Palluconi, D. F. (1990). Mars Observer's global mapping mission. *Eos, Transactions American Geophysical Union*, *71*(39), 1099-1107.

Albee, A. L., Palluconi, F. D., & Arvidson, R. E. (1998). Mars global surveyor mission: overview and status. *Science*, *279*(5357), 1671-1672.

Androwski, R. J., Flatt, K. M., & Schroeder, N. E. (2017). Phenotypic plasticity and remodeling in the stress-induced Caenorhabditis elegans dauer. *Wiley Interdisciplinary Reviews: Developmental Biology*, *6*(5), e278.

Axford, W. I. (1962). The interaction between the solar wind and the earth's magnetosphere. *Journal of Geophysical Research*, *67*(10), 3791-3796.

Byerly, L., Cassada, R. C., & Russell, R. L. (1976). The life cycle of the nematode Caenorhabditis elegans: I. Wild-type growth and reproduction. *Developmental biology*, *51*(1), 23-33.

Boyle R, Hughes-Fulford M. Space biology (cells to amphibians). In Handbook of Bioastronautics 2021 Aug 17 (pp. 205-217). Cham: Springer International Publishing.

Clément, G., & Clément, G. (2011). The neuro-sensory system in space. *Fundamentals of space medicine*, 95-142.

Cloutier, P. A., Law, C. C., Crider, D. H., Walker, P. W., Chen, Y., Acuña, M. H., ... & Ness, N. F. (1999). Venus-like interaction of the solar wind with Mars. *Geophysical research letters*, *26*(17), 2685-2688.

Connerney, J. E. P., Acuna, M. H., Wasilewski, P. J., Ness, N. F., Reme, H., Mazelle, C., ... & Cloutier, P. A. (1999). Magnetic lineations in the ancient crust of Mars. *Science*, *284*(5415), 794-798.

Crider, D. H., Acuña, M. H., Connerney, J. E., Vignes, D., Ness, N. F., Krymskii, A. M., ... & Winterhalter, D. (2002). Observations of the latitude dependence of the location of the Martian magnetic pileup boundary. *Geophysical Research Letters*, *29*(8), 11-1.

Curtis, S. A., & Ness, N. F. (1988). Remanent magnetism at Mars. *Geophysical research letters*, *15*(8), 737-739.

Dedolph RR, Dipert MH. The physical basis of gravity stimulus nullification by clinostat rotation. Plant physiology. 1971 Jun 1;47(6):756-64.

Dittrich WA. Drop tower physics. The Physics Teacher. 2014 Oct 1;52(7):415-7.

Ding, H. M., Wang, X., Mo, W. C., Qin, L. L., Wong, S., Fu, J. P., ... & Hua, Q. (2019). Hypomagnetic fields cause anxiety in adult male mice. Bioelectromagnetics, 40(1), 27-32.

Drake BG, Hoffman SJ, Beaty DW. Human exploration of Mars, design reference architecture 5.0. In2010 IEEE aerospace conference 2010 Mar 6 (pp. 1-24). IEEE.

Ferranti F, Del Bianco M, Pacelli C. Advantages and limitations of current microgravity platforms for space biology research. Applied Sciences. 2020 Dec 23;11(1):68.

Fu JP, Mo WC, Liu Y, Bartlett PF, He RQ. Elimination of the geomagnetic field stimulates the proliferation of mouse neural progenitor and stem cells. Protein & cell. 2016 Sep;7(9):624-37.

Geider, R. J., Delucia, E. H., Falkowski, P. G., Finzi, A. C., Grime, J. P., Grace, J., ... & Woodward, F. I. (2001). Primary productivity of planet earth: biological determinants and physical constraints in terrestrial and aquatic habitats. *Global change biology*, *7*(8), 849-882.

Giles, A. C., & Rankin, C. H. (2009). Behavioral and genetic characterization of habituation using Caenorhabditis elegans. *Neurobiology of learning and memory*, *92*(2), 139-146.

Gould JL. The Case for Magnetic Sensitivity in Birds and Bees (Such As It Is): Surprising concentrations of magnetite in the tissues of some animals may explain their sensitivity to the earth's magnetic field. American Scientist. 1980 May 1;68(3):256-67.

Groombridge, B., & Jenkins, M. (2002). *World atlas of biodiversity: earth's living resources in the 21st century*. Univ of California Press.

Guillen, A., Calcagno, P., Courrioux, G., Joly, A., & Ledru, P. (2008). Geological modelling from field data and geological knowledge: Part II. Modelling validation using gravity and magnetic data inversion. *Physics of the Earth and Planetary Interiors*, *171*(1-4), 158-169.

Häder, D. P., Hemmersbach, R., & Lebert, M. (2005). *Gravity and the behavior of unicellular organisms* (No. 40). Cambridge University Press.

Hammond, R. T. (2010). The necessity of torsion in gravity. *International Journal of Modern Physics D*, *19*(14), 2413-2416.

Hargens, A. R., & Richardson, S. (2009). Cardiovascular adaptations, fluid shifts, and countermeasures related to space flight. *Respiratory physiology & neurobiology*, *169*, S30-S33.

Hasenstein KH, Van Loon JJ, Beysens D. Clinostats and other rotating systems—design, function, and limitations. Generation and applications of extra-terrestrial environments on earth. 2015 May 31;14:147-56.

Hester, P. Y. (1999). Hypogravity's effect on the life cycle of Japanese quail.

Jakosky, B. (1998). *The search for life on other planets*. Cambridge University Press. Klaus, D. M. (2004). Gravitational influence on biomolecular engineering processes. *Gravitational and Space Biology*, *17*(2), 51-66.

Javer A, Currie M, Lee CW, Hokanson J, Li K, Martineau CN, Yemini E, Grundy LJ, Li C, Ch'ng Q, Schafer WR. An open-source platform for analyzing and sharing worm-behavior data. Nature methods. 2018 Sep;15(9):645-6.

Johnsen S, Lohmann KJ. Magnetoreception in animals. Physics today. 2008 Mar 1;61(3):29-35.

Kim, K. W., & Jin, Y. (2015). Neuronal responses to stress and injury in C. elegans. *FEBS letters*, *589*(14), 1644-1652.

Kirschvink JL, Winklhofer M, Walker MM. Biophysics of magnetic orientation: strengthening the interface between theory and experimental design. Journal of the Royal Society Interface. 2010 Apr 6;7(suppl_2):S179-91.

Klaus DM. Clinostats and bioreactors. Gravit Space Biol Bull. 2001 Jun 1;14(2):55-64.

Langel, R. A., & Estes, R. H. (1982). A geomagnetic field spectrum. *Geophysical Research Letters*, *9*(4), 250-253.

LeBlanc, A. D., Spector, E. R., Evans, H. J., & Sibonga, J. D. (2007). Skeletal responses to space flight and the bed rest analog: a review. *Journal of Musculoskeletal and Neuronal Interactions*, *7*(1), 33.

Lundin, R., Lammer, H., & Ribas, I. (2007). Planetary magnetic fields and solar forcing: Implications for atmospheric evolution. *Space Science Reviews*, *129*, 245-278.

Margie, O., Palmer, C., & Chin-Sang, I. (2013). C. elegans chemotaxis assay. *JoVE (Journal of Visualized Experiments)*, (74), e50069.

Markaki, M., & Tavernarakis, N. (2020). Caenorhabditis elegans as a model system for human diseases. *Current opinion in biotechnology*, *63*, 118-125.

McKay, C. P., & Stoker, C. R. (1989). The early environment and its evolution on Mars: Implication for life. *Reviews of Geophysics*, *27*(2), 189-214

Merrill, R. T., & McFadden, P. L. (1999). Geomagnetic polarity transitions. *Reviews of Geophysics*, *37*(2), 201-226.

Morey-Holton, E. R. (2003). The impact of gravity on life. In *Evolution on planet Earth* (pp. 143-159). Academic Press.

Moore CL. Technology development for human exploration of Mars. Acta Astronautica. 2010 Nov 1;67(9-10):1170-5.

Muheim, R., Boström, J., Åkesson, S., & Liedvogel, M. (2014). Sensory mechanisms of animal orientation and navigation. In *Animal movement across scales* (pp. 179-194). Oxford University Press.

Neufeld MJ, Charles JB. Practicing for space underwater: inventing neutral buoyancy training, 1963–1968. Endeavour. 2015 Sep 1;39(3-4):147-59.

Novikov, V. V., Yablokova, E. V., & Fesenko, E. E. (2018). *The Effect of a "Zero" Magnetic Field on the Production of Reactive Oxygen Species in Neutrophils. Biophysics, 63(3), 365–368.* doi:10.1134/s000635091803017x

Penley NJ, Schafer CP, Bartoe JD. The international space station as a microgravity research platform. Acta Astronautica. 2002 Jun 1;50(11):691-6.

Pfaff, R. F. (2012). The near-Earth plasma environment. *Space science reviews*, *168*, 23-112.

Pletser V. European aircraft parabolic flights for microgravity research, applications and exploration: A review. Reach. 2016 Mar 1;1:11-9.

Queirós L, Marques C, Pereira JL, Gonçalves FJ, Aschner M, Pereira P. Overview of chemotaxis behavior assays in Caenorhabditis elegans. Current protocols. 2021 May;1(5):e120.BibTeXEndNoteRefManRefWorks

Souza, K. A., Black, S. D., & Wassersug, R. J. (1995). Amphibian development in the virtual absence of gravity. *Proceedings of the National Academy of Sciences*, *92*(6), 1975-1978.

Spangenberg DB, Jernigan T, McCombs R, Lowe BT, Sampson M, Slusser J. Development studies of Aurelia (jellyfish) ephyrae which developed during the SLS-1 mission. Advances in Space Research. 1994 Aug 1;14(8):239-47.

Walton, K., Heffernan, C., Sulica, D., & Benavides, L. (2007). Changes in gravity influence rat postnatal motor system development: from simulation to space flight. *Gravitational and Space Research*, *10*(2).

Wang, T., & Amar, M. B. (2024). Physical forces drive C. elegans embryonic deformation. *International Journal of Non-Linear Mechanics*, *166*, 104833.

Wharton, D. A. (2007). *Life at the limits: organisms in extreme environments*. Cambridge University Press.

Zhang, B., Lu, H., Xi, W., Zhou, X., Xu, S., Zhang, K., ... & Guo, A. (2004). Exposure to hypomagnetic field space for multiple generations causes amnesia in Drosophila melanogaster. *Neuroscience letters*, *371*(2-3), 190-195.

Zhang B, Tian L. Reactive oxygen species: Potential regulatory molecules in response to hypomagnetic field exposure. Bioelectromagnetics. 2020 Dec;41(8):573-80.

Tables

Table 1. Morphological measurements. SS: Student t-test, AF: ANOVA-F, 1-b: Power

Treatment	**Generation 2**	**Generation 4**	**Generation 6**	Intergenerational test:
Earth: head	118.8 ± 6.4 µm	111.0 ± 9.9 µm	95.5 ± 4.5 µm	p=0.008, 1-b=0.9, G2v4 p=0.2, G2v6 p=0.01, G4v6 p=0.04 (one-way ANOVA)
Mars: head	118.7 ± 2.7 µm	98.9 ± 6.9 µm	83.2 ± 0.8 µm	p=0.002, 1-b=0.8, G2v4 p=0.5, G2v6 p=0.02, G4v6 p=0.2 (ANOVA on ranks)
Intertreatment test	SS: p=99, 1-b=0.83	SS: p=0.08, 1-b=0.96	SS: p=0.006, 1-b=1.0	
Earth: midbody	145.9± 7.4 µm	134.5 ± 9.4 µm	115.2 ± 3.5 µm	p=0.002, 1-b=1.0, G2v4 p=0.08, G2v6 p=0.02, G4v6 p=0.01 (one-way ANOVA)
Mars: midbody	131.8 ± 27.4 µm	119.7 ± 5.7 µm	98.7 ± 2.0 µm	P<0.001, 1-b=1.0, G2v4 p<0.001, G2v6 p<0.001, G4v6 p<0.001 (one-way ANOVA)
Intertreatment test	SS: p=0.44, 1-b=0.86	SS: p=0.03, 1-b=1.0	SS: p<0.001, 1-b=1.0	
Earth: tail	109.6 ± 5.6 µm	103.8 ± 11.1 µm	85.7 ± 3.3 µm	P=0.04, 1-b=0.54, G2v4 p=0.43, G2v6 p=0.05, G4v6 p=0.12 (one-way ANOVA)
Mars: tail	107.5 ± 32.6 µm	91.3 ± 4.2 µm	78.1 ± 1.7 µm	p=0.21, no test (ANOVA on ranks)
Intertreatment test	SS: p=0.92, 1-b=0.80	SS: p=0.06, 1-b=0.99	SS: p=0.07, 1-b=0.99	
Earth: worm length	1,009 ± 1 µm	1,297 ± 86 µm	973 ± 109 µm	Slope 33.5, intercept 902
Mars: worm length	952 ± 265 µm	1,202 ± 59 µm	1,003 ± 56 µm	Slope 19.8, intercept 987
Intertreatment test	SS: P=0.75, 1-b=0.82	SS: P=0.11, 1-b=0.95	SS: P=0.65, 1-b=0.84	AF: F=0.52, p=0.482, 1-b=0.59

Table 2. Worm curvature Measurement. SS: Student t-test, AF: ANOVA-F, 1-b: Power

Treatment	Generation 2	Generation 4	Generation 6	Intergenerational Test
Neck Curvature				
Earth	0.0040 ± 0.0005	0.0033 ± 0.0011	0.0039 ± 0.0005	p=0.21, 1-β=0.65, G2v4 p=0.08, G2v6 p=0.35, G4v6 p=0.12 (one-way ANOVA)
Mars	0.0052 ± 0.0011	0.0044 ± 0.0004	0.0053 ± 0.0002	p=0.002, 1-β=0.85, G2v4 p=0.15, G2v6 p=0.01, G4v6 p=0.09 (one-way ANOVA)
Intertreatment Test	SS: p=0.12, 1-β=0.60	SS: p=0.10, 1-β=0.82	SS: p=0.009, 1-β=0.95	AF: F=7.3, p=0.004, 1-β=0.90
Midbody Curvature				
Earth	0.0035 ± 0.0003	0.0024 ± 0.0007	0.0038 ± 0.0005	p=0.33, 1-β=0.62, G2v4 p=0.10, G2v6 p=0.50, G4v6 p=0.12 (one-way ANOVA)
Mars	0.0038 ± 0.0008	0.0038 ± 0.0003	0.0029 ± 0.0005	p=0.01, 1-β=0.80, G2v4 p=0.005, G2v6 p=0.07, G4v6 p=0.02 (one-way ANOVA)
Intertreatment Test	SS: p=0.55, 1-β=0.70	SS: p=0.005, 1-β=0.95	SS: p=0.11, 1-β=0.89	AF: F=6.0, p=0.01, 1-β=0.85
Tail Curvature				
Earth	0.0036 ± 0.0003	0.0026 ± 0.0007	0.0033 ± 0.0008	p=0.15, 1-β=0.72, G2v4 p=0.10, G2v6 p=0.30, G4v6 p=0.08 (one-way ANOVA)
Mars	0.0058 ± 0.0022	0.0035 ± 0.0006	0.0035 ± 0.0009	p=0.18, 1-β=0.60, G2v4 p=0.20, G2v6 p=0.33, G4v6 p=0.25 (one-way ANOVA)
Intertreatment Test	SS: p=0.09, 1-β=0.68	SS: p=0.18, 1-β=0.80	SS: p=0.30, 1-β=0.75	AF: F=2.8, p=0.22, 1-β=0.50

Table 3. Postural Alteration Analysis (Eigenworm Projections). SS: Student t-test, AF: ANOVA-F, 1-β: Power

Treatment	Generation 2	Generation 4	Generation 6	Intergenerational Test
Earth: Eigenworm 1	1.589 ± 0.163	1.651 ± 0.429	1.638 ± 0.164	p=0.10, 1-β=0.7, G2v4 p=0.12, G2v6 p=0.13, G4v6 p=0.20 (one-way ANOVA)
Mars: Eigenworm 1	1.959 ± 0.273	1.831 ± 0.321	2.012 ± 0.187	p=0.05, 1-β=0.8, G2v4 p=0.18, G2v6 p=0.03, G4v6 p=0.05 (one-way ANOVA)
Intertreatment Test	SS: p=0.08, 1-β=0.83	SS: p=0.10, 1-β=0.85	SS: p=0.04, 1-β=0.90	AF: F=3.4, p=0.047, 1-β=0.82
Earth: Eigenworm 4	0.788 ± 0.123	1.097 ± 0.254	0.821 ± 0.125	p=0.12, 1-β=0.65, G2v4 p=0.10, G2v6 p=0.08, G4v6 p=0.15 (one-way ANOVA)
Mars: Eigenworm 4	1.373 ± 0.386	1.035 ± 0.166	1.087 ± 0.112	p=0.04, 1-β=0.82, G2v4 p=0.07, G2v6 p=0.05, G4v6 p=0.03 (one-way ANOVA)
Intertreatment Test	SS: p=0.07, 1-β=0.78	SS: p=0.06, 1-β=0.88	SS: p=0.02, 1-β=0.95	AF: F=4.1, p=0.03, 1-β=0.88

Table 4. Summary and comparative statistic comparison of swimming frequencies of Day-1 *C. elegans* raised under Earth or Mars gravitational and magnetic conditions.

Treatment	**Generation 2**	**Generation 4**	**Generation 6**	ANOVA
Earth	2.0 ± 0.2 Hz	1.9 ± 0.2 Hz	2.0± 0.4 Hz	F(2,87)=5.97, p=0.0037
Mars	1.7 ± 0.3 Hz	1.8 ± 0.2 Hz	1.6 ± 0.3 Hz	F(2,87)=9.67, p=0.00016
Student t-test	P<0.001	P<0.001	P<0.001	

Table 5. Chemotaxis Index of *C. elegans* under Earth and Mars Conditions. SS: Student t-test, AF: ANOVA-F, 1-β: Power

Treatment	**Generation 2**	**Generation 4**	**Generation 6**	**Intergenerational Test**
Earth	0.888 ± 0.0223	0.801 ± 0.133	0.889 ± 0.0833	p=0.15, 1-β=0.6, G2v4 p=0.11, G2v6 p=0.90, G4v6 p=0.12 (one-way ANOVA)
Mars	0.874 ± 0.115	0.577 ± 0.0899	0.410 ± 0.0648	p<0.001, 1-β=0.9, G2v4 p=0.03, G2v6 p=0.001, G4v6 p=0.02 (one-way ANOVA)
Intertreatment Test	SS: p=0.816, 1-β=0.55	SS: p=0.005, 1-β=0.92	SS: p<0.001, 1-β=1.0	AF: F=6.2, p=0.004, 1-β=0.95

Figures

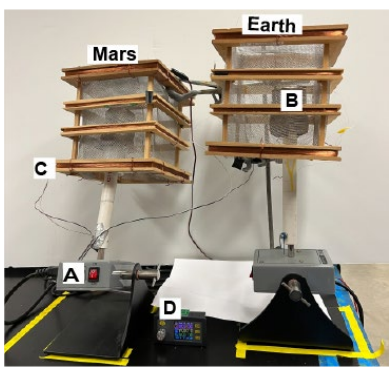

Figure 1. Simulation of Mars and Earth's magnetic and gravitational fields. Clinostats **(A)** angled to mimic the Mars' (3.71 m/s^2) or Earth's (9.81m/s^2) gravity. Worms were raised in *E. coli* seeded agar plates placed in cylinder **(B)** positioned at the center of the Helmholtz coils **(C)** powered by a power supply **(D)**.

Figure 2. Automated behavioral analysis of *C. elegans* using Tierpsy Tracker. The Basler acA4024-29um camera captures high-resolution video of the nematodes, allowing Tierpsy Tracker to accurately track their movements and quantify various behaviors and locomotion patterns.

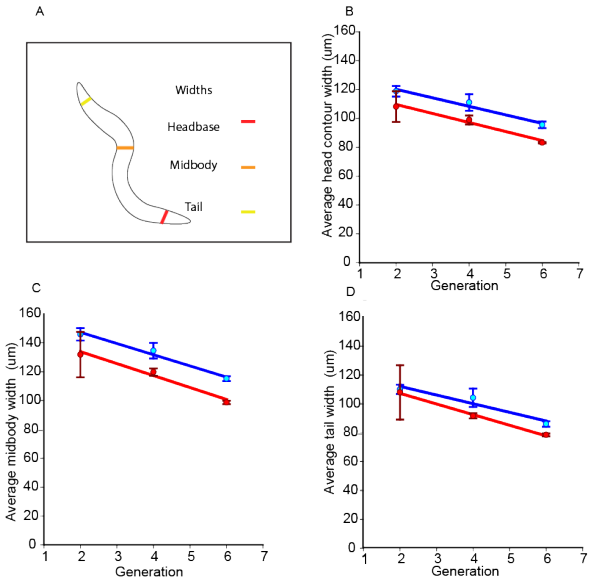

Figure 3. Diagram illustrating the regions of the *C. elegans* body where measurements of head contour width were taken. Red indicates the head region, orange denotes the mid-body region, and yellow represents the tail region(**A**). Morphological differences in worms grown under Earth and Mars conditions over generations 2, 4, and 6. A significant decrease in midbody width(**B**) head contour width (**C**) and average tail width (**D**) was observed in worms grown under Mars conditions compared to their Earth counterparts in the later generations ($P < 0.05$, Student's t-test). Error bars represent the standard deviation.

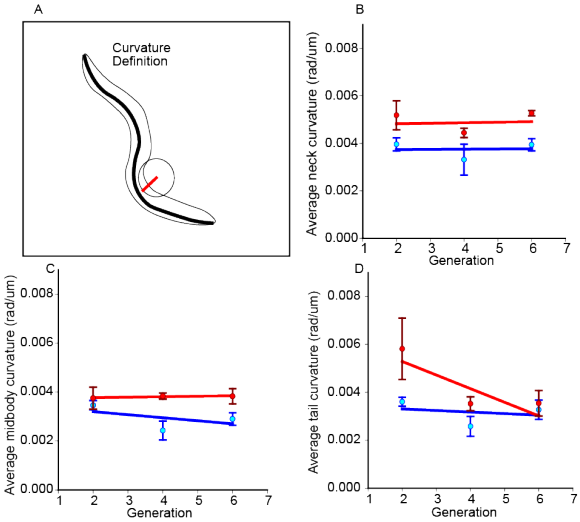

Figure 4. Curvature of *C. elegans* across generations 2, 4, and 6 under Earth and Mars conditions. (A) Representation of the measurement technique used to determine the contour width of *C. elegans* head region. The red line indicates the measured head contour width, while the blue lines outline the reference points along the body. The neck (B), midbody(C), and tail curvatures(D) are plotted for worms grown under Earth conditions (blue) and Mars conditions (red) for three generations (2, 4, 6). Worms grown under Mars conditions showed significantly higher curvatures in the neck, midbody, and tail at all generations measured ($P < 0.05$, Student's t-test). Error bars represent the standard deviation.

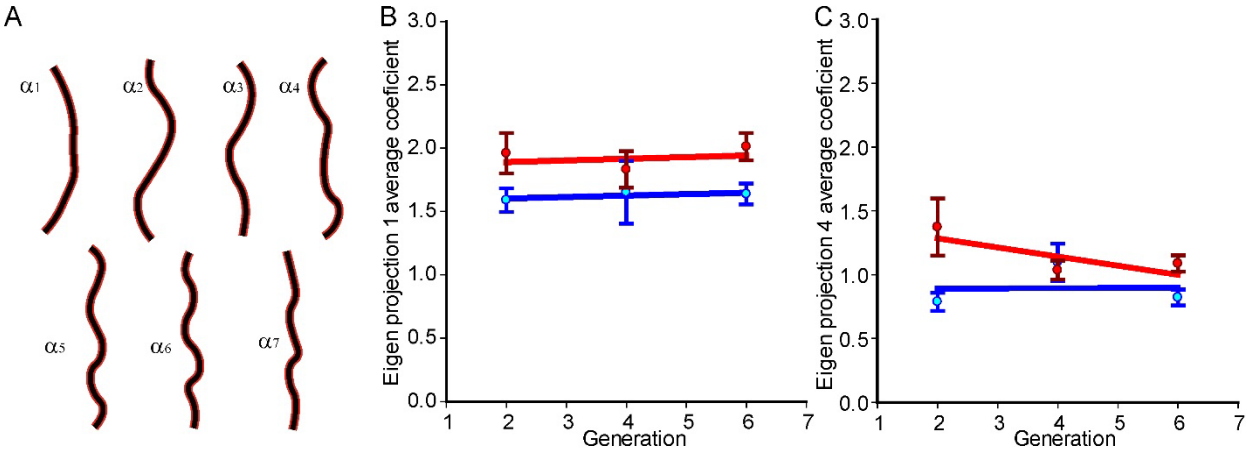

Figure 5. (A) The first seven Eigenworms (α1 to α7), representing the principal modes of shape variation in *C. elegans*. Each Eigenworm captures a distinct mode of shape variation, with α1 showing the primary bending motion and subsequent Eigenworms depicting finer, less dominant aspects of worm posture. **(B).** Average coefficients of the first Eigenworm projection (Eigen projection 1) across generations 2, 4, and 6 for worms grown under Earth (blue) and Mars (red) conditions. Both conditions show a slight increasing trend, suggesting consistent primary mode of shape variation with slight increases over successive generations. **(C).** Average coefficients of the fourth Eigenworm projection (Eigen projection 4) across generations 2, 4, and 6 for worms grown under Earth (blue) and Mars (red) conditions. Earth-grown worms

exhibit relatively stable coefficients, while Mars-grown worms show a decreasing trend, indicating a negative morphological response to the Mars environment.

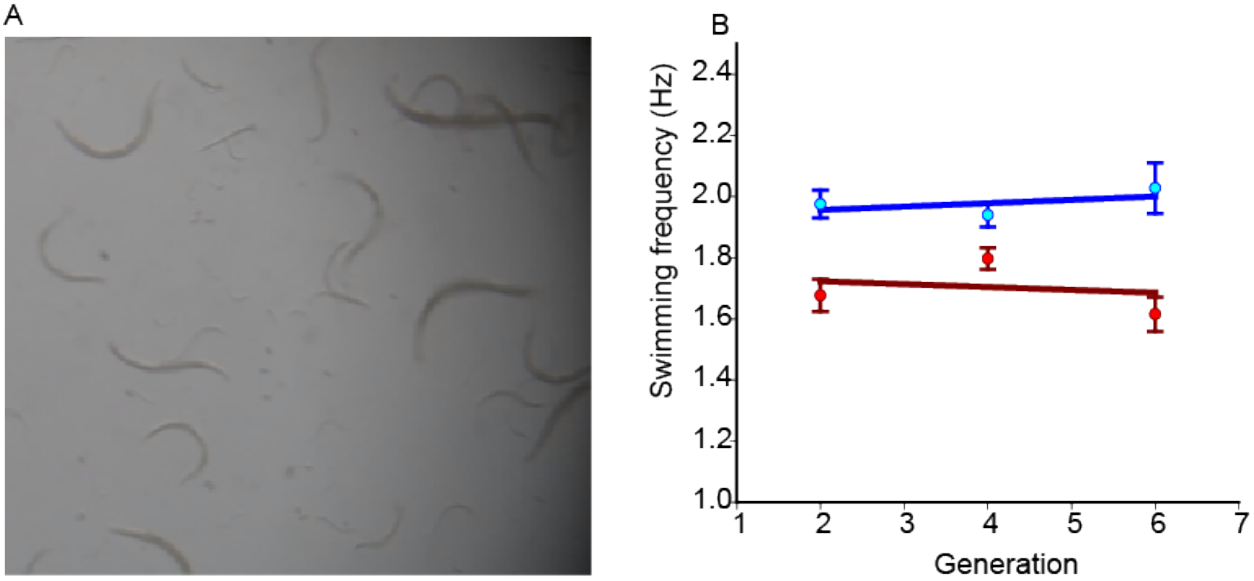

Figure 6. Swimming behavior of *C. elegans* under Mars conditions. (**A**) The image captures the defective swimming frequency of worms grown under simulated Mars conditions. Notable differences in swimming behavior are observed compared to their Earth-grown counterparts, indicative of the environmental impact on their locomotor activity. The impaired swimming patterns seen here reflect the significant reduction in activity measured across generations. (B) Swimming frequencies of *C. elegans* across generations 2, 4, and 6 under Earth and Mars conditions**.** The swimming frequency is plotted for worms grown under Earth conditions (blue) and Mars conditions (red) for three generations (2, 4, 6). Worms grown under Mars conditions showed a significant reduction in swimming frequency compared to their Earth counterparts at all generations ($P < 0.001$, Student's t-test). Error bars represent the standard deviation.

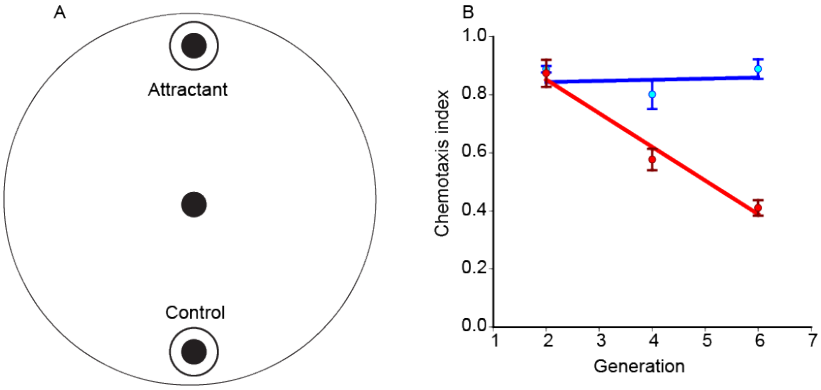

Figure 7. (A) Experimental setup of a chemotaxis assay**.** The setup for the chemotaxis assay includes a Petri dish with a central spot where *C. elegans* are placed. A gradient of attractant is applied from a designated area on the dish's periphery, while a control area with no attractant is established on the opposite side. Worm movement is tracked and recorded to determine the chemotaxis index, which reflects the worms' ability to navigate toward the attractant. **(B)** Chemotaxis index of *C. elegans* across generations 2, 4, and 6 under Earth and Mars conditions**.** The chemotaxis index is plotted for worms grown under Earth conditions (blue) and Mars conditions (red) for three generations (2, 4, 6). Worms grown under Earth conditions maintained a relatively constant chemotaxis index across generations, while worms grown under Mars conditions showed a declining trend. Generation 2 worms under Mars conditions had a chemotaxis index like those under Earth conditions, but significant reductions were observed in generations 4 and 6 for the Mars-grown worms ($P = 0.005$ and $P < 0.001$, respectively, Student's t-test). Error bars represent the standard deviation.