

Transgenerational epigenetic memory of salt preference in mixed strains of *Caenorhabditis remanei*

Author: Julie Bouvin

Bachelor of Science in Biology, Vrije Universiteit Brussel
Evolutionary genomics of Sex research group

Promotor: Dr. Paris Veltsos

Abstract

Organisms can pass on memories of environmental stress, such as changes in salinity, to their offspring via transgenerational epigenetic inheritance, without altering their DNA. Although this has already been studied in the hermaphroditic nematode *Caenorhabditis elegans*, this multigenerational memory remains unknown in obligate sexual nematodes. This thesis investigates how long such ancestral salt memory persists across four successive generations after synchronisation within a genetically diverse population of *Caenorhabditis remanei*. This was done by cultivating the parental generation under High, Normal (control) or Low salt conditions. Their offspring were then synchronised and maintained strictly on a standard control medium. We analysed their behaviour (chemotaxis) and mobility using a linear salt gradient test. The results show that ancestral exposure to Low salt causes a significant decrease in the chemotactic index compared to the control group ($p = 0.047$). This negative effect remained stable across the successive generations. High salt, by contrast, had no effect on this specific behaviour. Furthermore, the research showed that the overall mobility of the worms did not change significantly across the generations. These results demonstrate that the parental salt condition can induce a stable, multigenerational behavioural change.

Key words: Transgenerational epigenetic inheritance, *Caenorhabditis remanei*, Chemotaxis, Salinity stress, Obligate sexual nematodes.

Introduction

Since the beginning of evolutionary biology, a strict division has been made between genetic adaptation at population level and individual acclimatisation (Baugh and Day, 2020). Genetic adaptation is based on the Darwinian principle of natural selection, where variations in genomic sequences result in some individuals developing beneficial characteristics in a particular environment. Consequently, these individuals have the highest chance of survival and reproduction, and the genetic composition of a population changes over many generations, even though an individual's DNA remains unchanged during their lifetime (Darwin, 1859).

Acclimatisation, on the other hand, is a process at the individual level where an organism undergoes reversible physiological or morphological adaptations within a single generation in response to environmental variations. This enables the individual to optimise its fitness within the limits of its phenotypic plasticity, without any genetic changes occurring (Baugh and Day, 2020; Scheiner, 1993). Lamarck proposed that these adaptations to the environment were passed on to offspring

(Lamarck, 1809). This idea was long rejected by the emergence of the neo-Darwinian view, which considered the inheritance of acquired characteristics impossible within the framework of genetics (Huxley, 1942). This is partly due to August Weismann, who published his theory on 'the continuity of the germ line' in the late 19th century. He stated that only germ cells are responsible for the transmission of genetic information. A key component to his theory is the Weismann barrier, which suggests that the transmission of information from somatic cells to the germ line is inhibited, meaning that traits acquired during an individual's lifetime cannot be inherited (Weismann, 1893; Nilsson et al., 2020).

More recent breakthroughs in molecular biology have challenged this division between acclimatisation and adaptation by introducing the mechanism of epigenetic inheritance (Jablonka and Raz, 2009; Baugh and Day, 2020). Conrad Waddington introduced the term epigenetics in the 1940s as, for him, a necessary link between genetics and embryology. According to him, genes are not simply the fixed blueprint of a living organism, but their function is guided by complex interactions within the cell and regulated by their environment.

This laid the foundation for our modern understanding of how the environment influences our DNA (Van Speybroeck, 2002).

Currently, epigenetics is often defined as the study of heritable changes in gene function that do not alter the DNA sequence itself. It revolves around the interpretation of genetic information, how signals from the internal and external environment, such as stress or nutrition, determine which genes are expressed. In this sense, the core idea remains consistent with Waddington's vision, which focused on the complex networks between genes and their environment that determine the development of an organism (Jablonka and Lamb, 2002; Van Speybroeck, 2002).

To study these complex networks in practice, nematodes of the genus *Caenorhabditis* are ideal model organisms. In 1974, Sydney Brenner published a paper on the genetic manipulation of *Caenorhabditis elegans*, which laid the foundations for this new experimental model organism (Brenner, 1974; Chadarevian, 1998). This species has a short life cycle of three to four days, which makes it possible to efficiently conduct experiments across multiple generations (Riddle et al., 1998). It is a multicellular organism of which 60-80% of the genes have human homologues (The *C. elegans* Sequencing Consortium, 1998; Kaletta et al., 2006). Further, *C. elegans* reproduces through self-fertilisation of hermaphrodites or through cross-fertilisation between a hermaphrodite and a male (de Chadarevian, 1998).

Besides these genetic traits at the population level, *C. elegans* also offers unique insights into information processing at the individual level. This is because the species has a complex associative memory, meaning the worm learns to link specific salt concentrations to the presence of food (Kunitomo et al., 2013). For example, worms have different receptor sites for Cl⁻ and Na⁺ (Ward, 1973). These salt receptors are expressed by Amphid Sensory Neurons of subtype E (ASE). These neurons appear to be essential for detecting various substances, including salts, biotin and lysine (Ward et al., 1975). The left side, ASEL, is primarily sensitive to sodium, whilst the right side, ASER, is more sensitive to chloride and potassium (Pierce-Shimomura et al., 2001). Laser surgery confirms that these cells are essential and that, without ASE, the worm can barely navigate, while the elimination of

surrounding cells had no effect (Bargmann and Horvitz, 1991). However, the system is flexible, and upon loss of ASE, 'back-up' neurons such as ASG, ASI and ASK take over part of the sensory function. The sensory ability is therefore distributed across several specific cell types (Bargmann and Horvitz, 1991). In addition to basic sensing, these neural pathways facilitate associative memory. When individuals are cultivated at a specific salt concentration, they develop a chemotactic preference for that condition, which is a crucial strategy for locating food sources (Kunitomo et al., 2013).

While individual associative learning allows for immediate behavioral adaptation, it doesn't represent all of the organism's adaptive capacity. Recent studies have shown that this information can also be converted into a transgenerational signal via RNA interference (RNAi) by small RNA molecules (siRNAs) and histone modifications (Ashe et al., 2012; Rechavi et al., 2011). It has been shown that, in contrast to our understanding of the Weismann barrier, this is not always impermeable. Environmental information is, in fact, passed from the parent's somatic cells to the germ cells in *C. elegans* (Burton, 2017; Greer et al., 2011). As a result, offspring show an adapted response, even when they have not been exposed to the initial stressor. We see this, for example, in the study by Wan et al. (2022) when the parent generation (P₀) was fed a high-fat diet (egg yolk), this led to lipid accumulation. This effect was passed on to following generations via epigenetic pathways, specifically histone modifications. Both the first generation (F₁) and the second generation (F₂) stored significantly more fat, despite being fed a healthy diet themselves. Furthermore, research by Adam Klosin et al. (2017) shows that exposure to heat (25°C) has a long-lasting epigenetic effect. Due to specific histone modifications, offspring continue to react as if they were still in a warm environment for up to 14 generations, even when they are in actuality living in a cooler environment again.

Although these mechanisms are fundamental to our understanding of epigenetic inheritance in the hermaphroditic *C. elegans*, it is unclear whether these processes are maintained in the same way in species that reproduce obligately sexually. Additionally, *C. elegans* males are not good representatives of male biology in *Caenorhabditis*: they are only present in low numbers, and lack

specific mating techniques to facilitate sperm transfer, such as leaving behind a mating plug or temporarily paralysing the female (Garcia et al., 2007; Cutter, 2015). This study therefore focuses on the closely related species *Caenorhabditis remanei*, which reproduces obligately sexually (Cutter et al., 2006). A mixed population of *C. remanei* was used to facilitate genetic adaptation to different salt conditions over time.

Based on this, we hypothesise that parental exposure to high or low salt concentrations will induce a transgenerational preference in offspring that corresponds to the ancestral environment. Furthermore, we expect the epigenetic memory to persist across multiple generations, even after the initial environmental stimulus (particular salt concentration) has been removed.

Materials and methods

Worm maintenance

A genetically diverse mix of 8 *C. remanei* strains, provided by the lab of Marie-Ann Felix, was cultured on standard Nematode Growth Medium (NGM) at 18°C, 50% humidity and a 12-hour light/dark cycle. The worms were transferred to new plates every 3 days by suspension in 2 ml of M9 solution in the old plate, of which 300 µl was pipetted over to the new plate.

The worms were allowed to evolve over many generations on media with 3 NaCl concentrations: Low (0.9 g/400 ml), Normal (1.2 g/400 ml) and High (2.4 g/400 ml). The medium was prepared with 8 g agar and 1 g peptone in 400 ml distilled water, sterilised by autoclaving (1 h) and cooled to 55°C. Before plating, 400 µl of cholesterol (5 mg/ml), 400 µl of MgSO₄ (1 M), 400 µl of CaCl₂ (1 M) and 10 ml of KPO₄ buffer (pH 6.0) were added.

On the Petri dishes, 200 µl of *E. coli* OP50 culture was added and spread using a glass spreader under sterile conditions. After a minimum of 24 hours' incubation at room temperature to allow bacteria growth, the plates were stored at 4°C until use.

Synchronisation

To minimise behavioural variability, worm populations were synchronised using a bleach solution. This kills the adults, leaving only the eggs, which hatch at the same time. The procedure

ensures that all worms are at the same developmental stage during the experiment, and minimises behavioural variation. Worms were washed off the agar plate with 2 ml of M9 solution, transferred to a 15 ml Falcon tube which was filled with distilled water. After centrifugation (1000g, 3 min), the supernatant was removed. For lysis, 2–3 mL of bleach mix was added. For generations 7–15, the bleach mix contained 1.25 ml of 10M NaOH per 5 ml of NaClO (6–14% active chlorine) (Merck). From generation 16 onwards, the protocol transitioned to a master mix containing 5 ml NaClO, 1 ml 10M NaOH, and 9 ml H₂O per 25 ml, which was prepared fresh under sterile conditions near a flame. After 3–6 minutes of incubation, when approximately 70% of the adult worms had dissolved, the reaction was stopped by adding M9 to a total volume of 15 ml. After a second centrifugation (1000 g, 3 min) and removal of the supernatant, the pellet containing eggs was resuspended in 1 ml M9 and transferred to standard NGM plates.

Experimental setup

This synchronisation protocol was applied to each generation that was maintained long-term at specific salt concentrations (Low, Normal, High) (appendix). After synchronisation, the eggs were placed on standard NGM plates and cultured for 4 days. To investigate the duration of epigenetic memory, a portion of the synchronised worms were kept on standard NGM medium for several consecutive generations. Synchronisation was performed again at each intermediate generation, allowing the duration of the inherited memory to be analysed after the initial environmental stimulus had been removed.

Salt gradient plates

The salt gradients were prepared in square Petri dishes (12 × 12 cm). To create the gradient, the technique described by Weinberg et al. (1959) was used. The Petri dish was raised on one side using three coins to a height of approximately 6.5 mm, causing the dish to stand at an angle (see Figure 1). Next, 56 ml of low-salt NGM were added. Once the medium had completely solidified, the coins were removed and a second layer of 56 ml of high-salt NGM was added to fill the Petri dish evenly. The plates were then incubated at room temperature for at least 24 hours. During this period, diffusion between the two layers resulted in the formation of a

stable, linear salt gradient across the surface of the plate.

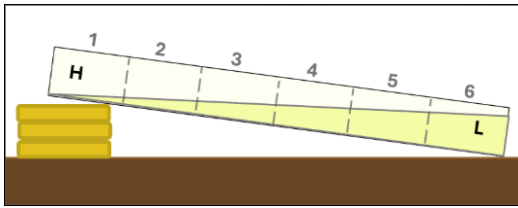


Figure 1: Salt gradient setup with labelled zones from High to Low (1-6)

The salt preference of the synchronised populations was measured by transferring synchronised worms, after 4 days, by washing the plates with 2 ml of M9. Then, 1 ml of this suspension was transferred to the central line of a gradient plate between zones 3 and 4 (see Figure 1). Excess fluid was absorbed with a paper tissue, allowing the worms to quickly start moving freely. After 60 min, the number of individuals per plate zone was counted using a stereomicroscope, with the final numbers per zone used to calculate the chemotaxis index.

Chemotaxis index

The chemotaxis index is a single number per gradient plate, which ranges from 1 to -1, the former indicating all worms prefer High salt, and the latter indicating all worms prefer Low salt. It was calculated by adding the number of worms in the high-salt zones (1 and 2), subtracting the number of worms in the low-salt zones (5 and 6), and dividing this by the total number of worms in these four zones (Tomioaka et al., 2006). The middle zones, 3 and 4, were not included because we assumed that they were either unable to move or had no preference for a particular salt concentration.

Mobility index

In addition, we investigated whether there is a difference in the proportion of mobile worms across different generations. This involves calculating a mobility index for each salt concentration. This index is determined by adding up the number of worms in the outer zones (zones 1, 2, 5 and 6) and dividing this by the total number of worms on the plate (zones 1 to 6) (Bargmann et al., 1993).

Statistical analysis

The statistical analysis of the data was performed using R (version 4.5.1) (R Core Team 2025) in

RStudio (version 2025.09.0). The following statistical procedures were applied independently to both the Chemotaxis Index and the Mobility Index. The dplyr package (version 1.1.4) was used to group the data. The data were first presented visually using box plots to obtain an overall overview of the distributions. In addition, dot plots with standard errors were created to better illustrate whether differences between groups are potentially statistically significant, and because for some groups the dataset is so small that a box plot could be misleading. This was done using ggplot2 (version 4.0.0) and combined via patchwork (version 1.3.2).

To determine which tests could be applied, the assumptions of linearity and variance were checked. The normality of the data (both overall and per generation for the ‘long-term salt’ groups) was tested using a Shapiro-Wilk test. The homogeneity of variances was checked using Levene’s test using the car package (version 3.2-3). Based on the results of these tests, a one-way ANOVA was performed on the data up to and including generation 3 after synchronisation ($\alpha = 0.05$). This was done for the chemotax index and for the mobility index. There was also a linear regression model applied to visualise the general trend across the generations.

Results

Prior to the main analysis, it was considered whether the data points from the 16th long-term generation onwards should be removed, as a different bleach solution was used from that point on. After removing this data, there were insufficient data points remaining to perform an analysis until generation 3 post-synchronisation, only the first generation post-synchronisation could still be statistically tested using a one-way ANOVA. The overall results remained relatively unchanged so it was decided to retain the full dataset (including the long-term generations beyond 16). Eliminating the data did not result in a significantly stronger effect, while keeping all data points increased the statistical power to be able to test the trends up to and including generation 3 after synchronisation.

Chemotax index per generation

A one-way ANOVA was performed for each generation after synchronisation, from 1 to 3, on salt preference. In the first generation after

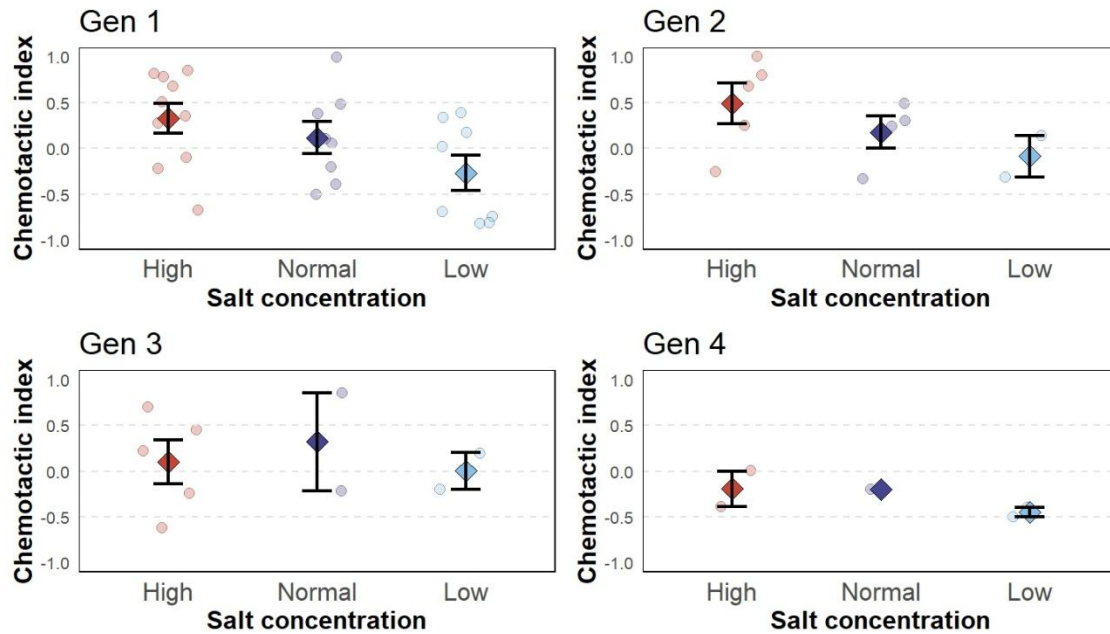


Figure 2: The chemotactic index across four consecutive generations after synchronisation, based on parental salt concentration, with the data points. The coloured squares represent the means, each with their standard error.

synchronisation, a marginally significant effect was found ($F = 2.98$, $p = 0.071$). For generation 2 ($p = 0.291$) and generation 3 ($p = 0.833$), the p-values were higher, indicating that the initial trend had disappeared.

In Figure 2, we clearly see a trend difference between the High and Low groups in the first generation. In addition, we see that all groups show a decline towards generation 4, which was not statistically analysed due to low sample size. This decline should be interpreted with caution as there are few data points. We also observe that the High salt group in particular declines the most across the generations.

Change in chemotax index over time

To analyse the effect of the long-term salt concentration and the number of generations following synchronisation on the chemotactic index, a multiple linear regression model was used. The control group (Normal) was used as the reference group, allowing the effects of the High or Low salt conditions to be compared directly with the baseline situation. Firstly, an interaction model was used to test whether the effect of the generations depended on the salt condition. This interaction proved to be non-significant ($F = 2.279$, $R^2 = 0.202$, $p = 0.063$). There was also no significant interaction found for the High group ($p = 0.427$) or the Low group ($p = 0.079$) compared to the control. This means that

there is no significant difference between the salt conditions in the trend across generations so we continued with a simpler multiple linear regression model without interaction.

This multiple linear regression model was, however, significant ($F = 3.514$, $R^2 = 0.183$, $p = 0.022$). This model shows that the average starting point for the control group (Normal) was 0.25, which was not statistically significant ($p = 0.140$). When comparing the High group with this control, we observed a slight increase (0.143), but this effect was also not significant ($p = 0.376$). Furthermore, when comparing the Low group with the control, a decrease was observed (-0.360) which was statistically significant ($p = 0.0473$). Finally, it appeared that the number of generations following synchronisation had no effect on the model. A slight decrease in the index was observed (-0.065), but this was again not significant ($p = 0.322$).

Figure 3 illustrates the difference in startingpoints of both the High and Normal groups compared to the Low salt group. This visually supports the statistically significant effect found in the multiple linear regression model. Additionally, the High salt group shows a downward trend across the generations, but this decline was not statistically significant. Due to the small sample size in generation 4, the results must be interpreted with caution.

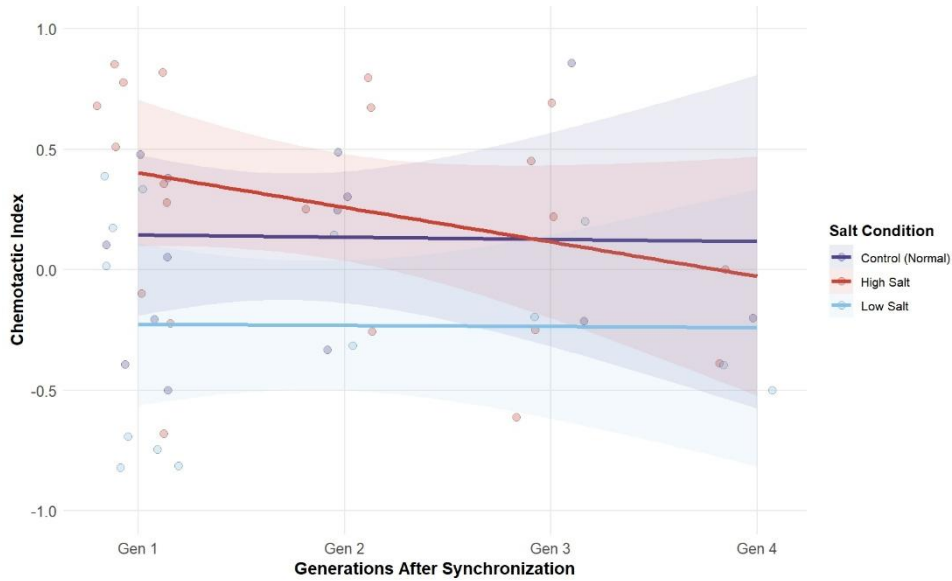


Figure 3: The effect of consecutive synchronisation on salt preference per parental salt concentration, shown by a linear regression line with the data points. The coloured lines represent the corresponding 95% confidence intervals.

Change in mobility index over time

To determine whether the number of generations following synchronisation had an effect on the mobility of the worms, the dataset was split based on the salt condition. A separate one-way ANOVA was then performed for each salt condition, comparing the average proportion of mobile worms between generations 1, 2 and 3 after synchronisation. Once again, generation 4 was not included after synchronisation due to a lack of data points.

In the high-salt group, the mobility index remained very stable across generations (average 37.1%). No

significant difference was found in the proportion of moving worms between generation 1 (37.8%), generation 2 (34.3%) and generation 3 (38.5%) ($F = 0.145$, $p = 0.866$). This trend was also observed within the Low salt group (average 35.1%). There was a slight increase in generation 2 (43.5%) compared to generation 1 (32.5%) and generation 3 (37.4%), but this effect was not statistically significant ($F = 0.307$, $p = 0.743$). Finally, the Normal salt group also showed no significant difference across the generations (average 26.9%) ($F = 0.590$, $p = 0.571$).

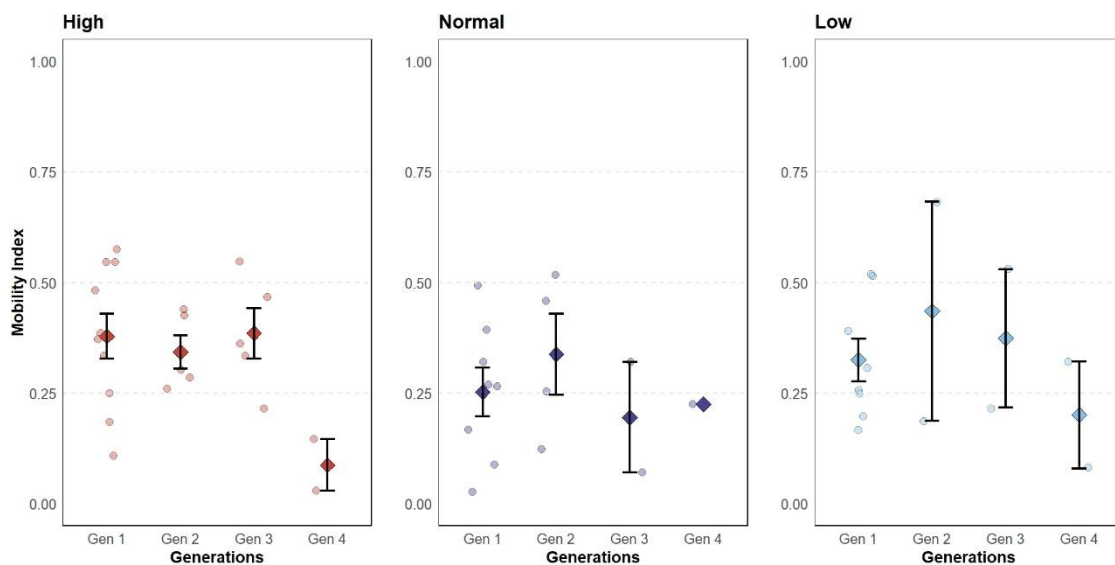


Figure 4: Proportion of moving worms plotted by generation for the three different salt conditions. The diamonds represent the group means, each with their standard error.

Figure 4 shows the mobility index for 4 consecutive generations after synchronisation per salt treatment. For the first three generations, the average mobility remains stable within each group. In generation 4, there is a decline in all salt conditions. This downward trend should be interpreted with caution. The decrease in data points for later generations results in significant variation, which may cause statistical unreliability.

Discussion

The goal of this thesis was to determine the extent to which an epigenetic memory for salt preference is passed on to consecutive generations within a mixed population of *C. remanei*. The initial One-Way ANOVA showed there was a marginally significant effect ($p = 0.071$) towards the expected direction for salt chemotaxis in the first generation only. Worms whose parents were raised on low salt tended to move towards low salt even though they were raised on a normal concentration after synchronisation. The chemotaxis index from the worms of the High salt treatment were also higher than the normal worms, but to a lower extent. It is possible that a larger sample size would result in a statistically significant effect. However the differences between salt treatments were not observed after 2 generations on normal salt.

While the separate One-Way ANOVA models suggest that the behavioral differences between treatments disappear by the second generation, shows the multiple linear regression model with an interaction term that all groups are declining at the same rate. This indicates that the low p -values in the separate ANOVAs of generation 2 and 3 are likely due to an insufficient sample size. This reduces the statistical power and is probably not due to the epigenetic memory being erased. Transgenerational epigenetic memory, particularly in the Low Salt group, therefore remains relatively stable over three generations.

Furthermore, analysis of the overall proportion of moving worms indicates that the worms' general response does not decline significantly as the number of generations after synchronisation increases. This indicates that time or the generation number does not have a negative impact on the worms' general preference behavior.

However, during the course of the experiment, it became clear that the synchronisation method used had a highly invasive impact on the population. The number of surviving worms decreased drastically with each successive generation, which in some cases even led to the complete extinction of a generation after synchronization, resulting in the loss of many data points. Furthermore, errors may also have crept in due to contamination of the M9 solution and the use of expired bleach. A new bleach was used from long-term generation 16 onwards which had no significant effect on the worms' salt preference, so it was good to use.

Due to a lack of data, this study cannot make definitive claims about transgenerational epigenetic memory. In order to draw statistically robust conclusions in the future, the experiment needs to be repeated more often. A next step could be to investigate after how many generations the memory actually begins to fade and look into differences between sexes in learned salt chemotaxis and its potential sex-specific inheritance of epigenetic memory. This could be tested by exposing only parental males to high- or low-salt and crossing them with females that were cultivated on a normal salt condition.

Acknowledgement

First of all, I would like to thank my supervisor, Paris Veltsos. He was extremely helpful and available at any time of the day. He not only taught us laboratory skills, but also the importance of keeping a detailed lab notebook. I would also like to thank Maeliss Hantson for the excellent collaboration on this project. We helped each other with questions and prepared each other's salt plates for maintenance and gradient plates whenever necessary. I would also like to thank Gabriele Campanile for helping me synchronise the worms and prepare gradient plates. Furthermore, I would like to thank Pouamoun Zouberou Njimbe, who took the time to come to the VUB to teach us essential laboratory skills. Finally, I would like to thank Ann Pauwels for preparing and autoclaving the agar.

Bibliography

Adam Klosin *et al.*, (2017). Transgenerational transmission of environmental information in *C. elegans*. *Science* **356**, 320-323. DOI:10.1126/science.aah6412

- Ashe A, Sapetschnig A, Weick E ... (2012) piRNAs Can Trigger a Multigenerational Epigenetic Memory in the Germline of *C. elegans* Cell; 150, 88-99 piRNAs Can Trigger a Multigenerational Epigenetic Memory in the Germline of *C. elegans*: Cell
- Asher D Cutter, Scott E Baird, Deborah Charlesworth (2006). High Nucleotide Polymorphism and Rapid Decay of Linkage Disequilibrium in Wild Populations of *Caenorhabditis remanei*, *Genetics*, Volume 174, Issue 2, Pages 901–913, <https://doi.org/10.1534/genetics.106.061879>
- Bargmann C, Horvitz H (1991). Chemosensory neurons with overlapping functions direct chemotaxis to multiple chemicals in *C. elegans*. *Neuron*, 7, 729-742 [https://doi.org/10.1016/0896-6273\(91\)90276-6](https://doi.org/10.1016/0896-6273(91)90276-6)
- Bargmann, C. I., Hartweg, E., & Horvitz, H. R. (1993). Odorant-selective genes and neurons mediate olfaction in *C. elegans*. *Cell*, 74(3), 515–527. [https://doi.org/10.1016/0092-8674\(93\)80053-H](https://doi.org/10.1016/0092-8674(93)80053-H)
- Baugh L Ryan, Day Troy (2020) Nongenetic inheritance and multigenerational plasticity in the nematode *C. elegans* eLife 9:e58498 Nongenetic inheritance and multigenerational plasticity in the nematode *C. elegans* | eLife
- Brenner S (1974). The genetics of *caenorhabditis elegans*, *Genetics*, Volume 77, Issue 1, Pages 71–94, <https://doi.org/10.1093/genetics/77.1.71>
- Burton, Nicholas O (2017). *Maternal Environment and Offspring Physiology: The Inheritance of Information Across a Generation*. Massachusetts Institute of Technology, PhD dissertation. *MIT Libraries*, <hdl.handle.net/1721.1/111294>.
- Cutter, AD (2015), ‘Caenorhabditis evolution in the wild’, *Bioessays*, 37 (9), 983-95.
- Darwin C (1859) On the Origin of Species by Means of Natural Selection
- de Chadarevian, S. (1998). Of worms and programmes: *Caenorhabditis elegans* and the study of development. *Studies in History and Philosophy of Science Part C: Studies in History and Philosophy of Biological and Biomedical Sciences*, 29(1), 81-105.
- Garcia, LR, B LeBoeuf, and P Koo (2007), ‘Diversity in mating behavior of hermaphroditic and male-female *Caenorhabditis nematodes*.’, *Genetics*, 175 (4), 1761-71.
- Greer, E., Maures, T., Ucar, D. *et al.* (2011). Transgenerational epigenetic inheritance of longevity in *Caenorhabditis elegans*. *Nature* 479, 365–371. <https://doi.org/10.1038/nature10572>
- Huxley (1942) *Evolution: The Modern Synthesis* New York: John Wiley and Sons.
- Jablonka E and Lamb, M.J. (2002), The Changing Concept of Epigenetics. *Annals of the New York Academy of Sciences*, 981: 82-96. <https://doi.org/10.1111/j.1749-6632.2002.tb04913.x>
- Jablonka E, Raz G (2009) Transgenerational epigenetic inheritance: prevalence, mechanisms, and implications for the study of heredity and evolution. *The Quarterly Review of Biology*. 84(2):131-176. DOI: 10.1086/598822. PMID: 19606595. Transgenerational Epigenetic Inheritance: Prevalence, Mechanisms, and Implications for the Study of Heredity and Evolution | *The Quarterly Review of Biology*: Vol 84, No 2
- Kaletta, T., Hengartner, M. (2006) Finding function in novel targets: *C. elegans* as a model organism. *Nat Rev Drug Discov* 5, 387–399. <https://doi.org/10.1038/nrd2031>
- Kunitomo, H., Sato, H., Iwata, R. *et al.* (2013) Concentration memory-dependent synaptic plasticity of a taste circuit regulates salt concentration chemotaxis in *Caenorhabditis elegans*. *Nat Commun* 4, 2210. <https://doi.org/10.1038/ncomms3210>
- Lamarck, J. B. (1809). *Philosophie Zoologique*.
- Nilsson, E. E., Maamar, M. B., & Skinner, M. K. (2020). Environmentally Induced Epigenetic Transgenerational Inheritance and the Weismann Barrier: The Dawn of Neo-Lamarckian Theory. *Journal of Developmental Biology*, 8(4), 28. <https://doi.org/10.3390/jdb8040028>
- Pierce-Shimomura, J., Faumont, S., Gaston, M. *et al.* (2001). The homeobox gene *lim-6* is required for distinct chemosensory representations in *C. elegans*. *Nature* 410, 694–698. <https://doi.org/10.1038/35070575>

- Rechavi O, Minevich G, Hobert O (2011) Transgenerational Inheritance of an Acquired Small RNA-Based Antiviral Response in *C. elegans* Cell, 2011; 147, 1248-1256 Transgenerational Inheritance of an Acquired Small RNA-Based Antiviral Response in *C. elegans*: Cell
- Riddle DL, Blumenthal T, Meyer BJ, Priess JR, editors (1997). *C. elegans* II. 2nd ed. Cold Spring Harbor : Cold Spring Harbor Laboratory Press; 1997. PMID: 21413221.
- Scheiner, S. M. (1993) "Genetics and Evolution of Phenotypic Plasticity." Annual Review of Ecology and Systematics 24: 35–68. Genetics and Evolution of Phenotypic Plasticity
- The *C. elegans* Sequencing Consortium (1998) Genome sequence of the nematode *C. elegans* : a platform for investigating biology. *Science* , 282 , 2012 –2018. 10.1126/science.282.5396.2012
- Tomioka M, Adachi T, Suzuki H, Kunitomo H, Schafer W, Line Y (2006). The Insulin/PI 3-Kinase Pathway Regulates Salt Chemotaxis Learning in *Caenorhabditis elegans*. *Neuron*, 51, p. 613-625 10.1016/j.neuron.2006.07.024 External Link
- Van Speybroeck, L. (2002). From epigenesis to epigenetics: the case of CH Waddington. *Annals of the New York Academy of Sciences*, 981(1), 61-81. <https://nyaspubs.onlinelibrary.wiley.com/doi/abs/10.1111/j.1749-6632.2002.tb04912.x>
- Wan QL., Meng X., Wang C. *et al.* (2022). Histone H3K4me3 modification is a transgenerational epigenetic signal for lipid metabolism in *Caenorhabditis elegans*. *Nat Commun* **13**, 768 <https://doi.org/10.1038/s41467-022-28469-4>
- Ward S (1973). Chemotaxis by the Nematode *Caenorhabditis elegans*: Identification of Attractants and Analysis of the Response by Use of Mutants, *Proc. Natl. Acad. Sci. U.S.A.* 70 (3) 817-821. <https://doi.org/10.1073/pnas.70.3.817>
- Ward, S., Thomson, N., White, J.G. and Brenner, S. (1975), Electron microscopical reconstruction of the anterior sensory anatomy of the nematode *caenorhabditis elegans*. *J. Comp. Neurol.*, 160: 313-337. <https://doi.org/10.1002/cne.901600305>
- Weinberg, E. D. (1959). Gradient Agar Plates. *The American Biology Teacher*, <https://doi.org/10.2307/4439185> 21(8), 347–350.
- Weismann, A. (1893). *The Germ-Plasm: A Theory of Heredity*. Google Books. https://books.google.be/books?hl=nl&lr=&id=nkwaAAAAYAAJ&oi=fnd&pg=PR23&ots=-2Cvaf2d8p&sig=Fupmlyv4bizMYu43t5ApGE9Ndvs&redir_esc=y#v=onepage&q&f=false
- R Core Team (2025). *_R: A Language and Environment for Statistical Computing_*. R Foundation for Statistical Computing, Vienna, Austria. <<https://www.R-project.org/>>.

Appendix

Table A1: Overview of the generations tested for each salt concentration, showing the duration of long-term exposure and the number of generations tested for preference following synchronisation.

| Longterm generation on salt | High salt (H) | Normal salt (N) | Low salt (L) |
|-----------------------------|---------------|-----------------|--------------|
| 7 | 1 | 1 | 1 |
| 8 | 1, 2, 3 | 1, 2 | 1, 2, 3 |
| 10 | 1, 2, 4 | 1, 2, 4 | 1, 4 |
| 11 | 1, 3 | 3 | 1, 3 |
| 12 | 1 | - | 1 |
| 16 | 1, 2, 3, 4 | 1 | 1, 2, 4 |
| 17 | 1, 2, 3 | 1, 2, 3 | 1 |
| 18 | 1, 2 | 1, 2 | - |
| 19 | 1 | 1 | 1 |

Table A2: An overview of the *C. remanei* strains used, including their biogeographical background.

| Strain | Landscape | Locality description | Substrate | Substrate comments |
|---------|-------------------|---------------------------------------|---------------------------|-----------------------------------|
| JU1084 | Rural garden | Japan, Shizuoka p., Kakegawa | vegetal litter/mix | decomposing fruits and vegetables |
| JU1086 | Forest | Japan, Shizuoka p., Kakegawa | vegetal litter/mix | leaf litter/bark/soil |
| JU2179 | Urban garden | Canada, Quebec, Montreal | rotting fruit (on ground) | Juglans nigra |
| JU2558 | Agricultural land | France, Grand Est, Illkirch | rotting fruit (on ground) | apple |
| JU2744 | Urban garden | Korea, Seoul | rotting fruit (on ground) | pumpkin? |
| JU2891 | Urban garden | Turkey, Kilimli | rotting fruit (on ground) | pear |
| TMR1001 | Rural garden | Germany, Mittelsachsen, Praebischuetz | rotting fruit (on ground) | apple |
| JU3419 | Forest | Japan, Miyagi p., Akiu | vegetal litter/mix | / |

Generatieve AI

| Toepassing | Tool | Wat heb je gedaan en waar in het document? |
|---|---------------|---|
| <input checked="" type="checkbox"/> Taalondersteuning | Deepl | De oorspronkelijke tekst heb ik zelf in het Nederlands geschreven en vervolgens met behulp van DeepL naar het Engels vertaald, omdat de promotor Engelstalig is. DeepL helpt daarmee ook voor de spelling en grammatica. Daarnaast heb ik gebruikgemaakt van de synoniemenfunctie van DeepL. Dit werd in het hele document gebruikt. |
| <input checked="" type="checkbox"/> Zoekmachine | Gemini | Gemini werd gebruikt om bepaalde temen of concepten uit te leggen die niet duidelijk waren uit de literatuur. |
| <input checked="" type="checkbox"/> Literatuuronderzoek | Deepl, Gemini | Voor het literatuuronderzoek is DeepL gebruikt om Engelstalige teksten te vertalen. Daarnaast is Gemini gebruikt om de interpretatie van complexe teksten te vergemakkelijken. Dit werd gebruikt in de introductie. |
| <input checked="" type="checkbox"/> Hulp bij korte invoer (e.g. zinnen vervolledigen, tekst assist) | Gemini | Gemini werd gebruikt om soms zinnen te herformuleren/in te korten. Dit werd in het hele document gebruikt. |
| <input checked="" type="checkbox"/> Genereren van programmeercode | Gemini | Voor de resultatenverwerking heb ik Gemini gebruikt als ondersteuning bij het schrijven van de R-code. Dankzij mijn kennis via 'data processing' kon ik de meeste plots zelfstandig maken, maar Gemini hielp bij het oplossen van foutmeldingen en complexe codeaanpassingen. Zo hielp het om via dplyr groepen aan te maken en de 'Normal'-groep als referentiegroep in te stellen, zonder handmatige aanpassingen in de dataset. Daarnaast hielp Gemini om de lay-out van de plots te verbeteren. |
| <input type="checkbox"/> Genereren van nieuwe onderzoeksideeën | | |
| <input type="checkbox"/> Genereren van tekstblokken | | |
| <input checked="" type="checkbox"/> Andere toepassingen | Gemini | Ik heb Gemini gebruikt om te controleren of mijn interpretatie van de statistische gegevens correct was. Daarnaast hielp het bij specifieke vragen over de statistische interpretatie. |