Epigenetic memory for salt concentration preference in *Caenorhabditis remanei*

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Abstract

We tested if salt conditioning affects chemotaxis and induces epigenetic memory in one strain of *Caenorhabiditis remanei*. Epigenetic memory refers to the heritable changes in behavior or gene expression that are induced by past environmental conditions without changes to DNA sequence. Worms were grown under high and low salt conditions and assayed on salt gradients, then, salt chemotaxis was recorded separately for the two sexes. No significant difference in salt preference was detected between salt conditioning treatments and the sexes responded in a similar way. In addition, there was no transgenerational memory for salt, as the chemotactic behavior remained the same across generations of passing through standard salt conditions. Overall, there was no salt-dependent preference or inherited behavioral effects under the tested conditions. These results contrast previously published strong effects for the salt conditioning affecting salt chemotaxis in *C. elegans*, and unpublished results for *C. remanei*. This is likely due to high variability and limited statistical power.

Keywords: Caenorhabditis, Sex differences, Salt chemotaxis, Epigenetic memory

Introduction

Caenorhabditis remanei are free-living nematodes inhabiting soil and rotting vegetation, where they consume bacteria (Diaz 2008). They reproduce by outcrossing, which gives them high natural genetic diversity and they have large effective population sizes (Teterina *et al.* 2023). These attributes make them valuable models for studying the influence of genetic variation on behavior and for testing evolutionary hypotheses about adaptive traits, like salt-preference.

This species has been chosen to be studied on because it has some valuable traits for these assays. Since we want to analyze females and males separately, we needed a species that reproduces only by outcrossing, like *C. remanei* (Teterina *et al.* 2023). Their short generation time, with an average maturation time of 1,25 days (Diaz 2008), allows to obtain lots of data fast. Furthermore, they have fast evolving phenotypic plasticity and within a few generations their phenotype can be modulated by external triggers (Sikkink *et al.* 2014), making them suitable for memory analysis on salt preference.

C. remanei is closely related to the model organism *C. elegans* that is broadly used for developmental biology, neural biology and evolutionary studies (Teterina *et al.* 2023; Haag *et al.* 2007). The reason they are good research organisms is because they have many qualities. Their generation time is short, they can reproduce by outcrossing and selfing and are simple to cultivate in a laboratory (Haag *et al.* 2007). Many aspects of *C. elegans* biology are expected to apply to *C. remanei* because these species are closely related (Teterina *et al.* 2023).

Salt-preference learning is controlled by two modules in *C. elegans*, namely the DAG/PKC-1 and the Insulin/IGF-1 signaling (IIS) pathways (Adachi *et al.* 2010).

DAG and PKC-1 are present at presynaptic regions, more specifically at the axon of ASER (Right Amphid Sensory neuron class E), where DAG production is regulated by EGL-8 and in turn modulates PKC-1. There are correlations present between the dynamics of DAG and the migration direction of worms after feeding. A decrease in salt concentration, increases DAG levels at the ASER axon, so well-fed worms move toward high salt concentrations. An increase in salt concentration, decreases DAG levels, leading worms to move toward low salt concentrations (Tomioka *et al.* 2022). Worms will thus remain in locations where they were fed.

The IIS pathway connects nutrient levels to behavior. This pathway is required for salt chemotaxis learning that uses calcium signaling in the ASER neuron (Murphy *et al.* 2013). The ASER neuron wherein DAF-2 and AGE-1 act senses salt. In this neuron, the strength and direction of salt chemotaxis is modulated by this DAF-2/AGE-1 activity level. INS-1, released from AIA interneurons, provides feedback onto ASER to regulate the signaling pathway and generate experience-dependent plasticity in chemotactic behavior (Tomoika *et al.* 2006).

C. elegans is also known to exhibit epigenetic memory, that can be perpetuated for several generations (Kunitomo *et al.* 2013). I am interested in exploring this in a species with separate sexes, such as *C. remanei*, including its inheritance along multiple generations. Epigenetic memory can be described as an epigenetic regulation where behavior induced earlier by a trigger, changes the expression of genes. This change in RNA causes heritable changes in phenotypes (D'Urso 2014). An example of epigenetic memory in *C. elegans* involves exposure to a virus which triggered the production of small-interfering RNAs, which silenced the viral sequence. In the offspring of these worms, the same sequence got silenced even though these individuals had never been exposed to the virus. The effect persisted for several generations with inheritance observed to last four to five generations in these experimental lineages (Rechavi 2011).

In *C. elegans*, an RNAi inheritance mechanism is present that may allow the inheritance of dietary history-dependent small RNA response across multiple generations. dsRNA (double-strand RNA) can spread through the whole organism and be transmitted from somatic cells to the germline (Rechavi *et al.* 2014). For RNAi effects to become heritable and not diluted across generations, the production of secondary siRNA (small interfering RNA) is needed in every generation. These siRNAs are generated after the initial RNA trigger (Ashe *et al.* 2012) and are amplified by RNA-dependent RNA polymerases in each generation, allowing persistent transmission of ancestral information. Maintenance of this RNA-based epigenetic inheritance requires continuous amplification of secondary siRNA by RNA-dependent RNA polymerases and their transmission through the germline. In the absence of reinforcement, the response is not maintained and gradually decays over the next generations, leading to the loss of the inherited state (Rechavi *et al.* 2017; Rechavi *et al.* 2014). This loss reflects dilution of small RNA signals rather than an active termination mechanism. Environmental stress has been shown to alter or reset ancestral small RNA-mediated inheritance in *C. elegans*, resulting in reduced transgenerational persistence of RNAi responses (Rechavi *et al.* 2014). Epigenetic inheritance mediated by RNAi typically persists for about three to five generations in *C. elegans* (Rechavi *et al.* 2017; Rechavi *et al.* 2014). *C. remanei* probably possesses these conserved RNAi components since this secondary siRNA pathway is conserved across nematodes (Shi *et al* 2013). Similar ecological pressures to *C. elegans* are also present in *C. remanei*, since they share comparable ecological niches (Kiontke *et al* 2006).

In this work I aim to confirm previously unpublished results of a conditioned response to salt concentration experience in C. remanei, demonstrate the response can be transmitted across generations (epigenetic memory) and estimate how many generations are required for the memory to fade. I also tested for differences between the sexes for this memory, because it may be a useful phenotype to link to the sex chromosome in future studies.

Materials and methods

Worms strains and culture

The worm strain used for this experiment was JU2891 (rK). It is a *C. remanei* strain that has been taken from an urban garden growing on rotten peers on the ground in Kilimli, Turkey and was provided by the Marie Ann Felix lab. It was cultured on standard nematode growth medium (NGM) with standard salt concentration (3g/L).

NGM medium

The media on which the worms would grow were made, for 400mL, by adding 1g of peptone and 8 g of agar. To this, NaCl was added in different quantities for high, low and normal salt concentration, respectively adding 2.4g, 0.9g and 1.2g. Next, water was added to reach 400mL. The mixture was then autoclaved at 100°C, 10 PSI for 1h, and then put at 50°C until pouring.

Right before use (for plates or gradients), 400 µL cholesterol, 400 µL 1M $CaCl_2$, 400 µL 1M $MgSO_4$ and 10 mL 2M KPO_4 were added.

Pouring plates

To pour plates, the medium (with added solutions) was poured into round petri dishes for maintenance or squared petri dishes to establish a salt concentration gradient.

To make plates, this mixture was poured into 10 cm diameter petri dishes in sterile conditions, under laminar air flow. Afterwards 200 µL of OP50 *E. coli* strain in 2x YT medium were added onto them using a multi-dispenser repeater pipette. The plates were maintained at room temperature for 24h for the bacteria to grow and then kept at 4°C until use.

Gradient plates

The gradient plates were used to visualize the impact of salt concentration on the worms. To make salt gradients, coins, square petri dishes and a peristaltic pump were used. First the petri dishes were divided into six sections; section one being on the lowest salt concentration side to section six being on the highest side. Section one was labelled as ‘L’ (Low) and section six as ‘H’ (High), these were indicated by using a marker (Figure A1). One side of the plates was then put on three coins (€2, €0.50 and €0.10), having a height of about six millimeters. They were put under both sides of the ‘H’ section so the low salt concentration agar poured into it would move towards the ‘L’ section. Using a pump, 5 pourings of 14 mL were made into each petri dish, which resulted in agar reaching the top of the non-elevated side of the petri dish. The low salt agar was pumped first. After it set, an equal amount of high salt agar was poured on the plate laid out flat. The petri dishes were stored for 24h before use to allow time for the gradient to fully form (Wimpenny *et al.* 1987).

Worms were transferred onto a gradient plate about 4 days after synchronization (see below) which ensured they were adult and generations were not mixed. The worms were placed in the middle of the gradient, on the line between sections three and four. After letting them move on the gradient for two hours, the worms present on each section were visualized using a microscope and counted. Only sections one, two, five and six were used for the data analysis. Worms on section three and four weren’t further considered because they could either be interpreted as unresponsive or damaged due to the transfer.

Worm transfer between plates

Worms were transferred from one plate to another within 4-6 days after being put on a new plate, which allowed enough time for a full generation to grow on a plate.

This transfer was done by cutting a square into the agar of the old plate, using a metal lab spatula, and putting it onto a new plate (chunking technique). The technique was later replaced by washing the old plate with 2 ml M9 and adding all of it to a new plate, so more worms are transferred and the plate reaches population capacity faster.

Synchronization

Synchronization kills all worms present on the plate, except for eggs, even eggs within pregnant worms. The eggs are then allowed to hatch in favorable conditions on a new plate so all emerging nematodes are the same age, which is very useful to remove behavioral variation due to age. The process starts by pipetting 2 mL M9 buffer (made out of 5,8g Na_2HPO_4, 3g KH_2PO_4, 0,5g NaCl, 1g NH_4Cl and water to reach 1L) on a plate with eggs and transferring this into a 15 mL falcon tube. To this, ddH_2O is added to reach 15 mL. This mix is then centrifuged at 1000 g for 3 min. at room temperature (±20°C). Afterwards, the supernatant is removed and 2 to 3 mL bleach mix (19mL ddH_2O, 5mL NaOCl and 1mL 10M NaOH) are added to the pellet. After 3-6 min incubation with occasional shaking, M9 solution is added to reach 15mL. This is centrifuged again (same settings) and the supernatant gets removed. Then, 10 mL M9 is added to the pellet and centrifuged again (same settings). After removing the supernatant, the pellet is resuspended into 1 mL M9 and transferred to a sterile petri dish.

For the preference experiment, a transfer to a normal salt petri dish represents a control group. Instead of transferring synchronized worms who grew on high salt concentration only on a high salt concentration dish, half of it got transferred to a normal sterile dish (Figure A2). The same happened for synchronized worms who grew on low salt concentration.

For the memory experiment, only the eight generation of worms growing on high salt concentration were used. After being synchronized they were transferred to normal salt concentration plates (Figure A3).

Data analysis

All data processing and statistical analysis were performed using RStudio version 4.4.1 (R Core Team 2025). Data were imported into R and processed using a script (available in appendix file 1). Chemotaxis indices were calculated from the counts per section of a gradient plate. Graphs and statistical visualization, like boxplots and trends, were produced by using the packages ggplot2, dplyr and gridExtra. The worm counts were extracted from each gradient sections (1-6) separately for males and females. For analysis, the sections were grouped into low salt (sections 1 and 2), neutral salt (sections 3 and 4) and high salt (sections 5 and 6). Only low and high salt sections were used for behavioral quantification, as worms in sections 3 to 4 were excluded from further analysis.

The chemotaxis index (CI) was used to quantify salt preference and was calculated for each replicate and sex separately as: $CI = (H-L)/(H+L)$. where H is the number of worms in high-salt region (sections 5 and 6) and L is the number of worms in the low-salt regions (sections 1 and 2). The chemotaxis index ranges from -1 to +1, with positive values indicating attraction to high salt, negative values indicating attraction to low salt and values around zero indicating no preference.

The chemotaxis index was analyzed across conditioning treatments (H, L, NH, NL) and across generations in the memory experiment. NH and NL represent control groups in which synchronized worms originating from high or low salt conditions were transferred to a normal salt environment before testing. All analyses were performed separately for males and females to account for sex-specific behavioral differences.

Normality of chemotactic index distributions was assessed using a Shapiro-Wilk test. As the data were not normally distributed, a non-parametric statistical method was applied. Differences in chemotaxis index between conditioning treatments were analysed by using a Kruskal-Wallis rank test for females and males separately.

To evaluate potential transgenerational effects, a Spearman’s rank correlation was used to test if monotonic relationships were present between generation number and chemotaxis index. In addition, a permutation test evaluated whether the observed trends across generations deviated from expectations.

Generative artificial intelligence (ChatGPT, OpenAI) was used as an assistive tool during the analysis of data. The AI was used to help for coding and plotting, based on user-provided descriptions.

Results

Salt preference

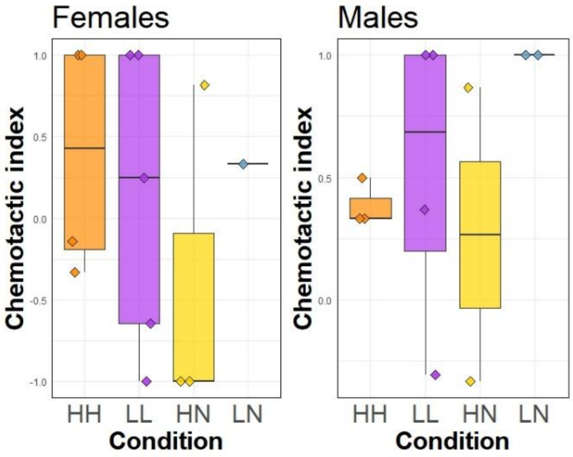

Figure 1: Boxplots of preference in females and males with their preference (-1 to 1) depending on their conditioning (HH: High salt to High salt, LL: Low salt to low salt, HN: High salt to normal salt, LN: Low salt to normal salt.).

Worm chemotactic index was very different among replicates but showed lots of overlap between different conditioning treatments for both sexes (Figure 1). The median chemotaxis index varies in both directions, with both positive and negative values across treatments and did not align with the expectations for their conditioning treatment.

The Shapiro-Wilk test resulted in p-values lower than 0,05, indicating a deviation from the normality of the distribution of chemotaxis indexes in both sexes. Therefore, a non-parametric test, the Kruskal-Wallis test was used. This revealed no significant differences in chemotaxis index across conditioning treatment in females ($\chi^2 = 2.16$, $p>0.05$) or males ($\chi^2 = 4.00$, $p>0.05$).

Memory

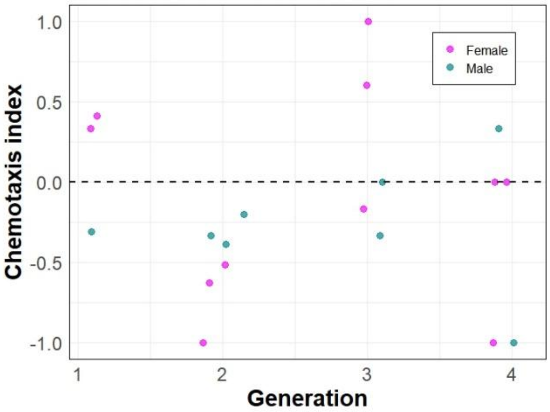

Figure 2: Chemotactic preference across generations for females (pink) and males (blue) with chemotaxis index going from -1,0 to 1,0)

Chemotactic indexes were highly variable within generations, with strong overlap between males and females. No visible trend of consistent increase or decrease in chemotactic index was detected across generations.

The Spearman’s rank correlation test resulted in a ρ (correlation coefficient) of approximatively zero and a p-value higher than 0,05, indicating no monotonic relationship between generation and chemotactic index. A permutation test confirmed that observed slopes were not significantly different from random expectations with a p-value of 0.78 for females and 0.98 for males, which indicates no detectable transgenerational memory, matching the chemotactic indices.

Data reliability

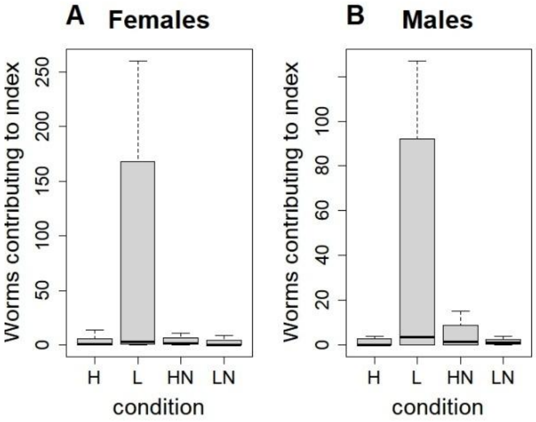

Figure 3: Female (A) and male (B) effective population size for high salt sections (H) and low salt sections (L) together (H+L)

The effective sample sizes (H+L counts) were strongly different across conditions (Figure 3). Low-salt treatments contributed many more worms, which increased the overdispersion in binomial models and likely amplified condition effects. The reliability of preference estimates are limited because of this variability and sampling imbalance.

Discussion

Preference

The expectations for the tested treatments were not met. Worms growing on high salt concentration were expected to prefer high salt. The same was expected for worms growing on low salt concentration (Figure 4) but neither of these preferences was detected.

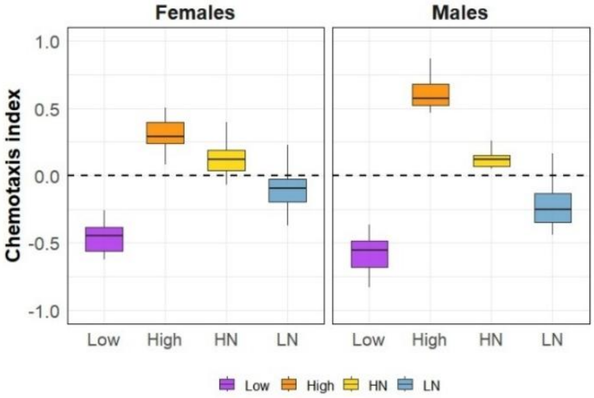

Figure 4: Expected results for preference in females (left) and males (right). Purple boxplots show results of worms with low salt conditioning, orange boxplots show results of worms with high salt conditioning. Yellow boxplots show results of worms on high salt conditioning put on normal salt concentration after synchronization. Blue boxplots show results of worms on low salt condition put on normals salt concentration after synchronization

The result is surprising because the influence of the environmental salt concentration has previously been shown to have a strong effect in salt chemotaxis in adult worms both in *C. elegans* (Saeki *et al.* 2001) and *C. remanei* (Pignatti, BSc thesis unpublished). If the expected effect had been observed, that would indicate that worm exposure during development can induce persistent behavioral changes in adults. This would support the idea that salt preference is plastic and shaped by environmental conditions during growth.

The absence of perceptible preference in this study is likely due to methodological and sampling limitations rather than a lack of behavioral differences. Lots of gradients contained low numbers of worms or showed limited dispersal across the assay plate, increasing variability in chemotaxis indices. As a result, individual worm positions had a disproportionate effect on the calculated chemotaxis index, increasing variance and reducing the ability to detect treatment-dependent differences.

Memory

If epigenetic memory persists across generations, worms conditioned to high salt for generations, were expected to have offspring which retain the high salt preference even after growing on normal salt concentration. This preference is expected to slowly decay over generations of growth on normal salt concentration (Figure 5).

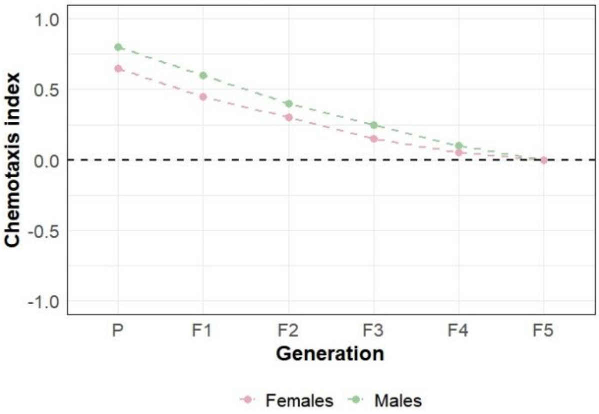

Figure 5: Expected results for high salt memory in females and males. Pink dots show memory in females and green dots show memory in males.

However, no trend of consistent increase or decrease was detected when plotting chemotactic indices across generations in normal salt (Figure 2). This suggests no memory could be detected across generations after conditioning to a high salt environment. Since there was no increased preference for high salt in the first generation to begin with, the analysis is meaningless as its first datapoint is against expectation. However, had there been a high salt chemotactic index in the first generation, a significant negative slope in a linear model of chemotactic index vs generation would indicate that there is cross generational epigenetic memory. In addition, given data from sufficient generations it might be possible to estimate the number of generations at which no epigenetic memory remains, by identifying the generation for which the chemotactic index does not significantly differ from zero.

Possible reasons for failure to obtain epigenetic memory have been discussed above. In addition, methodological factors may have contributed to variability in the results. Environmental stress has been shown to reset ancestral small RNA-mediated inheritance in *C. elegans*, resulting in reduced transgenerational persistence of RNAi responses (Houri-Ze'evi *et al.* 2021). Therefore experimental procedures introducing physiological perturbations, such as synchronization, may influence the stability or detectability of inherited epigenetic effects. An alternative way to test worms across generations should be employed such as a controlled mating protocol in which multiple males and females are placed together for a defined period to generate offspring. This is then followed by the removal of the parental generation. This approach allows collection of F1 progeny without the need for synchronization procedures such as bleaching, thereby minimizing potential methodological disturbance across generations.

Data reliability

Figure 3 shows how many worms contributed to the chemotaxis index per condition. It is clear that the LL condition (worms grown on low salt and synchronized on low salt) is represented by many more individual worms than the other conditions and there is a large imbalance in sample size between treatments. Chemotaxis is normalized to the total number of worms per assay so it is not directly biased by sample size. On the other hand conditions with fewer individuals are expected to have a higher variability because of higher sampling noise. Consequently, the estimates for LL are more precise than for conditions with lower sample size.

Possible reasons for the higher sample size at low salt condition include different survival or scoring attrition associated with salt concentration. In *C. elegans*, standard NGM salt concentration induces measurable physiological stress, shorten lifespan compared to low salt conditions and activates a stress response pathway, particularly in adult and aging worms (Pohl *et al.* 2025). Even though these effects have not been established directly in *C. remanei*, similar mechanisms could lead to reduced survival or increased scoring difficulty in worms that grew on higher salt concentrations.

Methodological issues

Results obtained from this research should be interpreted with caution, due to several methodological limitations during experimental execution. First, the timing between feeding, synchronization and placement of worms on gradient was highly inconsistent across replicates. Because chemotactic behavior is highly dependent on developmental and physiological stage in nematodes, this variability likely introduced plate-to-plate variation, reducing comparability between experimental conditions

In addition, sample sizes were generally low and uneven across treatments. One generation conditioned to low salt contributed disproportionately high numbers of worms, where other conditions contained very few individuals. the imbalance likely reflects differences in worm transfer methods. The chunking method, which was used for the preference assays, resulted in lower worm quantities compared to the M9 transfer method used for some memory assays. Consequently, some conditions were overrepresented, increasing variability and reducing reliability of preference estimates.

A possible issue with M9 transfer procedure is that the amount of solution (2mL) used for transfer was likely too high. This flooded the agar surface, creating reduced oxygen availability and potentially slowing down the larval development of the nematodes (Kitazume *et al.* 2018). This delay may in turn influence chemotactic response of the worms, since they are sensitive to physiological state.

Further, variability may also have been contributed by differences in worm handling and developmental staging. Sometimes synchronised worms were assayed before reaching sexual maturity. Since males sexually mature later (Diaz *et al.* 2008), their male-specific traits were not expressed yet, making visual sexing under the microscope more challenging. This leads to potential misclassification of males and probably contributed to male underrepresentation in an already low-abundance group, which limits the ability to detect sex-specific effects.

Some variability in assay conditions may have also influenced the results. About half of the plates used for memory assays showed signs of reduced structural integrity. This was likely due to incorrect agar-to-water ratio when making NGM. It probably affected the salt concentration of the plates. In addition, excess moisture following worm transfer to gradient using M9 method was not consistently removed. Plates that had not dried enough, likely impacted the crawling behavior of the worms as they have more difficulties moving through liquid than the agar surface. This reduced the reliability of some chemotaxis measurements. In later assays, with more dried gradients, worms moved across the plate faster, suggesting that earlier assays may have been underestimated chemotactic responses. Although this technical issue occurred, removing these datasets after inspection is not a robust solution for improving overall data quality. The number of available replicates is already limited, further removal of the data would reduce statistical power, increasing likelihood of type II errors and making it more difficult to detect biologically meaningful effects. Therefore, rather than excluding problematic plates, the results should be interpreted in light of increased variability and consider assay conditions as a source of experimental noise. These issues should be prevented at source instead of after data collection.

Also, the bacterial food preparation protocol was modified during the course of the experiment. Initially, bacterial lawns were applied in low quantities and put in the fridge shortly after preparation. In later experiments, larger bacterial lawns were used and incubated at room temperature for approximatively 24 hours before storing them in the fridge, this allowed bacterial growth. The differences likely resulted in variation of bacterial density between assays. Bacterial availability directly affects feeding state in nematodes, this may influence their chemotactic behavior. In *C. elegans* and related species, well-fed nematodes and starved ones can display opposite chemotaxis response to the same salt concentration (Saeki *et al*. 2001).

Finally, the bleach synchronization protocol was modified during data collection. The initial bleach solution was replaced due to low effectiveness, as the chemical had expired. The replacement solution was first used undiluted, like the first bleach solution, and was probably more potent, which likely increased worm mortality. It was later used in more diluted form for a shorter time period which worked better. However these changes may have introduced additional variability in survival across generations.

Conclusion

C. remanei salt preference had high variability across replicates, with no detectable evidence of consistent condition-dependent or sex-dependent differences in chemotactic behavior under the tested conditions. No evidence of transgenerational memory was observed in either females or males.

These results should however be interpreted in the context of high experimental variability. This includes, uneven sample sizes, differences in worm handling and assay condition and overdispersion across treatments. These factors probably reduced statistical power and hide some biological effects.

Overall, these findings suggest that while strong and consistent salt preference or inheritance effects were not detected in this dataset, improved experimental execution and increased sample size would be necessary to more reliably test chemotactic preference and memory in *C. remanei*.

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References

Adachi T, Kunitomo H, Tomioka M, e.al. (2010) Reversal of salt preference is directed by the insulin/PI3K and Gq/PKC signaling in *Caenorhabditis elegans*. *Neuron* 68:109–120. https://europepmc.org/article/MED/20837997

Ashe A, Sapetschnig A, Weick EM, e.al. (2012) piRNAs can trigger a multigenerational epigenetic memory in the germline of *Caenorhabditis elegans*. *Cell* 150:88–99. https://doi.org/10.1016/j.cell.2012.06.018

Diaz SA, Lindström J, Haydon DT, et al. (2008) Basic demography of *Caenorhabditis remanei* cultured under standard laboratory conditions. *Journal of Nematology* 40:167–178. https://pmc.ncbi.nlm.nih.gov/articles/PMC2664667/

D'Urso A, Brickner JH. (2014) Mechanisms of epigenetic memory. *Trends in Genetics* 30:230–236. https://doi.org/10.1016/j.tig.2014.04.004

Haag ES, Chamberlin H, Coghlan A, et al. (2007) Caenorhabditis evolution: if they all look alike, you aren't looking hard enough. Trends in Genetics 23:101-104. https://doi.org/10.1016/j.tig.2007.01.002

Kiontke K, Sudhaus W. (2006) Ecology of *Caenorhabditis* species. WormBook.

https://www.ncbi.nlm.nih.gov/books/NBK19723/

Kitazume, H., Dayi, M., Tanaka, R., & Kikuchi, T. (2018). Assessment of the behaviour and survival of nematodes under low oxygen concentrations. PLOS One, 13(5), e0197122. https://doi.org/10.1371/journal.pone.0197122

Kunitomo H, Sato H, Iwata R, e.al. (2013) Concentration memory-dependent synaptic plasticity of a taste circuit regulates salt concentration chemotaxis in *Caenorhabditis elegans*. *Nature Communications* 4:2210. https://doi.org/10.1038/ncomms3210

Murphy CT, Hu PJ. (2013) Insulin/insulin-like growth factor signaling in *Caenorhabditis elegans*. WormBook.. 2013_wormbook_murphy.pdf

Pignatti LS. (2025) Differences in salt chemotaxis between *Caenorhabditis* species and sexes. Bachelor's thesis, Vrije Universiteit Brussel. https://www.parisveltsos.com/research/research/researchBlog/students/luca_thesis.pdf

Pohl F, Egan BM, Schneider DL, e.al. (2025) Environmental NaCl affects *Caenorhabditis elegans* development and aging. *Genetics* 231:iyaf139. https://doi.org/10.1093/genetics/iyaf139

R Core Team (2025). _R: A Language and Environment for Statistical Computing_. R Foundation for Statistical Computing, Vienna, Austria. https://www.R-project.org/

Rechavi O, Houri-Ze'evi L, Anava S, e.al. (2014) Starvation-induced transgenerational inheritance of small RNAs in *Caenorhabditis elegans*. *Cell* 158:277–287. https://www.sciencedirect.com/science/article/pii/S009286741400806X

Rechavi O, Lev I. (2017) Principles of transgenerational small RNA inheritance in *Caenorhabditis elegans*. Current Biology 27:R720–R730. https://doi.org/10.1016/j.cub.2017.05.043

Rechavi O, Minevich G, Hobert O. (2011) Transgenerational inheritance of an acquired small RNA-based antiviral response in *Caenorhabditis elegans*. *Cell* 147:1248–1256. https://doi.org/10.1016/j.cell.2011.10.042

Saeki S, Yamamoto M, Iino Y. (2001) Plasticity of chemotaxis revealed by paired presentation of a chemoattractant and starvation in the nematode *Caenorhabditis elegans*. *Journal of Experimental Biology* 204:1757–1764. https://www.researchgate.net/publication/12018397_Plasticity_of_chemotaxis_revealed_by_paired_presentation_of_a_chemoattractant_and_starvation_in_the_nematode_Caenorhabditis_elegans

Shi Z, Montgomery TA, Qi Y, e.al. (2013) High-throughput sequencing reveals extraordinary fluidity of miRNA, piRNA, and siRNA pathways in nematodes. *Genome Research* 23:497–508. https://doi.org/10.1101/gr.149112.112

Sikkink KL, Reynolds RM, Ituarte CM, e.al. (2014) Rapid evolution of phenotypic plasticity and shifting thresholds of genetic assimilation in the nematode *Caenorhabditis remanei*. G3 4:1103–1112. https://doi.org/10.1534/g3.114.010553

Teterina AA, Willis JH, Lukac M, et al. (2023) Genomic diversity landscapes in outcrossing and selfing Caenorhabditis nematodes. PLoS Genetics 19:e1010879. https://doi.org/10.1371/journal.pgen.1010879

Tomioka M, Adachi T, Suzuki H, Kunitomo H, Schafer WR, Iino Y, et al. (2006) The insulin/PI 3-kinase pathway regulates salt chemotaxis learning in *Caenorhabditis elegans*. Neuron 51:613–625. https://doi.org/10.1016/j.neuron.2006.07.024

Tomioka M, Jang MS, Iino Y, et al. (2022) DAF-2c signaling promotes taste avoidance after starvation in Caenorhabditis elegans by controlling distinct phospholipase C isozymes. Communications Biology 5:1–8. https://doi.org/10.1038/s42003-021-02956-8

Wimpenny JWT, Waters P. (1987) The use of gel-stabilized gradient plates to map the responses of microorganisms to three or four environmental factors varied simultaneously. *FEMS Microbiology Letters* 40:263–267. https://doi.org/10.1111/j.1574-6968.1987.tb02036.x

Appendix

File A1: Raw data and codes used for this thesis. Available at: Electronic_Appendix MLM Hantson thesis.zip

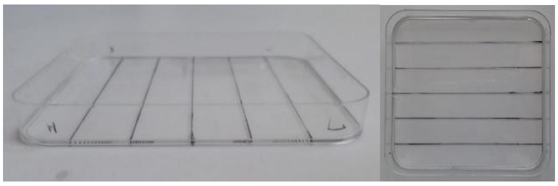

Figure A1: Side view of gradient plate with sections going from high (H) on the left and low (L) on the right (left panel). Top view of gradient plate with sections (right panel).

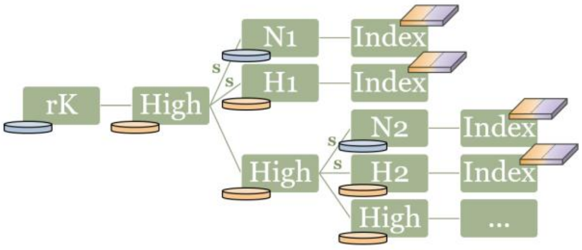

Figure A2: Experimental design of preference for high salt concentration. The 'S' stands for synchronization. Blue petri dishes represent ones with normal salt concentration. Orange petri dishes represent ones with high salt concentration. Orange and purple beams represent the gradient going from high (orange) to low (purple)

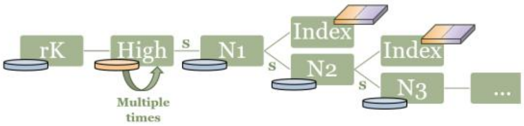

Figure A3: Experimental design of memory for low salt concentration. The 'S' stands for synchronization. Blue petri dishes represent ones with normal salt concentration. Orange petri dishes represent ones with high salt concentration. Orange and purple beams represent the gradient going from high (orange) to low (purple)

Toepassing	**Tool**	**Wat heb je gedaan en waar in het document?**
Literatuuronderzoek	ChatGPT + Anara	Support for searching scientific litterature Introduction
Hulp bij korte invoer (BV zinnen vervolledigen, tekst assist)	ChatGPT	Improve own texts to achieve a more scientific style Introduction Results Interpretation Conclusion
Genereren van programmeercode	ChatGPT	Help for writing and debugging codes for data analysis and figures Results

Table A1: Use of generative AI throughout the paper