UNIVERSITY OF SÃO PAULO
SCHOOL OF ARTS, SCIENCES AND HUMANITIES
GRADUATE PROGRAM IN COMPLEX SYSTEMS MODELING

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Is latitude associated with chronotype?

São Paulo

2024

Daniel Kachvartanian de Azevedo

Is latitude associated with chronotype?

Original version

Thesis presented to the School of Arts, Sciences and Humanities at the University of São Paulo, as a requirement for the degree of Master of Science by the Graduate Program in Complex Systems Modeling.

Area of concentration: Complex Systems.

Supervisor: Prof. Dr. Camilo Rodrigues Neto

São Paulo

2024

CATALOGUING IN PUBLICATION
(University of São Paulo. School of Arts, Sciences and Humanities. Library)
CRB 8-4936

Azevedo, Daniel Kachvartanian de
Is latitude associated with chronotype? / Daniel Kachvartanian de Azevedo ; supervisor, Camilo Rodrigues Neto. – 2024
43 p : il.

Thesis (Master of Science) – Graduate Program in Complex Systems Modeling, School of Arts, Sciences and Humanities, University of São Paulo.
Original version.

1. Complexity science. 2. Chronobiology. 3. Biological rhythms. 4. Chronotypes. 5. Circadian phenotypes. 6. Entrainment. 7. Latitude. I. Rodrigues Neto, Camilo, supervisor II. Title.

Thesis by Daniel Kachvartanian de Azevedo, under the title **Is latitude associated with chronotype?**, presented to the School of Arts, Sciences and Humanities at the University of São Paulo, as a requirement for the degree of Master of Science by the Graduate Program in Complex Systems Modeling, in the concentration area of Complex Systems.

Approved on ____________________ , __________ .

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Institution ____

Evaluation ____

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Institution ____

Evaluation ____

ACKNOWLEDGEMENTS

I would like to acknowledge and express my gratitude to the following persons and organizations. Without them, this work would not have been possible:

My partner in life, Salete Perroni (Sal).

My Mother, for her unconditional love.

My Sister and my Brother, for their love and companionship in life.

My two friends and partners in science, Alicia Rafaelly Vilefort Sales and Maria Augusta Medeiros de Andrade.

My friend and Professor Humberto Miguel Garay Malpartida, for his support; for his principles; and for his integrity, which was demonstrated when the need arose.

My supervisor, Professor Camilo Rodrigues Neto, for introducing me to and teaching me about the complexity science since 2012; for supervising my dissertation; for the patience and the virtue in taking on and mediating the process of transitioning my Master's supervision after the breakdown of relations with my former supervisor.

Professor Carlos Molina Mendes, for his speed, impartiality, patience, and virtuous approach in mediating the process of transitioning of my Master's supervision.

My fellow friends: Alex Azevedo Martins; Amanda Moreira; Augusto Amado, Carina (Cacau) Prado; Ítalo Alves Bezerra do Nascimento; Júlia Mafra; Letícia Nery de Figueiredo; Marcelo Ricardo Fernandes Roschel; Reginaldo Noveli; Sílvia Capelanes; and Vanessa Simon Silva.

USP's Support Program for Student Permanence and Education (PAPFE), which enabled me to get this far.

The Coordination for the Improvement of Higher Education Personnel (CAPES), for funding this work and enabling my presence in graduate studies.

This study was financed in part by the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior - Brasil (CAPES) - Finance Code 001. Grant number: 88887.703720/2022-00.

Nullius in verba[1]

[1] The Royal Society. (n.d.). *History of the Royal Society*. https://royalsociety.org/about-us/history/

ABSTRACT

Vartanian, D. (2024). *Is latitude associated with chronotype?* [Master's Thesis, University of São Paulo].

Theories on circadian rhythms are well-established in science, but there is still a need to test them in larger samples to gain a better understanding of the expression of temporal phenotypes. This thesis investigates the hypothesis that latitude influences chronotype expression, based on the idea that regions closer to the poles receive less sunlight over the year than equatorial regions. This difference suggests that equatorial areas have a stronger solar zeitgeber, which could lead to greater synchronization of circadian rhythms with the light-dark cycle, reducing the amplitude and diversity of circadian phenotypes, resulting in a higher propensity for morningness in those populations. To test this hypothesis, data from $120,265$ individuals from all regions of Brazil were analyzed, collected in 2017 based on the Munich ChronoType Questionnaire (MCTQ), a validated questionnaire to assess circadian phenotypes derived from individuals' sleep-wake cycles over the past four weeks. The analysis, using nested linear regression models, revealed a negligible effect of latitude on the variation in chronotype expression (Cohen's f^2: 0.00740), contrasting with recent studies. Although the hypothesis is reasonable and aligns with evolutionary theories of temporal biological systems, the results suggest that the phenomenon of entrainment is more complex than previously thought.

Keywords: Complexity science. Complex systems. Chronobiology. Biological rhythms. Chronotypes. Circadian phenotypes. Sleep. Entrainment. Latitude. MCTQ.

RESUMO

Vartanian, D. (2024). *A latitude está associada ao cronotipo?* [Dissertação de Mestrado, Universidade de São Paulo].

As teorias sobre ritmos circadianos estão bem estabelecidas na ciência, mas ainda há a necessidade de testá-las em amostras mais amplas para compreender melhor a expressão dos fenótipos temporais. Esta dissertação investiga a hipótese de que a latitude influencia a expressão dos cronotipos, baseada na ideia de que regiões próximas aos polos recebem menos luz solar ao longo do ano do que as regiões equatoriais. Esse diferencial sugere que áreas equatoriais possuem um zeitgeber solar mais forte, o que poderia levar a uma maior sincronização dos ritmos circadianos com o ciclo claro-escuro, reduzindo a amplitude e a diversidade de fenótipos circadianos, resultando em uma propensão maior ao cronotipo matutino. Para testar essa hipótese, foram analisados dados de 120.265 indivíduos de todas as regiões do Brasil, coletados em 2017 com base no Munich ChronoType Questionnaire (MCTQ), um questionário validado para medir fenótipos circadianos a partir do ciclo sono-vigília nas últimas quatro semanas. A análise, utilizando modelos de regressão linear aninhados, revelou um efeito negligenciável da latitude na variação da expressão dos cronotipos (f^2 de Cohen: $0,00740$), em contraste com estudos recentes. Embora a hipótese faça sentido e esteja alinhada com teorias evolutivas dos sistemas biológicos temporais, os resultados sugerem que o fenômeno de entraiment é mais complexo do que se imaginava.

Palavras-chaves: Ciência da complexidade. Sistemas complexos. Cronobiologia. Ritmos biológicos. Cronotipos. Fenótipos circadianos. Sono. Entrainment. Latitude. MCTQ.

CONTENTS

1 INTRODUCTION

> **Note**
>
> You are reading the work-in-progress of this thesis.
>
> This chapter should be readable but is currently undergoing final polishing.

There has been a long-standing debate in the chronobiology community regarding the relationship between latitude and circadian phenotypes (chronotypes) (Pittendrigh et al., 1991; Skeldon & Dijk, 2021; Zerbini et al., 2021), with many believing this association is already well-established. This hypothesis stems from the varying amounts of solar radiation experienced by populations across different latitudes. Since light exposure is a primary zeitgeber — a periodic environmental cue that influences or regulates biological rhythms — such variations, along with temperature variations, are thought to create noticeable differences in chronotype distributions globally. This thesis investigates the so-called latitude or environmental hypothesis in human circadian phenotypes, addressing the question: *Is latitude associated with chronotype?*

The central hypothesis is that *latitude is associated with human chronotype distributions*, with populations closer to the equator exhibiting, on average, a shorter or more morning-oriented circadian phenotype compared to those residing near the poles (Hut et al., 2013; Leocadio-Miguel et al., 2014, 2017; Pittendrigh et al., 1991; Randler, 2008; Randler & Rahafar, 2017; Roenneberg, Wirz-Justice, & Merrow, 2003). The primary objective is to model and test this hypothesis by critically examining whether a significant association and effect size exist between latitude and circadian phenotypes in the Brazilian population.

In the following chapters, the latitude hypothesis is explored using Popper's hypothetical-deductive method (Popper, 1979) and an improved approach to Null Hypothesis Significance Testing (NHST), rooted in the original Neyman-Pearson framework for data testing (Neyman & Pearson, 1928a, 1928b; Perezgonzalez, 2015). This exploration involves a series of analyses conducted on a large dataset comprising $120,265$ individuals, collected from the Brazilian population in 2017. The dataset is based on the Munich Chronotype Questionnaire (MCTQ) (Roenneberg, Wirz-Justice, & Merrow, 2003; Roenneberg et al., 2012), and contains information on sleep habits and demographic characteristics from various regions of Brazil.

The analyses utilize nested multiple regression models to evaluate the additional variance explained and the effect size when latitude is included as a predictor of chronotype. The results are then compared with those obtained from a restricted model that excludes latitude as a predictor. This method of prodecure builds on an article published by Leocadio-Miguel et al. (2017), which investigated the relationship between latitude and chronotype in the Brazilian population. The present study extends this work by utilizing a larger dataset, a more appropriate scale for measuring this phenomena (Leocadio-Miguel et al., 2014), and more advanced statistical methods to test the hypothesis. The results will contribute to the ongoing debate on the latitude-chronotype relationship, offering new evidences into how environmental factors influence human circadian rhythms.

The thesis is structured as a collection of articles. Chapters 2 through 4 comprise a series of essays and literature reviews related to the thesis topic. Chapter 5 presents the main investigation, including an article reporting the hypothesis test and discussing the central research question. Finally, Chapter 6 offers conclusions, limitations and directions for future research.

All analyses in this document are reproducible and were conducted using the R programming language (R Core Team, n.d.) along with the Quarto publishing system (Allaire et al., n.d.). Since this thesis focuses heavily on data, it is best viewed online. To access the digital version and supplemental materials, please visit https://danielvartan.github.io/mastersthesis. The thesis code repository is available on GitHub at https://github.com/danielvartan/mastersthesis). Finally, the research compendium can be accessed via The Open Science Framework at the following link: https://doi.org/10.17605/OSF.IO/YGKTS.

2 ON CHRONOBIOLOGY

Note

You are reading the work-in-progress of this thesis.

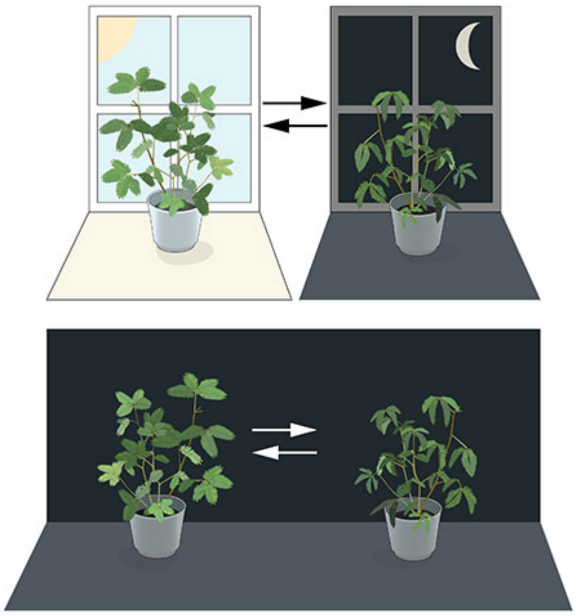
This chapter should be readable but is currently undergoing final polishing.

The dimension of time, manifest in the form of rhythms and cycles, like the alternating patterns of day and night as well as the annual transition of seasons, was consistently featured in the evolutionary journey of not only the human species but also all other life forms on our planet. These rhythms and cycles brought with them evolutionary pressures, resulting in the development of a temporal organization that allowed organisms to survive and reproduce in response to the conditions imposed by the environments they inhabited (Menna-Barreto, 2003; Pittendrigh, 1981). An example of this organization can be observed in the presence of different activity-rest patterns among living beings as they adapt to certain temporal niches, such as the diurnal behavior of humans and the nocturnal behavior of cats and some rodents (Foster & Kreitzman, 2005).

For years, scientists debated whether this organization was solely in response to environmental stimuli or if it was also present endogenously, internally, within organisms (Rotenberg et al., 2003). One of the early seminal studies describing a potential endogenous rhythmicity in living beings was conducted in 1729 by the French astronomer Jean Jacques d’Ortous de Mairan. De Mairan observed the movement of the sensitive plant (*mimosa pudica*) by isolating it from the light-dark cycle and found that the plant continued to move its leaves periodically (Figure 1) (de Mairan, 1729; Rotenberg et al., 2003). Charles Darwin also also wrote about the movement observed in plants and made thematic explorations of these intriguing “periodical phenomena” (Andrade & Beale, 2024). The search for this internal timekeeper in living beings only began to solidify in the 20th century through the efforts of scientists like Jürgen Aschoff, Colin Pittendrigh, Franz Halberg, and Erwin Bünning, culminating in the establishment of the science known as chronobiology, with a significant milestone being the Cold Spring Harbor Symposium on Quantitative Biology: Biological Clocks in 1960 (*chrónos*, from Greek, meaning time; and *biology*, pertaining to the study of life) (Cold Spring

Harbor Laboratory, n.d.; Rotenberg et al., 2003)[1]. However, the recognition of endogenous rhythmicity by the global scientific community truly came in 2017 when Jeffrey Hall, Michael Rosbash, and Michael Young were awarded the Nobel Prize in Physiology or Medicine for their discoveries of molecular mechanisms that regulate the circadian rhythm in fruit flies (*circā*, from Latin, meaning around, and *dĭes*, meaning day (Latinitium, n.d.) – a rhythm that expresses itself in approximately one day) (Nobel Prize Outreach AB, n.d.).

Figure 1 – Illustration of a circadian rhythm in the movement of the leaves of the sensitive plant (*mimosa pudica*) observed by Jacques d'Ortous de Mairan in 1729.



Source: Reproduction from Nobel Prize Outreach AB (n.d.).

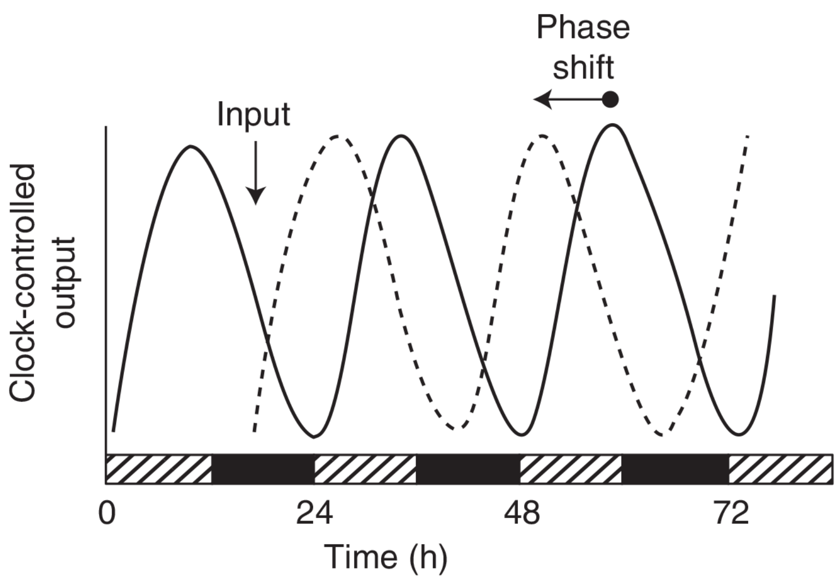
Science has already demonstrated and described various biological rhythms and their impacts on organisms. These rhythms can occur at different levels, whether at a macro level, such as the menstrual cycle, or even at a micro level, such as rhythms expressed within cells (Roenneberg & Merrow, 2016). Like many other biological phenomena, these are complex systems present in all living beings, i.e., an emergence created by a large number of connected and interecticve agents that exhibit adaptive characteristics, all without the need of a central control (Boccara, 2010). It is understood today that the endogeneity of rhythms has provided organisms with an anticipatory capacity,

[1] Some say the term *chronobiology* was coined by Franz Halberg during the Cold Spring Harbor Symposium on Quantitative Biology, vol. XXV (Menna-Barreto & Marques, 2023, p. 21).

allowing them to organize resources and activities before they are needed (Marques & Menna-Barreto, 2003).

Despite the endogenous nature of these rhythms, they can still be regulated by the external environment. Signals (cues) from the environment that occur cyclically and have the ability to regulate biological rhythmic expression are called zeitgebers (from the German *zeit*, meaning time, and *geber*, meaning donor (Cambridge University Press, n.d.)). These zeitgebers act as synchronizers by entraining the phases of biological rhythms (Khalsa et al., 2003; Kuhlman et al., 2018) (see Figure 2). Among the known zeitgebers are, for example, meal timing and changes in environmental temperature (Aschoff, 1981; Roenneberg & Merrow, 2016). However, the most influential of them is the light-dark cycle. It is understood that the day/night cycle, resulting from the rotation of the Earth, has provided the vast majority of organisms with an oscillatory system with a periodic duration of approximately 24 hours (Kuhlman et al., 2018; Roenneberg, Kuehnle, et al., 2007).

Figure 2 – Illustration of a circadian rhythm (output) whose phase is entrained in the presence of a zeitgeber (input). The rectangles represent the light-dark cycle.



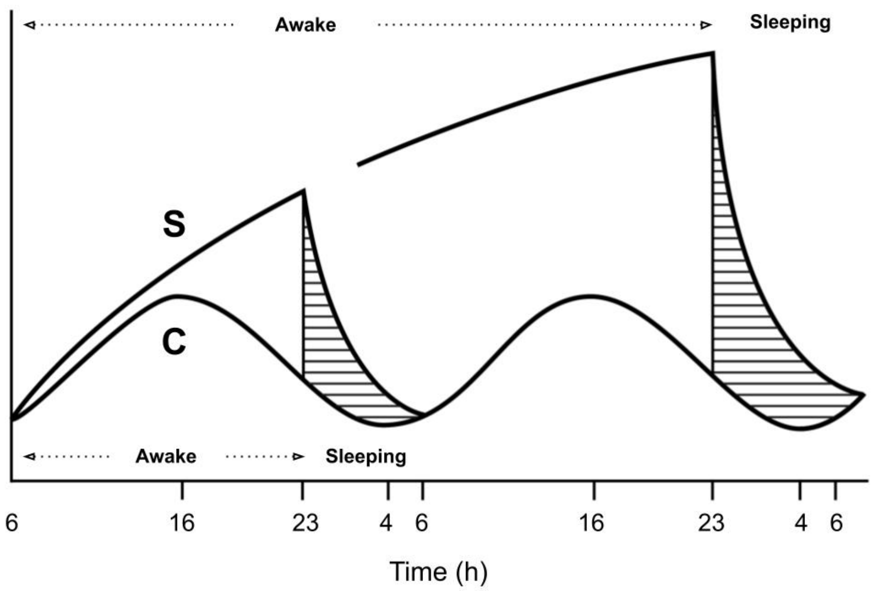
Source: Adapted from Kuhlman et al. (2018).

Naturally, the expression of this temporal organization varies from organism to organism, even among members of the same species, whether due to the different ways they are exposed to the environment or the differences in the expression of endogenous rhythmicity, which, in turn, results from gene expression (Roenneberg, Kumar, & Merrow, 2007). The interaction between these two expressions, external and internal, of the environment and genotype, generates a signature, an observable characteristic, which is called a phenotype (Frommlet et al., 2016).

The various temporal characteristics of an organism can be linked to different oscillatory periods. Among these are circadian phenotypes, which refer to characteristics observed in rhythms with periods lasting about a day (Foster & Kreitzman, 2005). Another term used for these temporal phenotypes, as the name suggest, is *chronotype* (Ehret, 1974; Pittendrigh, 1993). This term is also often used to differentiate phenotypes on a spectrum ranging from morningness to eveningness (Horne & Östberg, 1976; Roenneberg, Wirz-Justice, et al., 2019).

Sleep is a phenomenon that exhibits circadian expression. By observing the sleep characteristics of individuals, it is possible to assess the distribution of circadian phenotypes within a population, thereby investigating their covariates and other relevant associations (Roenneberg, Wirz-Justice, & Merrow, 2003). This is because sleep regulation is understood as the result of the interaction between two processes: a homeostatic process (referred to as the S process), which is sleep-dependent and accumulates with sleep deprivation, and a circadian process (referred to as the C process), whose expression can be influenced by zeitgebers, such as the light-dark cycle (Figure 3 illustrates these two process) (Borbély, 1982; Borbély et al., 2016). Considering that the circadian rhythm (the C process) is present in sleep, its characteristics can be estimated if the S process can be controlled.

Figure 3 – Illustration of the interaction between Process S (sleep-dependent process) and Process C (circadian rhythm process) in sleep regulation. The figure depicts two scenarios: one with 17 hours of wakefulness followed by 7 hours of sleep, and another with sleep deprivation, consisting of 41 hours of wakefulness followed by 7 hours of sleep. The y-axis represents the level of each process. The shaded areas indicate periods of sleep, along with the exponential decline of Process S.



Source: Adapted from Borbély (1982).

[Finish explaining about the MCTQ and its proxy for the chronotype]

3 ON COMPLEXITY SCIENCE

> **!** Important
>
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>
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Complex versus Complicated.

Complex systems are systems with many interconnected parts that exhibit emergent behavior.

Stable macroscopic patterns arising from **local interaction** of agents (Epstein, 1999).

In mathematical terms, the interactions of interest are non-linear (Holland, 2014).

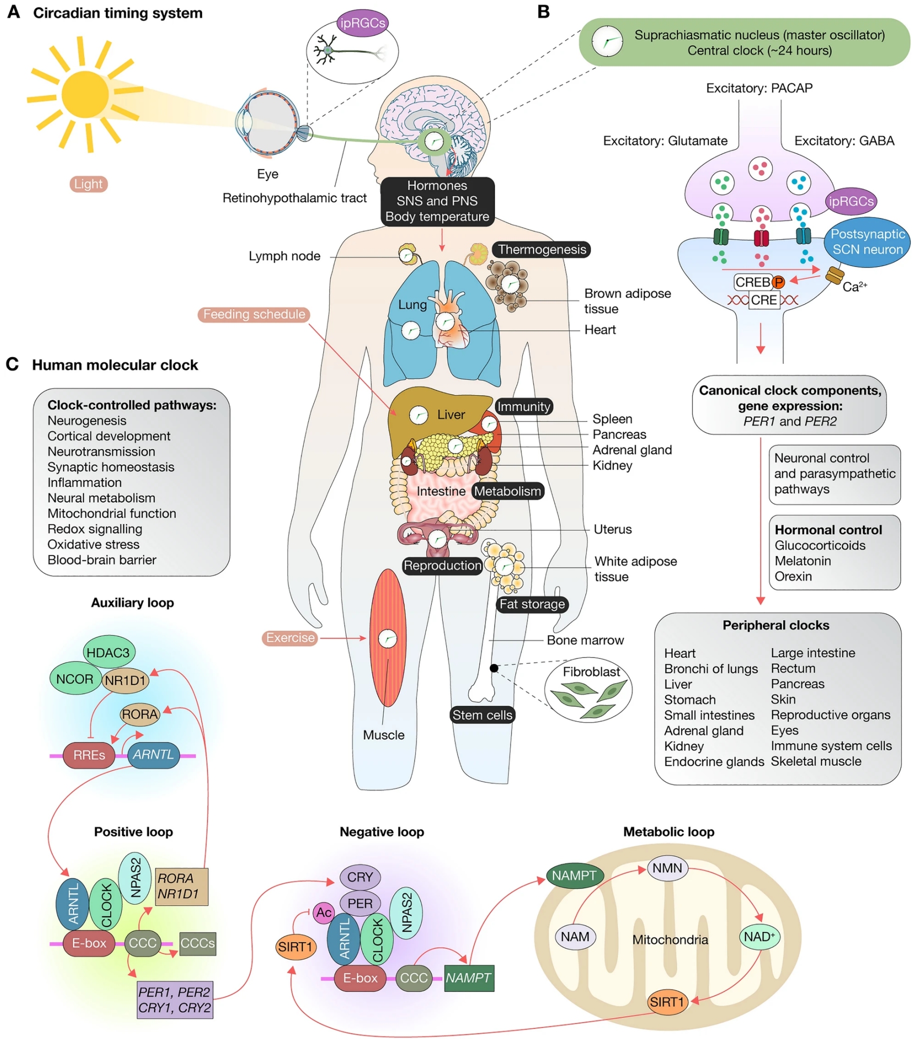
When the aggregate exhibits **properties not attained by summation** (Holland, 2014).

The behavior of each part don't explain how they behave collectively.

Emergence: new properties that arise from the interactions of the parts of a system, which are not present in the parts themselves. Collective behavior. Aggregate behavior.

You are dealing with an emergence phenomenon when there is no need to look under the hood (*Complexity explorer lecture*, 2023).

Figure 4 – An illustration depicting how the human circadian clock system works. (A) The circadian timing system receives light signals through the eyes, which are processed by the brain's central clock, the suprachiasmatic nucleus (SCN). This clock helps to orchestrate peripheral clocks, regulating body functions like hormone release, metabolism, and temperature. Other factors like feeding schedules and exercise also influence this process, ensuring the synchronization of body systems like immunity and metabolism. (B) Specific brain cells and chemicals manage this timing by adjusting the activity of neurons, which helps reset the clock and keeps it in tune with day-night cycles. These neurons also influence clocks in other body parts via neuronal and hormonal signals. (C) At a molecular level, a network of genes and proteins operates in a feedback loop, maintaining near 24-hour cycles. This ensures that various body systems, such as metabolism and immune function, follow daily rhythms.



Source: Reproduction of Pérez-Villa et al. (2023).

The result of a *juggling act* of billions of years of evolution. There are no specific functions. There's no proposed goal.

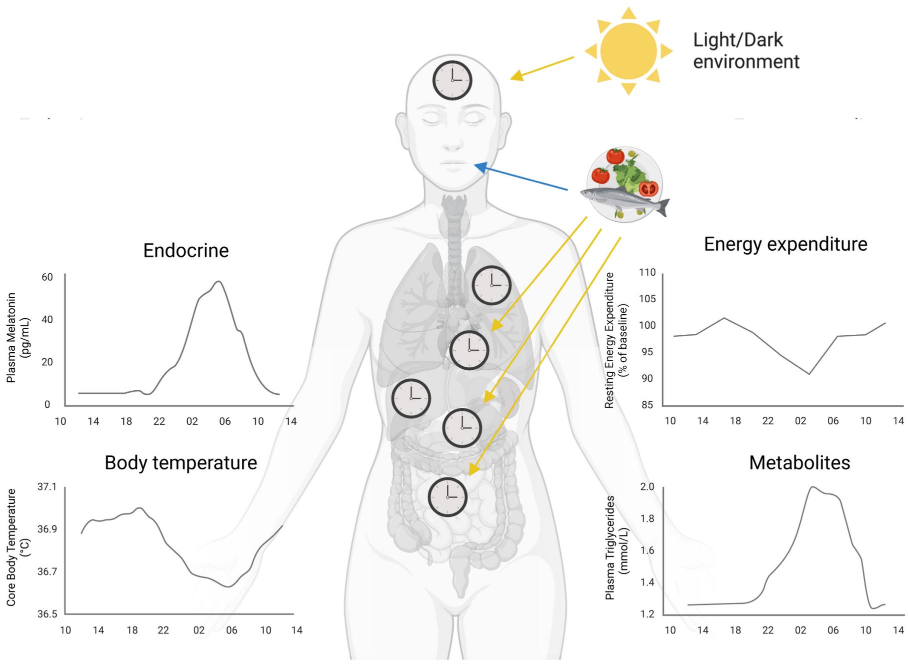
Levels of description (Nicolis & Nicolis, 2012). Starting in the cell nucleus with the molecular clock, then the cell, the tissue, the organ following to stable macroscopic patterns of circadian rhythms in behavior and physiology.

If you think about it, the extensive clock control is like a finely-tuned choreography. Everything is organized to happen at the right time, just like a **Rube Goldberg machine** (Merrow & Roenneberg, 2020).

Circadian clocks regulate and/or modulate functions at all levels, ranging from gene expression and physiology to behavior and cogitation (Roenneberg, Kuehnle, et al., 2007).

It's an emergent phenomenon. It's not a property of the parts themselves. It's a property of the system as a whole.

Figure 5 – This illustration shows how circadian rhythms influence key aspects of human metabolism. These rhythms impact hormone levels, body temperature, energy use, and metabolite concentrations in the body, independent of external factors like environment and behavior. The light/dark cycle and food intake help synchronize these internal clocks located throughout the body, regulating physiological processes like resting energy expenditure and melatonin secretion.



Source: Reproduction of Flanagan et al. (2021).

"[…] involve great numbers of parts undergoing a kaleidoscopic array of simultaneous interactions." (Holland, 1992)

In complex adaptive systems, **emergent properties** often occur when coevolving signals and boundaries generate **new levels of organization** (Holland, 2012).

A system of many interacting parts where the system is **more than just the sum of its parts** (Mark Newman in Mitchell (2013)).

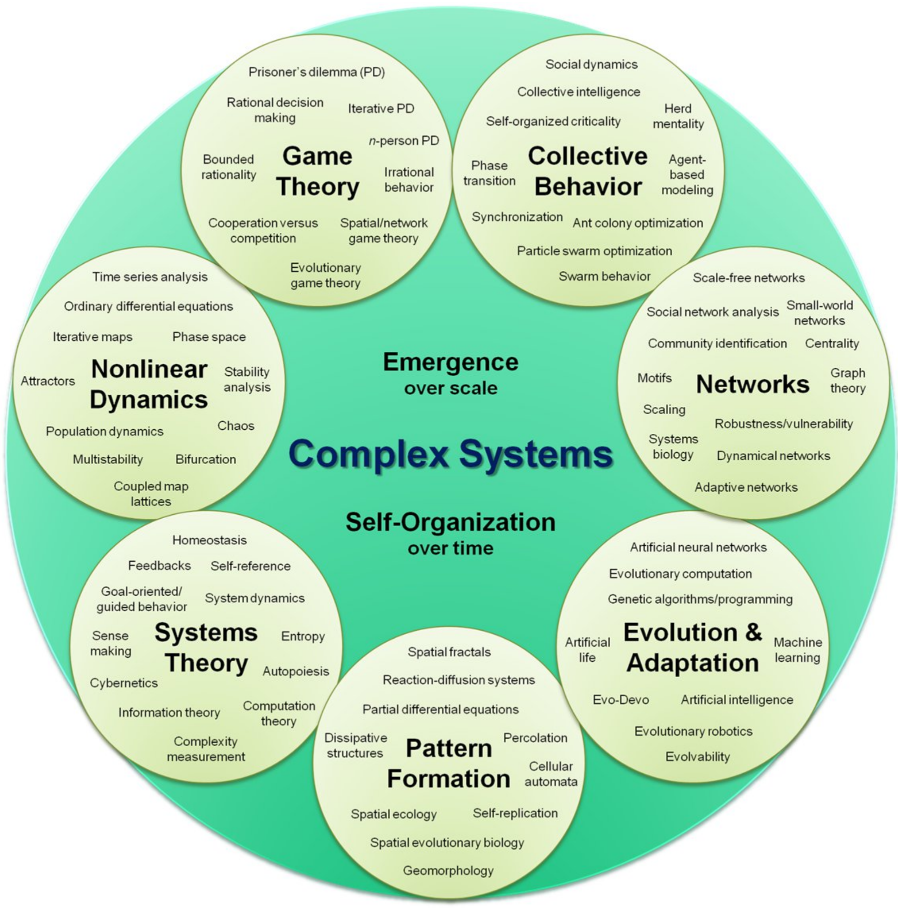
A system that involves a large number of parts undergoing a kaleidoscopic array of simultaneous interactions, exhibiting **aggregate behavior** that cannot be simply derived from the actions of the individual parts (Holland, 1992).

Systems with many connected agents that interact and exhibit self-organization and **emergence behavior**, all without the need for a central controller (Camilo Rodrigues Neto).

Dialectics at its finest (my working definition).

Complexity science seeks to **explain emergent phenomena** or mechanisms that “screen-off” their constituent parts and thereby allow new levels of description and understanding (Krakauer & Wolpert, 2024).

Figure 6 – This visual map organizes complex systems science into seven main areas. The fields of Nonlinear Dynamics, Systems Theory, and Game Theory represent its historical foundations, while newer areas include Pattern Formation, Evolution and Adaptation, Networks, and Collective Behavior. Together, these areas explore how complex systems emerge and self-organize over time and scale.



Source: Reproduction of Sayama (2015).

Other concepts: chaos, power laws & factor sparsity, feedback loops, robustness, equilibrium states, path dependence, leverage points

Approaches to study and model circadian clocks.

4 ON THE LATITUDE HYPOTHESIS

> **!** Important
>
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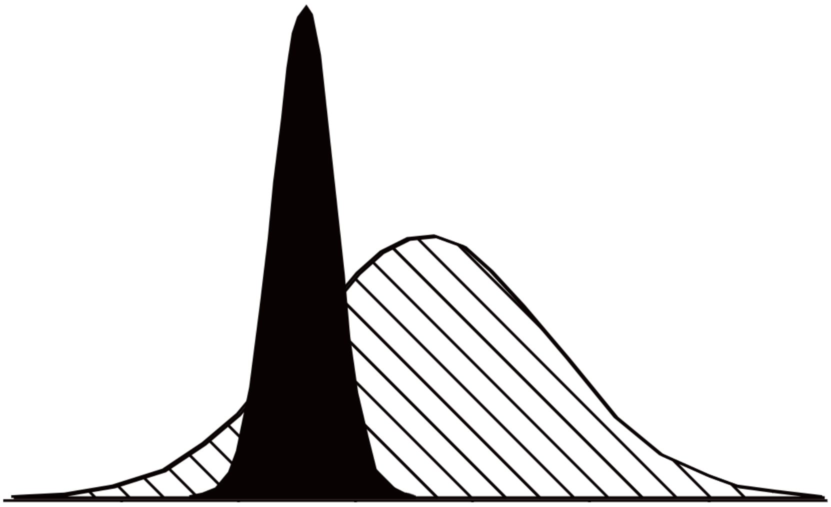
Although many theories related to sleep and circadian rhythms are well-established in science, it is still necessary to verify and test them in larger samples to obtain a more accurate picture of the mechanisms related to the ecology of sleep and chronotypes. This project undertakes this commitment with the aim of investigating a hypothesis that is still relatively untested but widely accepted in chronobiology, which suggests that latitude is associated with the regulation of circadian rhythms (Hut et al., 2013; Leocadio-Miguel et al., 2014, 2017; Pittendrigh et al., 1991; Randler, 2008; Randler & Rahafar, 2017; Roenneberg, Wirz-Justice, & Merrow, 2003).

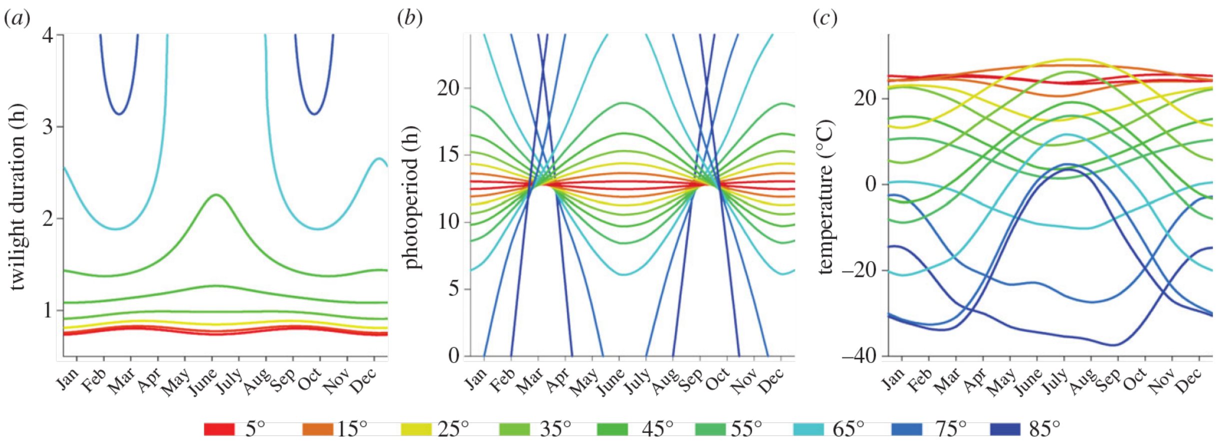
The latitude hypothesis is based on the idea that regions located at latitudes close to the poles, on average, experience less annual sunlight exposure compared to regions near the equator. Therefore, it is deduced that regions near latitude 0° have a stronger solar zeitgeber, which, according to chronobiology theories, should lead to a greater propensity for the synchronization of circadian rhythms in these populations with the light-dark cycle. This would reduce the amplitude and diversity of circadian phenotypes found due to a lower influence of individuals' characteristic endogenous periods (Figure 7 illustrates this effect). This would also give these populations a morningness characteristic when compared to populations living farther from the equator, where the opposite would occur – greater amplitude and diversity of circadian phenotypes and an eveningness characteristic compared to populations living near latitude 0° (Roenneberg, Wirz-Justice, & Merrow, 2003).

Figure 7 – Different chronotype distributions, influenced by strong and weak zeitgebers – black for strong and hatched for weak. An illustration of the effect hypothesized by the latitude hypothesis.



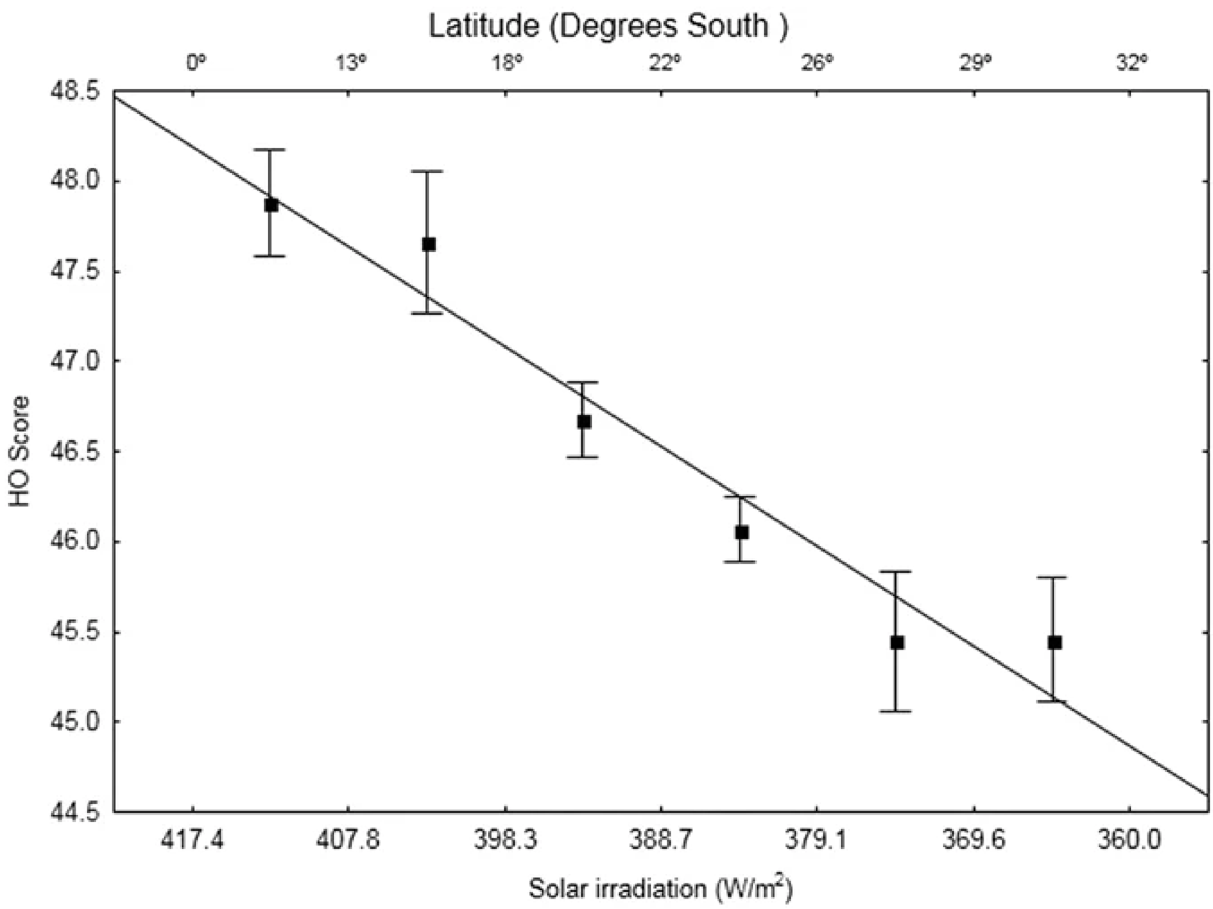
Source: Adapted from Roenneberg, Wirz-Justice, and Merrow (2003).

Figure 8 – Annual changes in (a) twilight duration, (b) daylight hours, and (c) temperature across different latitudes. Each color shows a specific latitude range, illustrating how these factors vary throughout the year.



Source: Reproduction from Hut et al. (2013).

Figure 9 – Mean HO scores (±SE) along a latitudinal cline, showing corresponding annual average of solar irradiation level (W/m2).



Source: Reproduction from Leocadio-Miguel et al. (2017).

“This last fact contradicts the otherwise plausible interpretation of our data that strength of Zeitgeber (and hence precision) is greatest when the light/dark ratio is 1:1. The strength of the Zeitgeber certainly depends on the light/dark ratio [5] and it may be expected to influence precision, too. However, unexplained additional factors must complicate the picture. It is unknown whether the fluctuations in precision have an ecological significance.” (Aschoff et al., 1972)

“Latitude and longitude are key factors that influence how light–dark cycle interacts with the circadian rhythm of humans. Therefore, it is reasonable to assume that chronotype distribution of a population may vary as a result of different geographical location.” (Leocadio-Miguel et al., 2014)

“However, it is also our experience that some mathematically sophisticated scientists may lack the conceptual frame that links the mathematical procedures to the substantive scientific task in a particular case” (Cohen et al., 2002).

Effect sizes. Statistical ritual versus statistical thinking (Gigerenzer, 2004).

A p-value is not evidence of the existence of an effect., it's only indirect evidence at best. Large samples and the p-value problem (Lin et al., 2013). P-values as an exponential model of data sizes (Gómez-de-Mariscal et al., 2021). A small p-value does not imply that there is an important effect; it only tells us something about the plausibility of the effect. You need to have a meaningful effect to have an interesting result, and the p-value doesn't tell you that.

Confidence intervals for an effect size measure in multiple linear regression (Algina et al., 2007). Setting hypotheses with effect sizes: minimal effect size (Perezgonzalez, 2015).

The original article had two issues regarding data testing, commonly related to null hypothesis significance tests (NHST). It is not within the scope of this article to discuss all the issues regarding NHST; for that, I recommend checking Perezgonzalez (2015). The two main methodological issues regarding the hypothesis test in the original article were:

1. Using the p-value instead of the effect size as a criterion for rejecting or accepting the alternative hypothesis;
2. Failing to integrate a minimum effect size in the alternative hypothesis. A test without the latter can create serious distortions in the interpretation of the results, since even a negligible effect could lead to the acceptance of the alternative hypothesis.

Considering the particular emphasis that the solar zeitgeber has on the entrainment of biological rhythms (as demonstrated in many experiments), it would not be reasonable to assume that the latitude hypothesis could be supported without at least a non-negligible effect size. Although the latitude association claimed by many authors implies not just a linear relationship but also a significant effect on chronotype, considering the biological construct of chronotype, the approach that incorporates a minimum effect size (MES) is not new. In fact, when Neyman and Pearson published their schema for data testing, a MES was required.

Even assuming a very low threshold, similar to the claim of 2% variance explained by latitude as a predictor, the hypothesis does not hold. As shown by Leocadio-Miguel et al. (2017), adding 0.00388 to the R^2 results in Cohen's $f^2 = (0.06352 - 0.05964)/(1 -$

$0.06352) = 0.004143174$, which is negligible by all standards. Again, the conclusion drawn by the authors of this study was not based on sound statistical thinking.

y-axis illusion. unbalanced data. Horner & Östberg scale from 16-86.

Solar irradiation used as a proxy for latitude.

5 IS LATITUDE ASSOCIATED WITH CHRONOTYPE?

> **Note**
>
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>
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> **Warning**
>
> The results shown here are **preliminary**, so please take them with a grain of salt.
>
> The data has not yet been fully cleaned, balanced, and cross-referenced with the secondary databases. Think of these results as a low-resolution preview of the final results. The step-by-step analysis can be seen in the appendices section.

> **Target journal**
>
> 1. Scientific Reports (IF 2022: 4.6/JCR | A1/2017-2020).

> **Note**
>
> The following study was performed by Daniel Vartanian (DV), Mario Pedrazzoli (MP) and Camilo Rodrigues Neto (CR).
>
> **DV** contributed to the design and implementation of the study. **DV** and **MP** collected the data. **DV** and **CR** performed the statistical analysis. **DV** wrote the manuscript. All authors discussed the results and revised the final manuscript.
>
> *Future reference*: Vartanian, D., Pedrazzoli, M., & Rodrigues Neto, C. (2024). A biological approach for the latitudinal cline of the chronotype. *Scientific Reports*.

Chronotypes are temporal phenotypes (Ehret, 1974; Pittendrigh, 1993). Observable traits, like weight and eye color. Our current understanding of these traits is that they are linked to our environment and are the result of evolution pressures for creating an inner temporal organization (Aschoff, 1989; Paranjpe & Sharma, 2005), a way that organisms found to anticipate events. Having such an important function in nature, these internal rhythms need to be closely aligned with environmental changes. The agents that shift these oscillations towards the environment are called zeitgebers and the shift phenomenon is called entrainment (Roenneberg, Daan, & Merrow, 2003; Roenneberg et al., 2010). The main zeitgeber

for humans is light exposure, particularly the light of the sun (Khalsa et al., 2003; Minors et al., 1991; Roenneberg, Kumar, & Merrow, 2007). Considering the major role of light on entrainment, several studies hypothesized that the latitude shift of the sun could influence or even define the chronotypes of different populations (Horzum et al., 2015; Hut et al., 2013; Leocadio-Miguel et al., 2014, 2017; Pittendrigh et al., 1991; Randler & Rahafar, 2017). For example, populations that live close to the equator would be, on average, more entrained to the light-dark cycle and have morning-leaning characteristics. Here we test this hypothesis using a biological measure, the chronotype state, provided by the Munich ChronoType Questionnaire (Roenneberg, Wirz-Justice, & Merrow, 2003). We tested the latitude hypothesis on a sample with $76,744$ subjects living in different latitudes in Brazil. Our results show that, even with a wide, big, and aligned sample, the latitude is associated only with negligible effect sizes. The entrainment phenomenon appears to be much more complex than previously imagined, opening new questions and contradictions that need to be further investigated.

5.1 MAIN TEXT

5.1.1 **Introduction**

Humans can differ from one another in many ways. These observable traits, like hair color or height, are called phenotypes and are also presented in the way that our body functions.

A chronotype is a temporal phenotype (Ehret, 1974; Pittendrigh, 1993). This word is usually used to refer to endogenous circadian rhythms, i.e., rhythms which periods that are close to a day or 24 hours (*circa diem*). The current body of knowledge of Chronobiology, the science that studies biological rhythms, indicates that the evolution of these internal oscillators is linked to our oscillatory environment, like the day and night cycle, which, along with our evolution, created environmental pressures for the development of a temporal organization (Aschoff, 1989; Paranjpe & Sharma, 2005). A way in which an organism could predict events and better manage its needs, like storing food for the winter.

A temporal system wouldn't be of much use if it could not follow environmental changes. To those environmental signals that can regulate the biological rhythms are given the name zeitgeber (from the German Zeit, time, and Geber, giver). These zeitgebers produce inputs in our bodies that can shift and align those rhythms. This phenomenon is called entrainment (Roenneberg, Daan, & Merrow, 2003; Roenneberg et al., 2010).

The main zeitgeber known today is the light, particularly the sun's light (Khalsa et al., 2003; Minors et al., 1991; Roenneberg, Kumar, & Merrow, 2007). Considering its influence in entraining the biological temporal system, several studies hypothesize that the latitudinal shift of the sun, related to the earth's axis, would produce, on average, different temporal traits in populations that live close to the equator line when compared to populations that live close to the planet's poles (Horzum et al., 2015; Hut et al., 2013; Leocadio-Miguel et al., 2014, 2017; Pittendrigh et al., 1991; Randler & Rahafar, 2017). That is because the latter ones would have greater oscillations in sun activity and an overall weak solar zeitgeber. This is the latitude hypothesis, that can also appear as the environmental hypothesis of circadian rhythm regulation.

Recently there have been attempts to test the latitude hypothesis in different settings, but, at least in humans, none of them have been successful in seeing a significant effect size related to the latitudinal cline. Some of these approaches worked with secondary data and with small samples. One of the most serious attempts of testing this hypothesis was made by Leocadio-Miguel et al. (2017). They measured the chronotype of $12,884$ Brazillian subjects on a wide latitudinal spectrum using the Morningness–Eveningness Questionnaire (MEQ). Their results showed a negligible effect size. One possible reason for this is that the MEQ measures psychological traits and not biological states (Roenneberg, Pilz, et al., 2019), i.e., the circadian oscillation itself, therefore, it's not the best way to answer the question (Leocadio-Miguel et al., 2014).

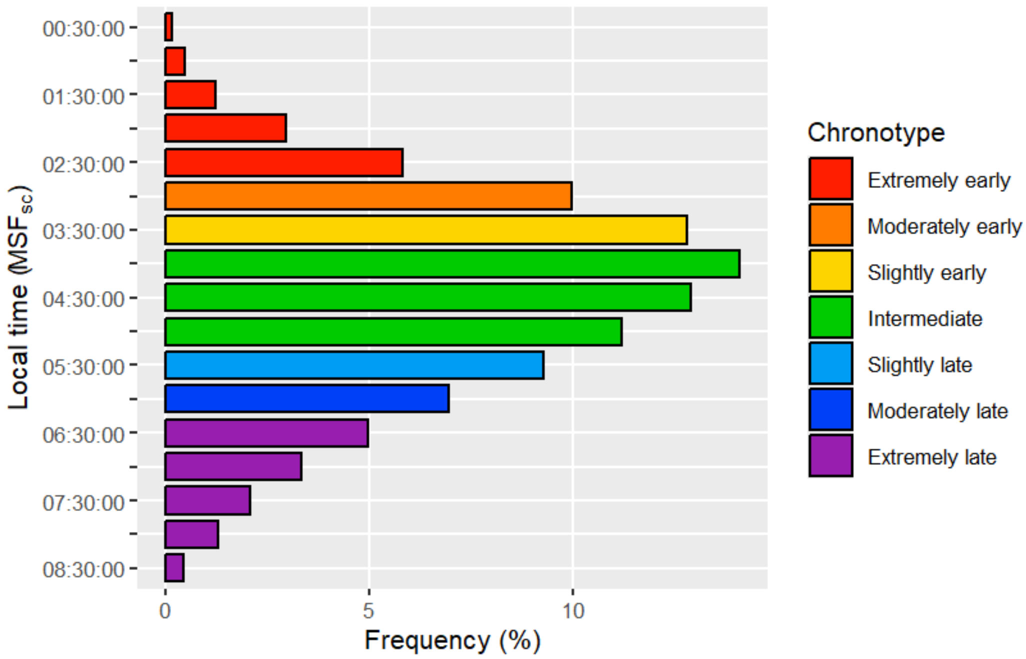
This article brings a novel attempt to test the latitude hypothesis, using, this time, a biological approach provided by the Munich ChronoType Questionnaire (MCTQ) (Roenneberg, Wirz-Justice, & Merrow, 2003). Furthermore, the test was carried out on the biggest chronotype sample ever collected in a same country. A sample made of $76,744$ subjects, all living in the same timezone in Brazil, with only one week of difference between questionnaire responses.

5.1.2 **Results**

The local time of the sleep corrected midpoint between sleep onset and sleep end on work-free days (MSF_{sc}), MCTQ proxy for measuring the chronotype, had an overall mean of 04:28:35. The distribution curve is shown in Figure 10.

That's the midsleep point of Brazilian subjects with an intermediate/average chronotype. One can imagine, following the 7-9h sleep recommendation for healthy adults of the American Academy of Sleep Medicine (AASM) (Watson et al., 2015), that this average person would, if he/she had no social obligations, typically wake up at about 08:28:35.

Figure 10 – Distribution of the local time of the sleep corrected midpoint between sleep onset and sleep end on work-free days (MSF_{sc}), MCTQ proxy for measuring the chronotype. The categorical cut-offs follow a quantile approach going from extremely early $(0| - 0.11)$ to the extremely late $(0.88 - 1)$.



Source: Created by the author. Based on data visualization found in Roenneberg, Wirz-Justice, et al. (2019).

The MSF_{sc} curve had a skewness of 0.284 and a kurtosis of 2.773. However, the distribution was not normal accordingly to Kolmogorov-Smirnov test (D $= 0.03717$; p-value $= 2e - 16$) and D'Agostino Skewness test (Z3 $= 31.525$; p-value $= 2.2e - 16$) (see D'Agostino & Belanger, 1990; also Thode, 2002, 46, p. 101).

A linear regression model was created with MSF_{sc} as the response variable and with age and sex as predictors ($\text{R}^2 = 0.05373$; $\text{F}(2, 76741) = 2180$, p-value $= 2e - 16$), the two most known predictors for chronotype (Roenneberg, Kumar, and Merrow (2007)). A Box-Cox transformation of the response variable was needed to attend to the linear regression model assumptions ($\lambda = -1.1111$; $\text{MSF}_{\text{sc}}^{\lambda-1}/\lambda$). All coefficients were significantly different than 0 (p-value $= 2e - 16$) and, accordingly to D'Agostino Skewness test, the residuals were normal (Z3 $= -1.1906$; p-value $= 0.23383$). Residual homoscedasticity was verified by a Score Test for Heteroskedasticity ($\chi^2 = 0.00$; p-value $= 1$). No collinearity was found between the predictor variables (variance inflation factor: age $= 1.0012$; sex $= 1.0012$).

Another model was created on top of the first one, adding the latitude as a predictor variable ($\text{R}^2 = 0.060698$; $\text{F}(3, 76740) = 1650$, p-value $= 2e-16$). All coefficients were significantly different than 0 (p-value $= 2e-16$) and the residuals were normally distributed accordingly to the D'Agostino Skewness test, (Z3 $= 0.0742$; p-value $= 0.94085$). Residual homoscedasticity was verified by a Score Test for Heteroskedasticity ($\chi^2 = 0.00$; p-value $= 1$). No collinearity was found between the predictor variables (variance inflation factor: age $= 1.0065$; sex $= 1.0016$; latitude $= 1.0056$). The longitude was not used as a predictor because it presented colinearity with the latitude variable.

An F test for nested models showed a significant reduction of the residual sum of squares ($\text{F}(1, 76740) = 568.94$, p-value $= 2e - 16$), meaning that the latitude seems to produce an effect on the chronotype. However, when estimating Cohen's f^2 effect size, the result was negligible (Cohen, 1992) $((0.06069 - 0.05373)/(1 - 0.06069) = 0.00740)$.

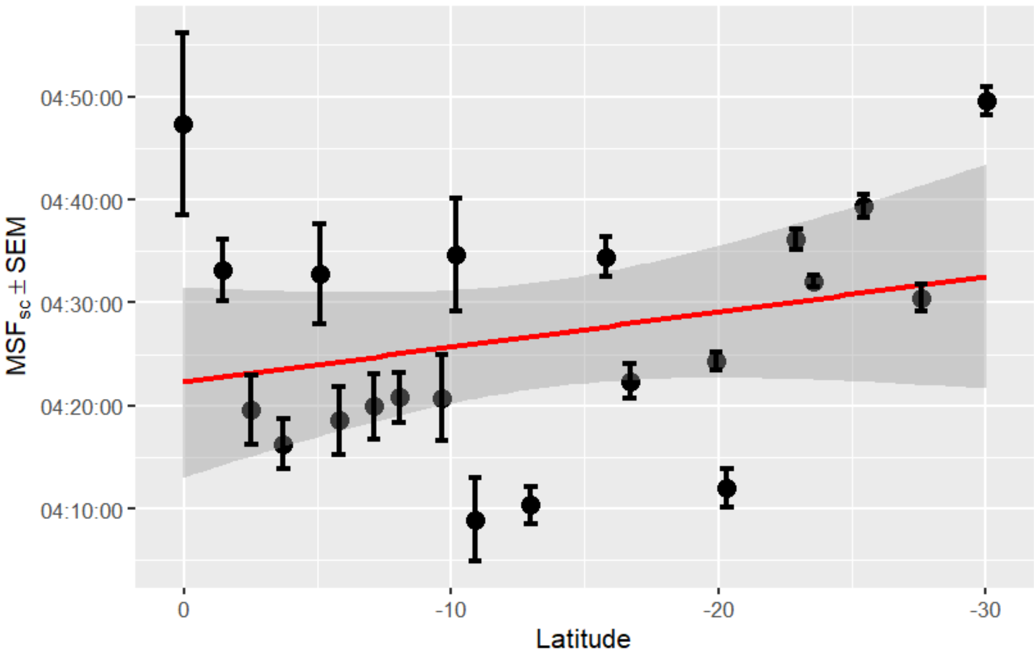
5.1.3 **Discussion**

It's important to note that a causal and linear relationship between latitude and chronotype is an *a priori* assumption. The objective of this study is to test or falsify this hypothesis.

For the latitude hypothesis to be true, there must be a significant association between these two variables when the most common covariates are controlled. Predictive models, in this case, are an adequate method for testing this.

The results show that even with a wide latitudinal spectrum and with a big and aligned sample of biological states the latitude effect does not reveal itself in a non-negligible size. Several studies indicate the existence of this effect on the chronotype (Hut et al., 2013; Leocadio-Miguel et al., 2017; Pittendrigh et al., 1991; Randler, 2008; Randler & Rahafar, 2017; Roenneberg, Wirz-Justice, & Merrow, 2003), but, at this time, at least in humans, no empirical evidence can support this claim. Our results are very similar to Leocadio-Miguel et al. (2017), which also found a negligible effect size (Cohen's $f^2 = 0.004143174$). The inconsistency of the latitude effect can be visualized in Figure 11.

Figure 11 – Distribution of mean aggregates of the local time of the sleep corrected midpoint between sleep onset and sleep end on work-free days (MSF_{sc}), MCTQ proxy for measuring the chronotype, in relation to latitude decimal degree intervals. Higher values of MSF_{sc} indicate a tendency toward a late chronotype. The red line represents a linear regression, and the shaded area indicates a pointwise 95% confidence interval. Attention is needed when interpreting this chart. Note that the y-axis is limited, and the difference between the highest and lowest values is negligible considering the MCTQ scale.



Source: Created by the author. Based on data visualization found in Leocadio-Miguel et al. (2017).

Despite the lack of evidence, is not uncommon to hear talks insisting that this effect is real and already confirmed. We suspect that this behavior may be derived from a lack of understanding of statistical models and techniques. Although it may be logical and aligned with the overall theory for the evolution of biological temporal systems, it's our role as scientists to eliminate contractions, not pursue them.

The absence of a strong entrainment with the solar zeitgeber shows that the entrainment phenomenon is more complex than we previously imagined. Other hypotheses for the human circadian entrainment, like the entrainment to self-selected light, proposed by Anna Skeldon and Derk-Jan Dijk (2021), need to be tested and may produce significant results. Methods and techiniques for complex systems, like causal loop diagrams and agent-based models (ABM), may help to unravel this phenomenon more properly.

It's important to notice that the results shown here are preliminary. The data still needs some cleaning and to be balanced with Brazil's latest population census. The latitude coordinates used in the analysis are related to subjects' residential state capital and, hence, have low resolution. Even with these results, it may be that a significant latitude effect can still appear at the end of the research.

Despite the several strengths that the dataset used in this study has, it is also important to notice its weaknesses and limitations. The fact that all the subjects were measured in the Spring season is one of them. Since the objective is to catch individuals in different seasonal patterns, the ideal moment to collect this kind of data is in the wintertime, when there is a greater insolation gradient between the equator and the poles. Another one is that this dataset can be influenced by the presence of a Daylight Saving Time (DST) event. This latter issue is explored in more detail in the methods section.

5.2 METHODS

5.2.1 **Measurement instrument**

Chronotypes were measured using the core version of the standard Munich ChronoType Questionnaire (MCTQ) (Roenneberg, Wirz-Justice, & Merrow, 2003). MCTQ is a widely validated and widely used self-report questionnaire for measuring the sleep-wake cycle and chronotypes (Roenneberg, Pilz, et al., 2019). It quantifies the chronotype as a state, a biological circadian phenotype, using as a proxy the local time of the sleep corrected midpoint between sleep onset and sleep end on work-free days (MSF_{sc}). A sleep correction (SC) is made when a possible sleep compensation related to a lack of sleep on workdays is identified (Roenneberg, 2012).

Subjects were asked to complete an online questionnaire based on the MCTQ Portuguese translation created by Till Roenneberg & Martha Merrow for the EUCLOCK project (Roenneberg & Merrow, 2006) (statements mean cosine distance $= 0.921$). They were also asked to provide sociodemographic (e.g., age, sex), geographic (e.g., full residential address), anthropometric (e.g., weight, height), and work/study routine-related data. A deactivated version of the questionnaire can be seen at https://bit.ly/brchrono-form.

5.2.2 Sample

The sample is made up of $76,744$ Brazilian subjects. It was obtained in 2017 from October 15th to 21st by a broadcast of the online questionnaire on a popular Sunday TV show with national reach (Rede Globo, 2017). This amount of data collected in such a short time gave the sample a population cross-sectional characteristic.

A survey conducted in 2019 by the Brazilian Institute of Geography and Statistics (IBGE) (2021) found that 82.17% of Brazilian households had access to an internet connection. Therefore, this sample is likely to have a good representation of Brazil's population. Only residents of Brazilian states in the UTC-3 timezone, aged 18 years or older, were included in the final sample.

In order to verify if the sample size was adequate for the study of the phenomenon under investigation, a power analysis was conducted for nested multiple regression models using the G*Power software (Faul et al., 2007). The analysis used the parameters presented in Leocadio-Miguel et al. (2017) article for a multiple linear regression with 10 tested predictors and only 10 conceived predictors, considering a significance level of 0.05 (α) and a power of 0.95 ($1 - \beta$). The result showed that a sample of $5,895$ individuals would be necessary to test the hypothesis.

Daylight Saving Time (DST) began in Brazil at midnight on October 15th, 2017. Residents from the Midwest, Southeast, and South regions were instructed to set the clock forward by 1 hour. We believe that this event did not contaminate the data since it started on the same day of the data collection. It's important to notice that MCTQ asks subjects to relate their routine behavior, not how they behaved in the last few days. A possible effect of the DST on the sample is the production of an even later chronotype for populations near the planet's poles, amplifying a possible latitude effect. However, this was not shown on the hypothesis test.

Based on the 2022 census (Instituto Brasileiro de Geografia e Estatística, n.d.-a), Brazil had 52.263% of females and 47.737% of males with an age equal to or greater than 18 years old. The sample is skewed for female subjects, with 66.297% of females and 33.703% of male subjects.

The subjects' mean age is 32.015 years (SD $= 9.252$; Max. $= 58.786$). Female subjects have a mean age of 31.787 years (SD $= 9.364$; Max. $= 58.786$) and male subjects 32.464 years (SD $= 9.012$; Max. $= 58.772$). For comparison, based on the 2022 census (Instituto Brasileiro de Geografia e Estatística, n.d.-b), Brazil's population with an age equal to or greater than 18 years old had a mean age of 44.277 years (SD $= 17.221$), with a mean age of 44.987 years (SD $= 17.511$) for female subjects and a mean age of 43.499 years (SD $= 16.864$) for male subjects.

Considering the five regions of Brazil, the sample is mostly skewed for the Southeast, the most populated region. According to Brazil's 2022 census (Instituto Brasileiro de Geografia e Estatística, 2022), the Southeast region is home to 41.784% of Brazil's population, followed by the Northeast (26.910%), South (14.741%), North (8.544%), and Midwest (8.021%) regions. 62.454% of the sample is located in the Southeast region, 11.797% in the Northeast, 17.861% in the South, 1.682% in the North, and 6.205% in the Midwest region. Note that a lack of subjects in the North and Midwest region is justified by the sample timezone inclusion criteria (UTC-3).

The sample latitudinal range was 30.211 decimal degrees (Min. $= -30.109$; Max. $= 0.10177$) with a longitudinal span of 16.378 decimal degrees (Min. $= -51.342$; Max. $= -34.964$). For comparison, Brazil has a latitudinal range of 39.024 decimal degrees (Min. $= -33.752$; Max. $= 5.2719$) and a longitudinal span of 39.198 decimal degrees (Min. $= -34.793$; Max. $= -73.991$).

The results shown in this article are just a preliminary view of the data analysis. The latitudes and longitudes of each subject are represented by the coordinates of his/her state's capital (a low resolution). The final results will have the latitude and longitude coordinates based on subjects' postal codes and will also use a balanced dataset following the latest Brazil census.

5.2.3 Data wrangling

The data wrangling and analysis followed the data science program proposed by Hadley Wickham and Garrett Grolemund (Wickham, 2016). All processes were made with the help of the R programming language (R Core Team, n.d.), RStudio IDE (Posit Team, n.d.), and several R packages. The tidyverse and rOpenSci package ecosystem and other R packages adherents of the tidy tools manifesto (Wickham & Bryan, 2023) were prioritized. The MCTQ data was analyzed using the `mctq` rOpenSci peer-reviewed package (Vartanian, 2023). All processes were made in order to provide result reproducibility and to be in accordance with the FAIR principles (Wilkinson et al., 2016).

5.2.4 Hypothesis testing

The study hypothesis was tested using nested models of multiple linear regressions. The main idea of nested models is to verify the effect of the inclusion of one or more predictors in the model variance explanation (i.e., the R^2) (Allen, 1997). This can be made by creating a restricted model and then comparing it with a full model. Hence, the hypothesis can be schematized as follows.

$$\begin{cases} \text{H}_0 : \Delta \text{ Adjusted } \text{R}^2 \leq \text{MES} \quad \text{or} \quad \text{F-test is not significant} \\ \text{H}_a : \Delta \text{ Adjusted } \text{R}^2 > \text{MES} \quad \text{and} \quad \text{F-test is significant} \end{cases}$$

In order to test a possible latitude association in predicting the chronotype, the full model was the restricted model with the addition of the latitude variable. The restricted model had the local time of the sleep corrected midpoint between sleep onset and sleep end on work-free days (MSF_{sc}) as the response variable, MCTQ proxy for the chronotype, with sex and age as predictors.

A residual analysis was made to ensure the validity of the models before the hypothesis test. The hypothesis was tested using a 0.05 (α) significance level.

To favor the alternative hypothesis (H_a), not only the R^2 of the full model must be significantly larger than the R^2 of the restricted model, but the effect size must be at least considered small. To evaluate the effect size, Cohen's f^2 and his categorical parameters for size were used (Cohen, 1992). That means that, in order to favor (H_a), the effect size must be at least equal to or greater than 0.0219.

5.2.5 **Data availability**

The data that support the findings of this study are available from the corresponding author [DV]. Restrictions apply to the availability of these data, which were used under the approval of a Research Ethics Committee (REC) linked to the Brazilian National Research Ethics Committee (CONEP), hence it cannot be publicly shared. Data are, however, available from the author upon reasonable request and with CONEP approval.

5.2.6 **Code availability**

The research compendium of the project is available under the MIT license at https://github.com/danielvartan/mastersthesis. The code has all the steps from the raw data to the test results.

5.3 ACKNOWLEDGMENTS

Financial support was provided by the Coordination for the Improvement of Higher Education Personnel (CAPES) (Grant number: 88887.703720/2022-00).

5.4 ETHICS DECLARATIONS

5.4.1 **Competing interests**

The author declares that the study was carried out without any commercial or financial connections that could be seen as a possible competing interest.

5.5 ADDITIONAL INFORMATION

This manuscript shows only preliminary results and should not be considered as a document ready for journal submission.

See the appendices section for supplementary information.

Correspondence can be sent to Daniel Vartanian (danvartan@gmail.com).

5.6 RIGHTS AND PERMISSIONS

6 CONCLUSION

> ❗ Important
>
> You are reading the work-in-progress of this thesis.
>
> This chapter is currently a dumping ground for ideas, and I don't recommend reading it.

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6.1 LIMITATIONS

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6.2 FUTURE PERSPECTIVES

Initially, the thesis project proposed a global modeling approach to the phenomenon. This approach was maintained due to the scope of a master's degree program. As can be seen from the results of this and other studies, no non-negligible effect regarding latitude and chronotype was identified. It is possible, however, that this phenomenon, if it exists, could be observed through a local approach using an agent-based model. This approach would allow the simulation of an environment where agents have varying light exposure and considering their endogenous rhythms and the phase-response curve of the circadian clock to light. The data from this thesis could be used to calibrate validate the model.

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