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**Is latitude associated with chronotype?**

São Paulo

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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

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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.

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*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 $65,824$ individuals from all regions of Brazil were analyzed, collected in 2017 based on the Munich ChronoType Questionnaire (MCTQ). The analysis, using nested linear regression models, revealed a negligible effect of latitude on the variation in chronotype expression (Cohen's $f^2 = 0.012137120$), 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 $65.824$ indivíduos de todas as regiões do Brasil, coletados em 2017 com base no Munich ChronoType Questionnaire (MCTQ). A análise, utilizando modelos de regressão linear aninhados, revelou um efeito negligenciável da latitude na variação da expressão dos cronotipos ($f^2$ de Cohen $= 0.012137120$), 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 imagina.



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

## CONTENTS

# 1 INTRODUCTION

There has been a long-standing debate in the chronobiology community regarding the relationship between latitude and circadian phenotypes (chronotypes) (Bohlen & Simpson, 1973; Leocadio-Miguel et al., 2017; Pittendrigh et al., 1991; Skeldon & Dijk, 2021; Zerbini et al., 2021), with many assuming that this association is well-established. The hypothesis is based on the varying amounts of solar radiation experienced by populations across different latitudes. Since light exposure serves as a primary zeitgeber — a periodic environmental cue that influences or regulates biological rhythms (Pittendrigh, 1960) — such variations, along with temperature differences, are thought to result in observable 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 living near the poles (Bohlen & Simpson, 1973; Hut et al., 2013; Leocadio-Miguel et al., 2017; Pittendrigh et al., 1991; Randler, 2008; Randler & Rahafar, 2017; Roenneberg, Wirz-Justice, & Merrow, 2003). The primary objective of this study is to model and test this hypothesis by critically examining whether a significant association exists between latitude and circadian phenotypes in the Brazilian population.

This study emerged from an insightful debate with my former supervisor, sparked by results published in 2017 in *Nature Scientific Reports* (Leocadio-Miguel et al., 2017). In this paper, the authors conclude, as the theory suggests, that there is a significant association between latitude and chronotype in the Brazilian population. However, the results were not as clear-cut as suggested, and the methodology used to test the hypothesis was not optimal. This thesis revisits the hypothesis using an improved statistical approach, aiming to provide a more accurate and reliable answer to the research question.

In the following chapters, the latitude hypothesis is explored using Popper's hypothetical-deductive method (Popper, 1979) and an enhanced 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 $65\,824$ 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 includes data on sleep habits and demographic characteristics from all of Brazil's states.

It is important to emphasize that this thesis does not aim to propose or discuss the mechanisms underlying the latitude-chronotype relationship. Instead, it focuses solely on the statistical association between them. If a cause-effect relationship exists, it must be preceded by, at the very least, an association — something this thesis aims to uncover.

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 does not have latitude as a predictor. This method of procedure builds on the method used in Leocadio-Miguel et al. (2017). The results will contribute to the ongoing debate on the latitude-chronotype relationship, offering new evidences into how environmental factors influence human circadian rhythms.

In accordance with the graduate program regulation, this thesis follows an article-based format, inspired by the structure of Reis (2020)'s PhD thesis. Chapters 2 through 4 consist of a series of essays and literature reviews related to the thesis topic, while Chapter 5 presents the core investigation, including an article detailing the hypothesis test and addressing the central research question. Finally, Chapter 6 offers conclusions, discusses limitations, and proposes directions for future research. Additionally, supplementary materials are provided to offer a richer, more comprehensive understanding of the research, and the reader is encouraged to explore them in detail.

All analyses in this thesis are fully reproducible and were conducted using the R programming language (R Core Team, n.d.) alongside the Quarto publishing system (Allaire et al., n.d.). Given the thesis's data-centric nature, it is best experienced online. To view the digital version visit https://danielvartan.github.io/mastersthesis.

The thesis code repository is available on GitHub at `{=latex} \\*` https://github.com/danielvart and 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

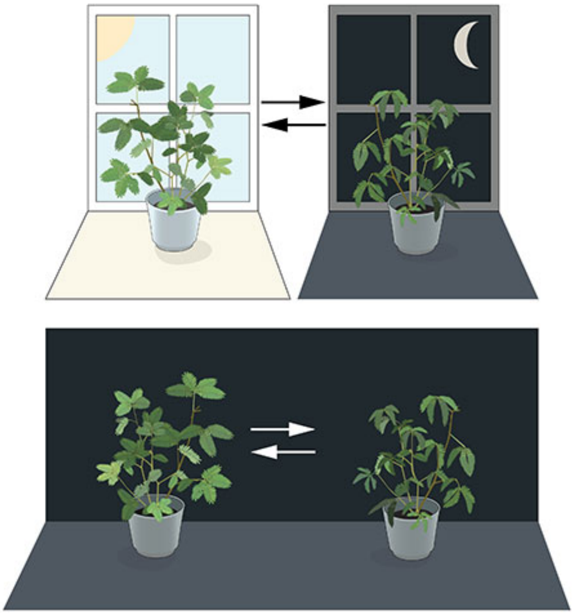
The dimension of time, manifest in the form of rhythms and cycles, like the alternating patterns of day and night as well as the annual transition of seasons, was consistently featured in the evolutionary journey of not only the human species but also all other life forms on our planet. These rhythms 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 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

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

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.

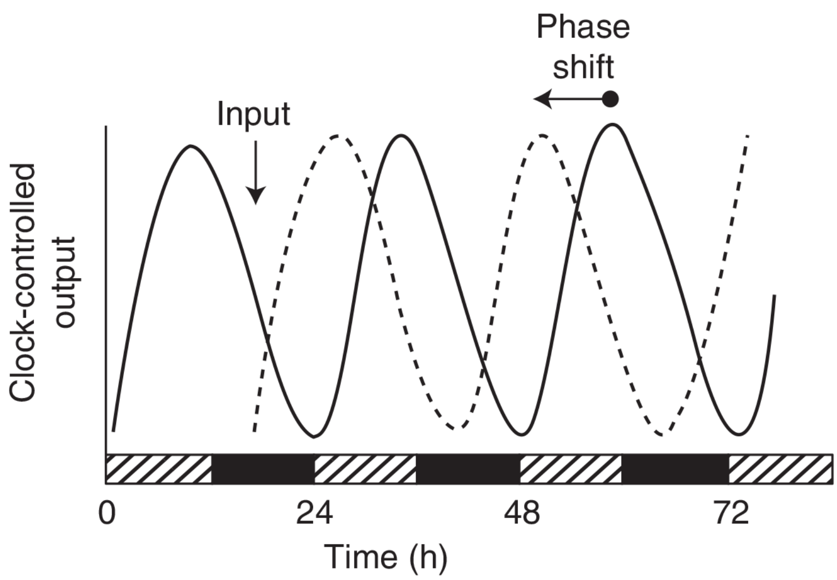


Source: Reproduction from Nobel Prize Outreach AB (n.d.).

Science has already showed and described various biological rhythms. 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., systems with a large number of connected parts that presents stable macroscopic patterns (emergences, in this case, the rhythms) arising from local interactions or the collective behavior of its parts, giving the system properties not attained by the aggregate summation (Epstein, 1999; Holland, 2014). It is understood today that the endogeneity of rhythms has provided organisms with an anticipatory capacity, 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 in nature 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 the rhythms (Khalsa et al., 2003; Kuhlman et al., 2018) 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 (or, simply, light exposure). 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.



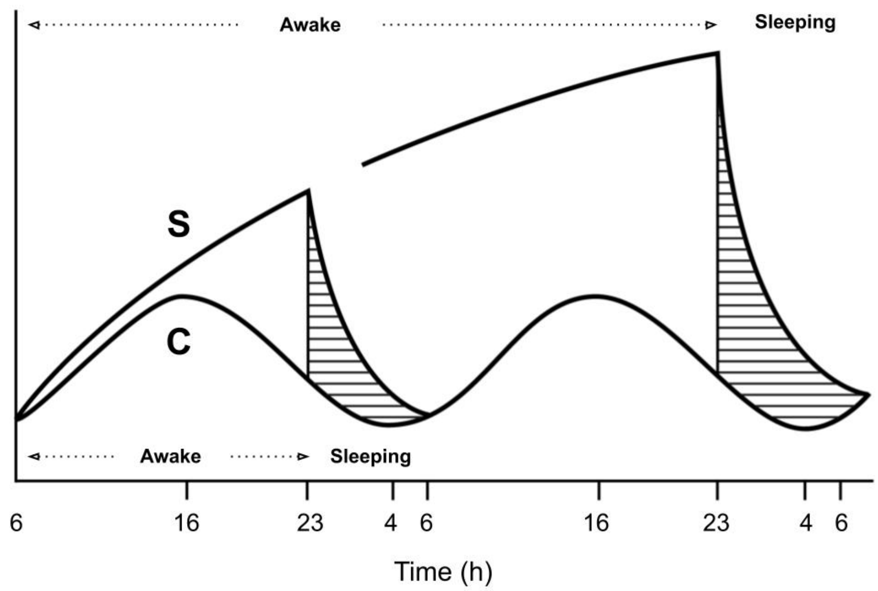

Source: Adapted from Kuhlman et al. (2018).

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.

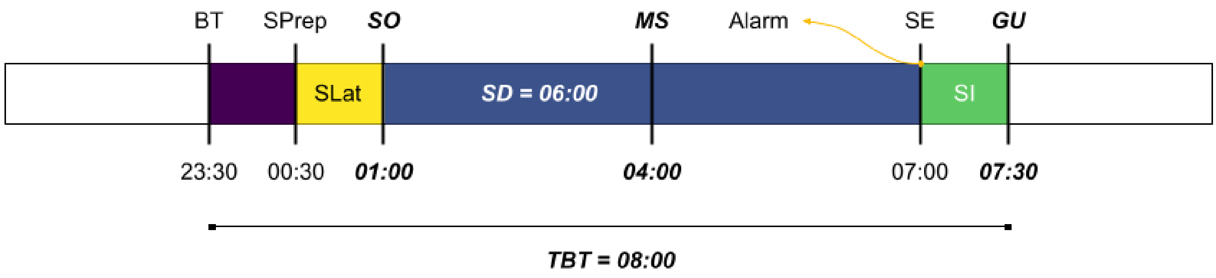
Figure 3 – Illustration of the interaction between Process S (sleep-dependent process) and Process C (circadian rhythm process) in sleep regulation. The figure depicts two scenarios: one with $17$ hours of wakefulness followed by $7$ hours of sleep; and another, with sleep deprivation, consisting of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




Source: Adapted from Borbély (1982).

Building on this idea, Roenneberg, Wirz-Justice, and Merrow (2003) developed the Munich Chronotype Questionnaire (MCTQ) to measure the circadian phenotype through sleep patterns. The MCTQ asks individuals about their sleep habits, such as the times they go to bed and wake up on workdays and work-free days. Based on this information, the MCTQ calculates the local time of the midpoint of sleep on work-free days Figure 4 and, if sleep deprivation is detected on workdays, adjusts the measurement accordingly. This midpoint, reflecting sleep without social obligations, is thought to represent the unabridged expression of the circadian rhythm. Given its basis in the two processes of sleep regulation, the MCTQ provides a good proxy for measuring the circadian phenotype (or C process) (Leocadio-Miguel et al., 2014).

Figure 4 – Variables of the Munich ChronoType Questionnaire scale (a sleep log). In its standard version, these variables are collected in the context of workdays and work-free days. BT = Local time of going to bed. SPrep = Local time of preparing to sleep. SLat = Sleep latency *or* time to fall asleep after preparing to sleep. SO = Local time of sleep onset. SD = Sleep duration. **MS** = Local time of mid-sleep. SE = Local time of sleep. Alarm = A logical value indicating if the respondent uses an alarm clock to wake up. SE = Local time of sleep end. SI = “Sleep inertia” (despite the name, this variable represents the time the respondent takes to get up after sleep end). GU = Local time of getting out of bed. TBT = Total time in bed.




Source: Create by the author.

For this thesis, the MCTQ serves as the instrument for measuring subjects’ chronotypes (circadian phenotypes). The study uses a dataset of $65\,824$ Brazilian respondents from an online survey conducted by the author in 2017, which includes geographical data such as postal codes. This data enables the examination of the potential association between chronotype and geographic factors, particularly latitude and longitude. The research ultimately seeks to determine whether latitude plays a role in shaping chronotype, contributing to our understanding of circadian rhythms in relation to geographic variables.

# 3 ON COMPLEXITY SCIENCE

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).

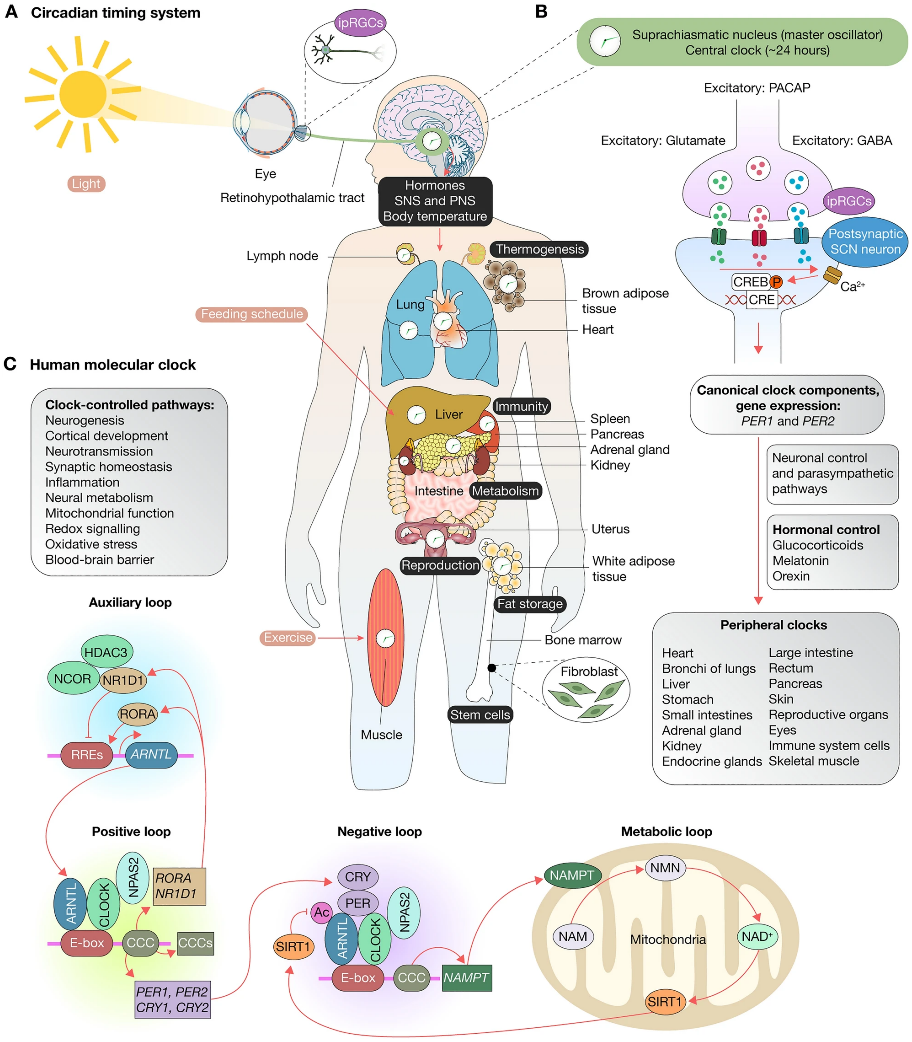
When the aggregate exhibits **properties not attained by summation** (Holland, 2014).

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 5 – 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.

"[…] 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).

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

Approaches to study and model circadian clocks.

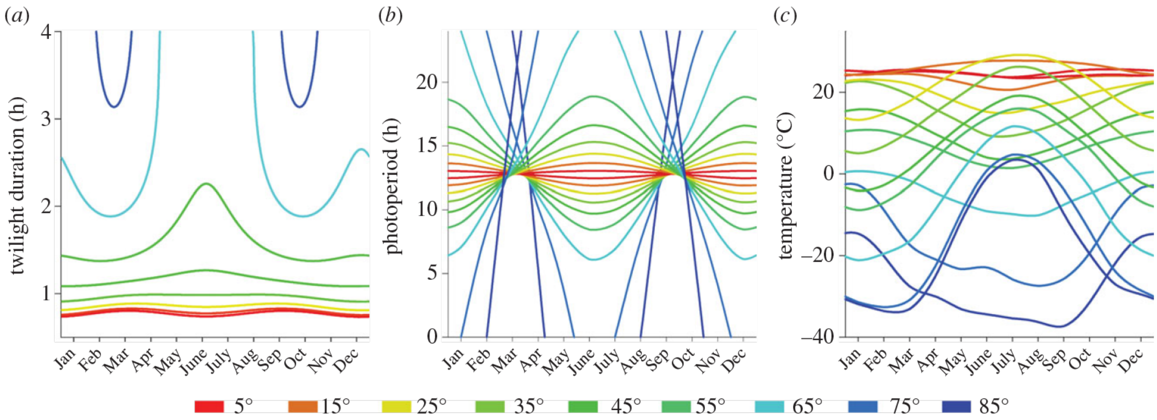
## 4 ON THE LATITUDE HYPOTHESIS

The first mention of this hypothesis in English scientific literature dates back to at least 1973, as noted by Bohlen and Simpson (1973), with earlier hints of the idea from Erhard Haus and Franz Halberg in 1970 (Haus & Halberg, 1970, p. 101), building on discussions initiated by Jürgen Aschoff (Aschoff, 1969). Since then, numerous studies have explored this topic, yielding somewhat conflicting results (a systematic review is provided by Randler and Rahafar (2017)).

The hypothesis, also called *environment hypothesis*, posits that regions closer to the poles receive, on average, less annual sunlight compared to regions near the equator Figure 6. Consequently, regions around latitude 0° are thought to have a stronger solar zeitgeber. According to chronobiological theories, this stronger zeitgeber would enhance the synchronization of circadian rhythms with the light-dark cycle, resulting in lower variability and amplitude of circadian phenotypes. This reduced influence of individual endogenous periods is illustrated in Figure 7.

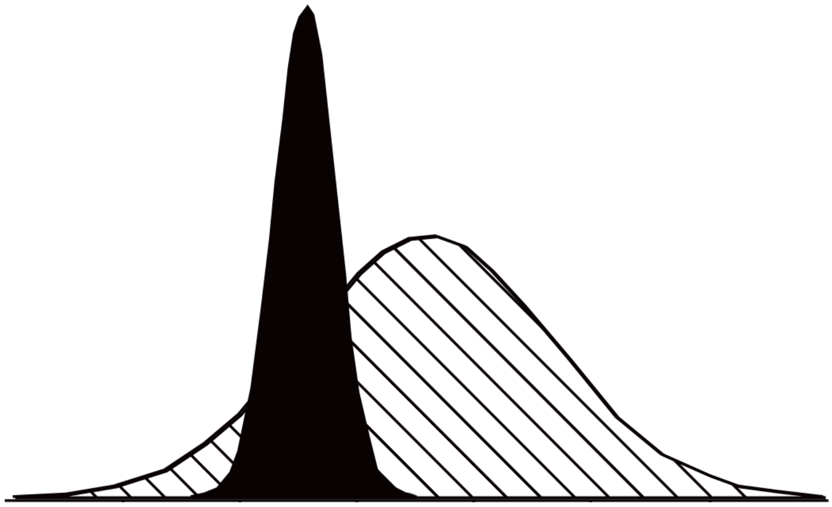
In contrast, populations near the poles experience a weaker solar zeitgeber, leading to greater variability and amplitude of circadian phenotypes. This disparity translates into differences in chronotype: equatorial populations tend to exhibit a morningness orientation, while populations at higher latitudes tend toward eveningness (Bohlen & Simpson, 1973; Roenneberg, Wirz-Justice, & Merrow, 2003).

Figure 6 – Annual changes in (a) twilight duration, (b) daylight hours, and (c) temperature across different latitudes. Each color shows a specific latitude, illustrating how these factors vary throughout the year.




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

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.

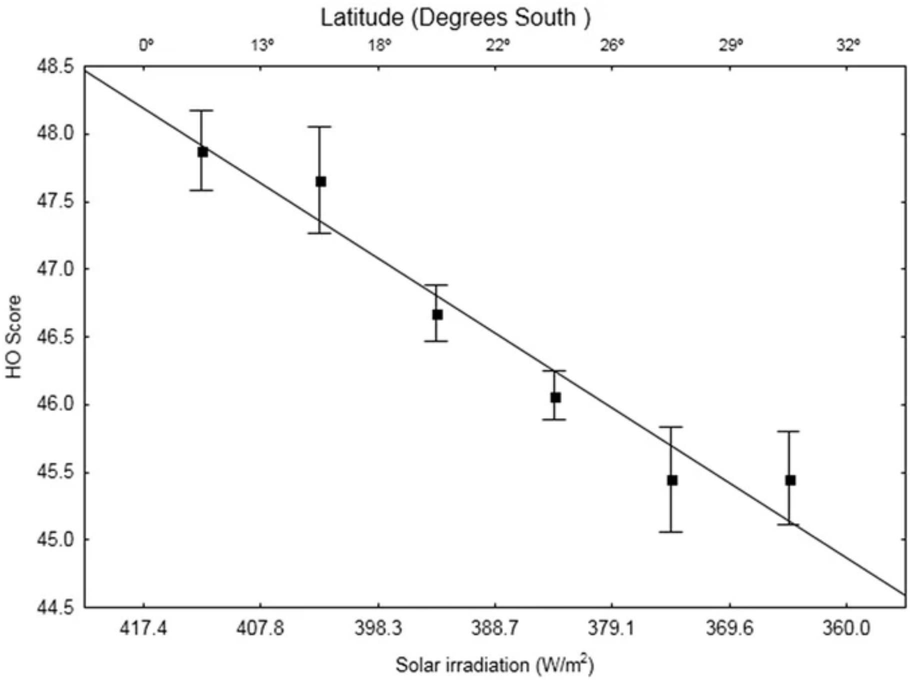


Source: Adapted from Roenneberg, Wirz-Justice, and Merrow (2003).

Some authors claim to found this association, but a closer look at the data reveals that it is not as clear as it seems. For example, Leocadio-Miguel et al. (2017) found a significant association between latitude and chronotype in a sample of $12,884$ Brazilian participants. However, the effect size was negligible, with latitude explaining only about $0.388\%$ of the variance in chronotype Figure 8. 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.

The findings of Leocadio-Miguel et al. (2017) are not consistent with the hypothesis that latitude is a strong predictor of chronotype, as the reported effect size is too small to be considered practically significant (Cohen, 1988). This highlights a common limitation of studies relying on Null Hypothesis Significance Testing (NHST) (Perezgonzalez, 2015). A p-value does not measure the effect size; instead, it represents the probability of observing the data (or something more extreme) assuming the null hypothesis is true, thus quantifying the likelihood of a type I error.

Figure 8 – The mean scores (±SE) on the Horne & Östberg (HO) chronotype scale (Horne & Östberg, 1976) are presented across a latitudinal gradient, along with the corresponding annual average solar irradiation levels (W/m²). The HO scale comprises 19 items, with total scores ranging from 16 to 86; lower scores indicate a stronger evening orientation, while higher scores reflect a greater morning orientation. Notably, the y-axis exaggerates the visual impact of the differences, as it represents a range of only about 4.5 points, which may overstate the perceived significance of the effect.




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

Several factors may invalidate this hypothesis, such as local clock time and social constraints (Skeldon & Dijk, 2021). To gain a more accurate understanding of the mechanisms underlying chronotype expression, it remains crucial to test this hypothesis in larger samples. This study aims to address that gap. In the following sections, the hypothesis will be tested using one of the largest chronotype datasets, to the author's knowledge, with geocoding information integrated for a comprehensive analysis. The approach will adhere to sound statistical principles, incorporating a minimum effect size in the alternative hypothesis, as originally proposed by Neyman and Pearson (Neyman & Pearson, 1928a, 1928b).

The following study was designed for publication in the journal *Scientific Reports* (IF 2023: 3.8/JCR | CAPES: A1/2017-2020) and structured in accordance with the journal's submission guidelines.

# 5 IS LATITUDE ASSOCIATED WITH CHRONOTYPE?

## 5.1 ABSTRACT

**Chronotypes are temporal phenotypes that reflect our internal temporal organization, a product of evolutionary pressures enabling organisms to anticipate events. These intrinsic rhythms are modulated by zeitgebers — environmental stimuli that entrain these biological oscillations, with light exposure being the primary mechanism. Given light's role in these systems, previous research hypothesized that latitude might significantly influence chronotypes, suggesting that populations near the equator would exhibit more morning-leaning characteristics due to more consistent light-dark cycles, while populations near the poles might display more evening-leaning tendencies with a potentially freer expression of intrinsic rhythms. To test this hypothesis, we analyzed chronotype data from a large sample of** $65\,824$ **subjects across diverse latitudes in Brazil. Our results revealed that latitude show only negligible effect sizes on chronotype, indicating that the entrainment phenomenon is far more complex than previously conceived. These findings challenge simplified environmental models of biological timing and underscore the need for more nuanced investigations into the mechanisms underlying temporal phenotypes, opening new avenues for understanding the intricate relationship between environmental cues and individual circadian rhythms.**

## 5.2 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).

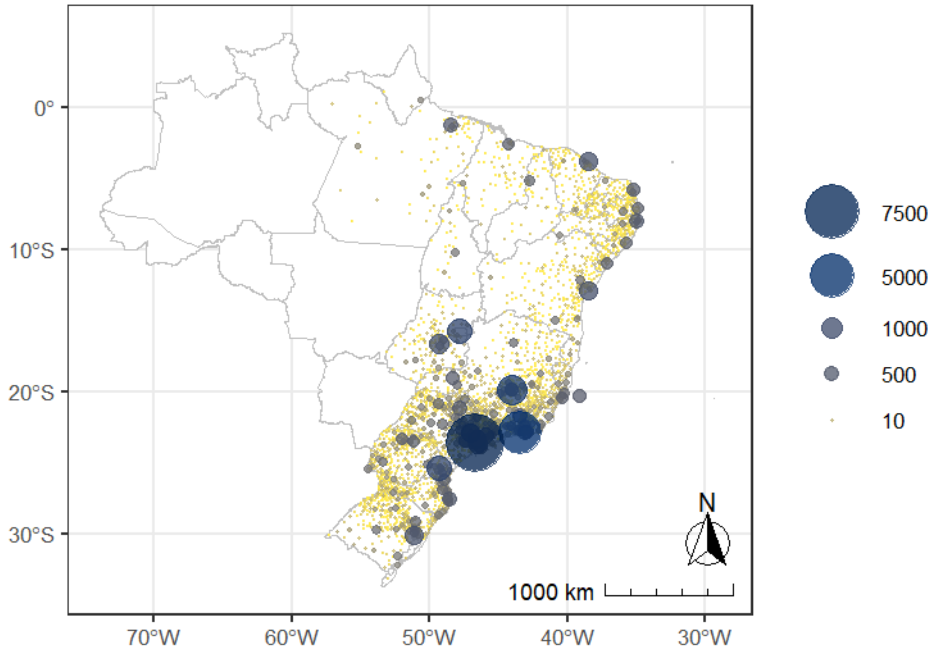
The main zeitgeber known today is the light, particularly the sun's light (Khalsa et al., 2003; Minors et al., 1991; Roenneberg, Kumar, & Merrow, 2007). Considering its influence in entraining the biological temporal system, several studies hypothesize that the latitudinal shift of the sun, related to 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 $65\,824$

subjects, all living in the same timezone in Brazil, with only one week of difference between questionnaire responses Figure 9. The unfiltered valid sample comprises $115\,166$ participants from all Brazilian states.

Figure 9 – Geographical distribution of the sample used in the analysis: ($n = 65\,824$). Each point represents a municipality, with its size proportional to the number of participants and its color indicating participant density. The sample includes Brazilian individuals aged 18 or older, residing in the UTC-3 timezone, who completed the survey between October 15 and 21, 2017. Size and color scale are in $\log_{10}$.



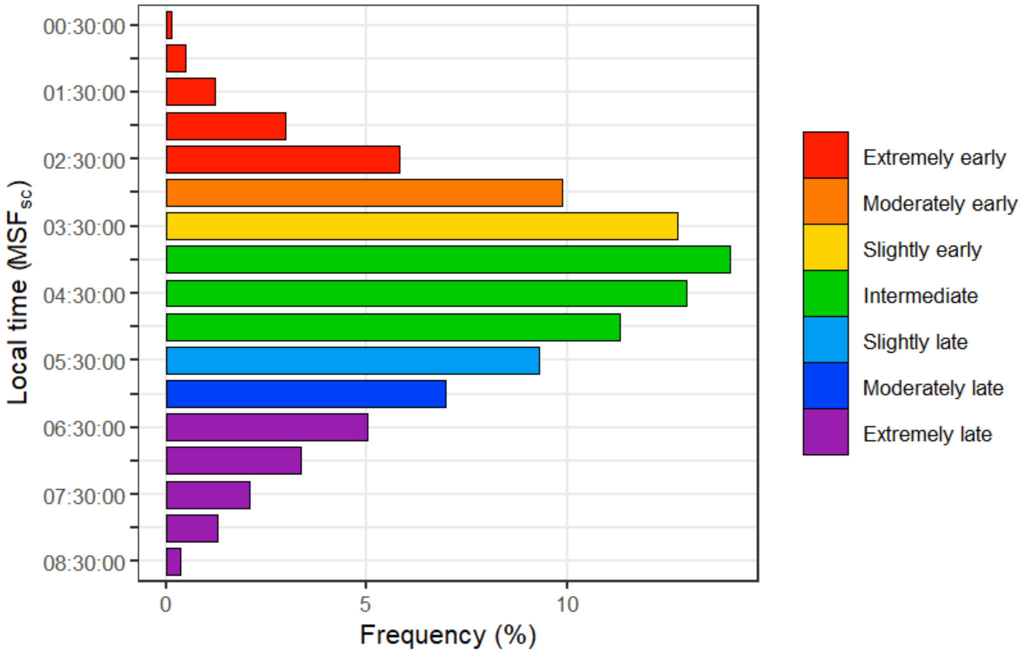

Source: Created by the author.

## 5.3 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 for the sleep-corrected midpoint between sleep onset and sleep end on work-free days ($MSF_{sc}$), a proxy for chronotype. Chronotypes are categorized into quantiles, ranging from extremely early ($0|-0.11$) to 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 ($R^2 = 0.05373$; $F(2, 76741) = 2180$, 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 ($\lambda = -1.1111$; $MSF_{sc}^{\lambda-1}/\lambda$). 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 ($R^2 = 0.060698$; $F(3, 76740) = 1650$, 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 (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) $((0.06069 - 0.05373)/(1 - 0.06069) = 0.00740)$.

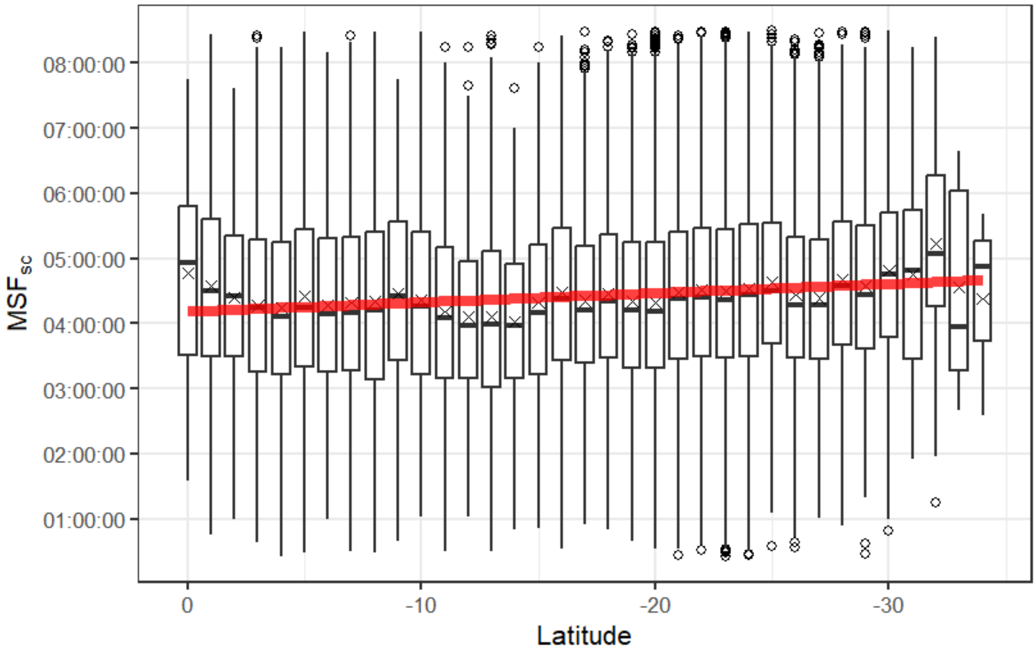
## 5.4 DISCUSSION

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 – Boxplots of mean $MSF_{sc}$ values aggregated by 1° latitude intervals, illustrating the relationship between latitude and chronotype. $MSF_{sc}$ represents the local time of the sleep-corrected midpoint between sleep onset and sleep end on work-free days, a proxy for chronotype. Higher $MSF_{sc}$ values indicate later chronotypes. The × symbol points to the mean. The red line represents a linear regression. The differences in mean/median values across latitudes are minimal relative to the Munich ChronoType Questionnaire (MCTQ) scale.

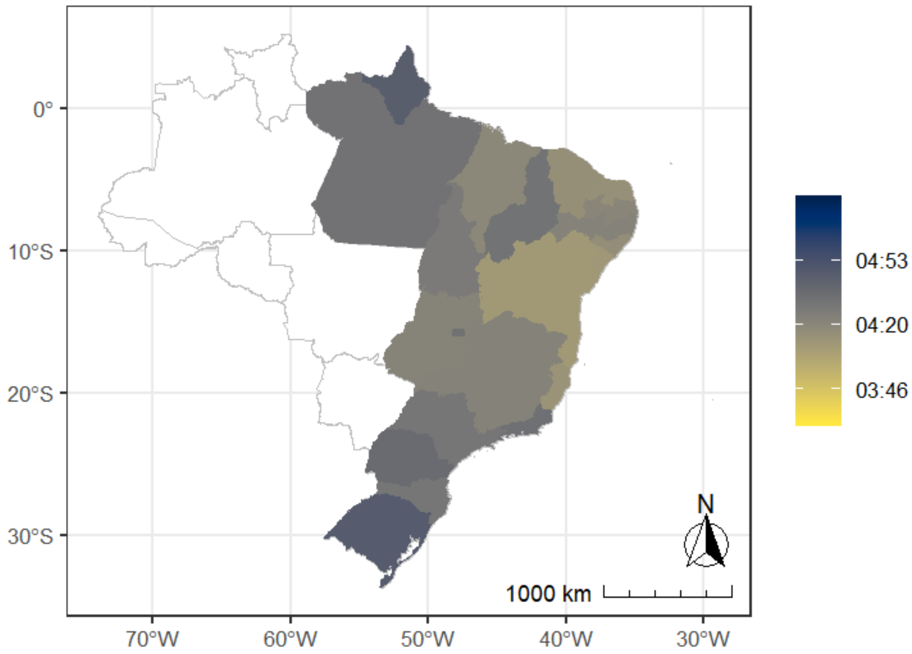



Source: Created by the author.

It's also important to point out that this seemly weak relationship shown in Figure 11 is of very similar magnitude from the one Leocadio-Miguel et al. (2017) found (see Leocadio-Miguel et al. (2017), Figure 2), with the difference that it isn't masked by a distorted y-axis — see the supplemental materials to view a resescaled version of the mentioned figure.

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.

Figure 12 – Geographical distribution of mean $MSF_{sc}$ values by Brazilian state. The figure shows the mean mid-sleep on free days sleep-corrected (MSF~sc) values aggregated by state, illustrating how chronotype varies with latitude in Brazil. $MSF_{sc}$ is a proxy for chronotype, representing the midpoint of sleep on work-free days, adjusted for sleep debt. Higher $MSF_{sc}$ values correspond to later chronotypes. The color scale was not transformed and it has as limits the first and third quartile (interquartile range). While the map suggests a potential latitudinal pattern, differences in mean $MSF_{sc}$ values across states are small and fall within a narrow range relative to the scale of the Munich ChronoType Questionnaire (MCTQ), limiting the significance of these variations.




Source: Created by the author.

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.

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.

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.

## 5.5 METHODS

### 5.5.1 **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.

### 5.5.2 **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 $0.05$ ($\alpha$) 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 of Brazil 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. $= -73.991$).

### 5.5.3 **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).

### 5.5.4 **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.

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

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 ($\text{MSF}_{\text{sc}}$) 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 $0.05$ ($\alpha$) significance level.

To favor the alternative hypothesis ($\text{H}_a$), not only the $\text{R}^2$ of the full model must be significantly larger than the $\text{R}^2$ 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 ($\text{H}_a$), the effect size must be at least equal to or greater than $0.0219$.

### 5.5.5 **Data availability**

Some restrictions apply to the availability of the main research data, which contain personal and sensitive information. As a result, this data cannot be publicly shared. Data are, however, available from the author upon reasonable request.

Unrestricted data can be access on the research compendium via The Open Science Framework at the following link: https://doi.org/10.17605/OSF.IO/YGKTS.

### 5.5.6 **Code availability**

All analyses are fully reproducible and were conducted using the R programming language alongside the Quarto publishing system. The `renv` package was used to ensure that the R environment used can be restored (see `renv.lock`).

The code repository is available on GitHub at https://github.com/danielvartan/mastersthesis), and the research compendium can be accessed via The Open Science Framework at the following link: https://doi.org/10.17605/OSF.IO/YGKTS

## 5.6 ACKNOWLEDGMENTS

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## 5.7 ETHICS DECLARATIONS

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

## 5.8 ADDITIONAL INFORMATION

See the supplementary material for more information.

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

## 5.9 RIGHTS AND PERMISSIONS

# 6 CONCLUSION

This study revealed, using the largest dataset on chronotype in a single time zone, as far as the existing literature suggests, balanced to reflect the population at the time of collection, that the latitude hypothesis is not supported by the data (Cohen's $f^2 = 0.012137120$). The findings align with those of Leocadio-Miguel et al. (2017), who reported a similar effect size (Cohen's $f^2 = 0.004143174$). However, the earlier study did not consider a minimum effect size criterion, leading to a misleading conclusion.

Several factors could explain the lack of an association between latitude and chronotype — or, as Jürgen Aschoff might have phrased it, the absence of "ecological significance" (Aschoff et al., 1972). For instance, if latitude does affect the circadian system, the effect may be too small to detect or could be overshadowed by other influences, such as social habits, work schedules, or the use of artificial light (Bohlen & Simpson, 1973). Additionally, the difference in solar exposure across latitudes may be insufficient to produce a meaningful effect on the circadian system, which is highly sensitive to light. Even minor variations in light exposure can yield significant physiological responses, suggesting that latitude alone may not be a strong determinant of chronotype.

These results suggest that the relationship between latitude and the circadian system is far more complex than anticipated. In human contexts, the perception of such an effect may have arisen from statistical misinterpretations, driven by ritualistic reliance on Null Hypothesis Significance Testing (NHST) and confirmation bias, rather than a critical evaluation of the data.

## 6.1 LIMITATIONS

While this study provides valuable insights, it is essential to acknowledge certain limitations that may influence the interpretation of the findings. First, the data collection occurred predominantly during a single week in spring, as summer approached, which limited the photoperiod variability between regions. A better approach would involve data collection across different seasons, particularly during winter, when photoperiod differences are more pronounced between equatorial and polar regions.

Additionally, the use of the Munich Chronotype Questionnaire (MCTQ), while a validated instrument, introduces the potential for recall and social desirability biases inherent to self-reported measures. However, the large sample size likely mitigates these biases, as predicted by the law of large numbers (DeGroot & Schervish, 2012, p. 352). Furthermore, at the time of data collection, the MCTQ had not yet been officially validated in Portuguese (this was only introduced in 2020 by Reis et al. (2020)), which may have introduced minor inconsistencies, though its nature as a sleep log suggests this impact was minimal.

Another factor to consider is the timing of data collection relative to the start of Daylight Saving Time (DST) in Brazil. On the day data collection commenced (October 15th, 2017 – $80.15$ of the data were collected on this day), a significant portion of respondents had just adjusted their clocks forward by one hour. While this could theoretically influence their responses, the questions were specifically designed to capture daily routines, which were not affected by the DST adjustment at that moment. Furthermore, any potential effect of DST would likely strengthen the latitude hypothesis; however, this was not supported by the data.

These limitations, while noteworthy, do not undermine the study's findings but rather highlight areas for refinement in future research.

## 6.2 DIRECTIONS FOR FUTURE RESEARCH

This thesis proposed using a global modeling approach to investigate the latitude-chronotype relationship. However, as demonstrated by the results of this study and others, no significant effect of latitude on chronotype was identified. That said, it remains possible that if such a phenomenon exists, it could be captured through a localized approach, such as agent-based modeling. This approach would simulate an environment where agents are exposed to varying light levels, while accounting for their endogenous rhythms and the circadian clock's phase-response curve to light. The data from this thesis could serve to calibrate and validate this model.

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