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Daniel Kachvartanian de Azevedo

Is latitude associated with chronotype?

São Paulo
2024

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Corrected 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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Examination committee

Committee chair:

Prof. Dr.	Camilo Rodrigues Neto
Institution	School of Arts, Sciences, and Humanities, University of São Paulo

Examiners:

Prof. Dr.	Tiago Gomes de Andrade
Institution	Faculty of Medicine, Federal University of Alagoas
Evaluation	Approved

Prof. Dr.	Domingos Alves
Institution	Ribeirão Preto Medical School, University of São Paulo
Evaluation	Approved

Prof. Dr.	Marcelo de Souza Lauretto
Institution	School of Arts, Sciences, and Humanities, University of São Paulo
Evaluation	Approved

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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].

Although significant progress has been made in understanding circadian rhythms, further research with larger and more diverse samples is needed to deepen our understanding of temporal phenotypes and their variability. This thesis examines the relationship between latitude and human chronotype expression, investigating whether variations in annual sunlight exposure between equatorial and non-equatorial regions influence circadian phenotypes. The underlying premise suggests that a stronger solar zeitgeber near the equator should promote greater entrainment to the light/dark cycle, potentially reducing phenotype diversity and favoring morningness in equatorial populations. To test this hypothesis, data from 65.824 individuals distributed across a $33.85026°$ latitute range in Brazil were analyzed. Data collection employed the Munich ChronoType Questionnaire (MCTQ) during a single spring week (October 15–21, 2017), minimizing seasonal variations in photoperiod across regions. The analysis employed nested regression models weighted according to population proportions at the time of data collection. Contrary to expectations, results revealed no meaningful relationship between latitude and chronotype (Cohen's $f^2 = 0.0030818242, 95\%$ CI$[0, 0.0121371208]$), consistent with recent findings in the field. All analytical procedures, from raw data processing through effect size estimation, were conducted using reproducible methods. These findings contribute to our evidence-based understanding of circadian rhythm regulation while challenging established assumptions in chronobiology research. While this study does not refute the hypothesis outright, the association between latitude and chronotype should remain an open scientific question rather than settled knowledge until robust evidence confirms it.

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].

Embora avanços significativos tenham sido feitos na compreensão dos ritmos circadianos, pesquisas adicionais com amostras maiores e mais diversas são necessárias para aprofundar o entendimento sobre os fenótipos temporais e sua variabilidade. Esta dissertação examina a relação entre latitude e a expressão do cronotipo humano, investigando se variações na exposição anual à luz solar entre regiões equatoriais e não equatoriais influenciam os fenótipos circadianos. A premissa subjacente sugere que um *zeitgeber* solar mais forte ao equador promove uma maior *entrainment* com o ciclo claro/escuro, potencialmente reduzindo a diversidade fenotípica e favorecendo a matutinidade em populações equatoriais. Para testar essa hipótese, foram analisados dados de 65.824 indivíduos distribuídos ao longo de um intervalo latitudinal de $33,85026°$ no Brasil. A coleta de dados foi realizada com o Munich ChronoType Questionnaire (MCTQ) durante uma única semana de primavera (15–21 de outubro de 2017), minimizando variações sazonais no fotoperíodo entre as regiões. A análise empregou modelos de regressão aninhados ponderados de acordo com as proporções populacionais no momento da coleta. Contrariando as expectativas, os resultados não indicaram uma relação significativa entre latitude e cronotipo (f^2 de Cohen $= 0,0030818242$, 95% IC$[0; 0,0121371208]$), em consonância com achados recentes da área. Todos os procedimentos analíticos, desde os dados brutos até a estimativa do tamanho do efeito, foram conduzidos por meio de métodos totalmente reprodutíveis. Esses achados contribuem para uma compreensão baseada em evidências da regulação dos ritmos circadianos, ao mesmo tempo que desafiam pressupostos estabelecidos na pesquisa em cronobiologia. Ainda que este estudo não refute completamente a hipótese, a associação entre latitude e cronotipo deve permanecer uma questão científica em aberto, em vez de ser considerada um conhecimento consolidado, até que evidências robustas a confirmem.

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 human circadian phenotypes (chronotypes) (e.g., Bohlen & Simpson, 1973; Randler, 2008; Leocadio-Miguel et al., 2017; Wang et al., 2023), 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 (Aschoff, 1960; 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; Horzum et al., 2015; Leocadio-Miguel et al., 2014, 2017; Randler, 2008). The primary objective of this study is to model and test this hypothesis by critically examining whether a meaningful 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 the journal *Scientific Reports* (Leocadio-Miguel et al., 2017). In this paper, the authors conclude that there is a meaningful association between latitude and chronotype in the Brazilian population, consistent with theoretical predictions. However, the results were not as clear-cut as presented, 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 tested using Popper's hypothetical-deductive method (Popper, 1972/1979) 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 involves a series of analyses conducted on a large dataset of $65,824$ individuals, collected from the Brazilian population in 2017. The dataset is based on the Munich Chronotype Questionnaire (MCTQ) (Roenneberg et al., 2003, 2012), and includes data on sleep habits and geeographical 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 concerning only human populations. An association is a necessary precursor to any causal relationship—and this thesis aims to determine whether such an association exists.

The analyses utilized nested multiple regression models to assess the variance explained by latitude in predicting chronotype. 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 evidence on the influence of environmental factors on 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, 3, and 4 consist of essays and literature reviews related to the thesis topic that provide essential background for understanding the research. Chapter 5 presents the core investigation, including an article detailing the hypothesis test and addressing the 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. 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 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

2 ON CHRONOBIOLOGY

The dimension of time, manifest in the form of rhythms and cycles, such as the alternation of day and night and the annual transition of seasons, has consistently influenced the evolutionary trajectory of humans and all other life forms on our planet. These rhythms and cycles brought with them evolutionary pressures, resulting in the development of a temporal organization enabling organisms to survive and reproduce in response to the conditions imposed within their environments (Aschoff, 1989a; Paranjpe & Sharma, 2005; Pittendrigh, 1981, 1993). 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 crepuscular or nocturnal behavior of cats and certain rodents (Aschoff, 1989b; Kronfeld-Schor et al., 2017).

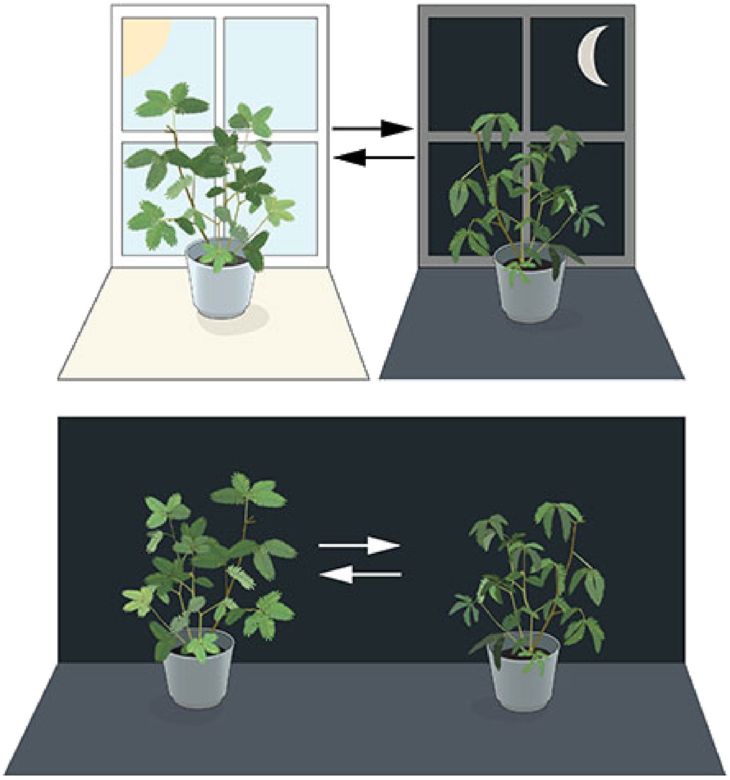
For years, scientists debated whether this organization was solely in response to environmental stimuli or if it was also present endogenously, internally, within organisms (Shackelford, 2022). One of the seminal studies describing a potential endogenous rhythmicity in living beings was conducted in 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) (Mairan, 1729; Shackelford, 2022). 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 (Cold Spring Harbor Laboratory, n.d.; Shackelford, 2022)[1,2]. 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

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

[2] From the Greek *chrónos*, meaning time/duration, and *biology*, pertaining to the study of life (Merriam-Webster, n.d.).

Physiology or Medicine for their discoveries of molecular mechanisms that regulate the circadian rhythm[3] in fruit flies (Nobel Prize Outreach AB, n.d.).

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



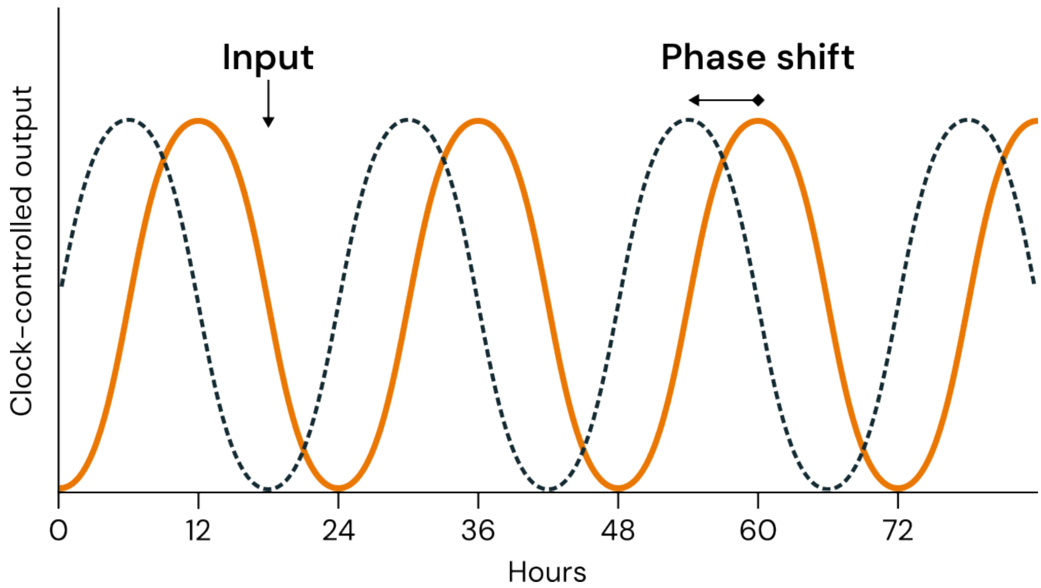
Source: Reproduced from Nobel Prize Outreach AB (n.d.).

Various biological rhythms have already been shown and described by science. These rhythms can occur at different description levels, whether at a higher level, such as the menstrual cycle (Ecochard et al., 2024), or even at a lower level, such as rhythms expressed within cells (Buhr & Takahashi, 2013; Sartor et al., 2023). Like many other biological phenomena, these are emergent properties of complex systems found in all living beings—stable macroscopic patterns arising from the collective behavior of the system's parts, resulting in properties not attainable by the aggregate summation (Epstein, 1999; Holland, 2014). Today, it is understood that endogenous rhythms provide organisms with an anticipatory capacity, enabling them to pre-emptively organize resources and activities (Aschoff, 1989b).

[3] From the Latin *circā*, meaning around, and *dĭes*, meaning day (Latinitium, n.d.)—a rhythm with an approximately 24-hour period.

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[4]. These zeitgebers act as synchronizers by entraining the phases of the rhythms (Khalsa et al., 2003; Minors et al., 1991) (Figure 2). Among the known zeitgebers are, for example, meal timing (Flanagan et al., 2021) and changes in environmental temperature. However, the most influential of them is the light/dark cycle (or, simply, light exposure) (Aschoff, 1960; Pittendrigh, 1960; Roenneberg & Merrow, 2016). 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 (Aschoff, 1989b; Roenneberg, Kuehnle, et al., 2007).

Figure 2 – Illustration of a circadian rhythm entrained (Phase-advanced, indicated by a leftward shift) by a zeitgeber (Input).



Source: Adapted by the author from Kuhlman et al. (2018, Figure 2B).

The expression of this temporal organization varies among organisms, even within the same species (Duffy et al., 2011; Silvério et al., 2024). These variations can be attributed to differences in how organisms experience their environment or to differences in their endogenous rhythmicity, a characteristic ultimately influenced

[4] From the German *zeit*, meaning time, and *geber*, meaning donor (Cambridge University Press, n.d.).

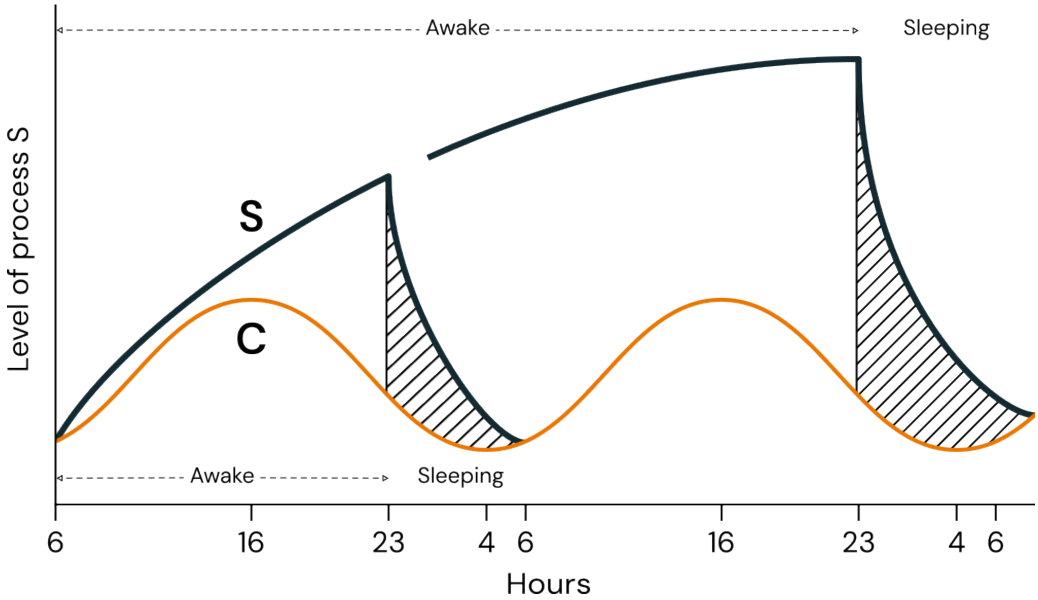
by gene expression (Roenneberg, Kumar, & Merrow, 2007). The interplay between environmental influences and genetic predisposition results in an observable characteristic: the phenotype (Frommlet et al., 2016).

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 suggests, 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 et al., 2003). This is because sleep is understood to result from the interaction of two processes: a homeostatic process (The S process), which is sleep-dependent and accumulates with sleep deprivation, and a circadian process (The C process), whose expression can be influenced by zeitgebers such as the light/dark cycle (Borbély, 1982; Borbély et al., 2016). These two processes are illustrated in Figure 3. Because the circadian rhythm is a component of sleep, its characteristics can be inferred by isolating its effects from those of the S process.

Figure 3 – Illustration of the interaction between Process S (Homeostatic/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, under sleep deprivation, with 41 hours of wakefulness followed by 7 hours of sleep. The hatched areas indicate periods of sleep, illustrating the exponential decline of Process S.

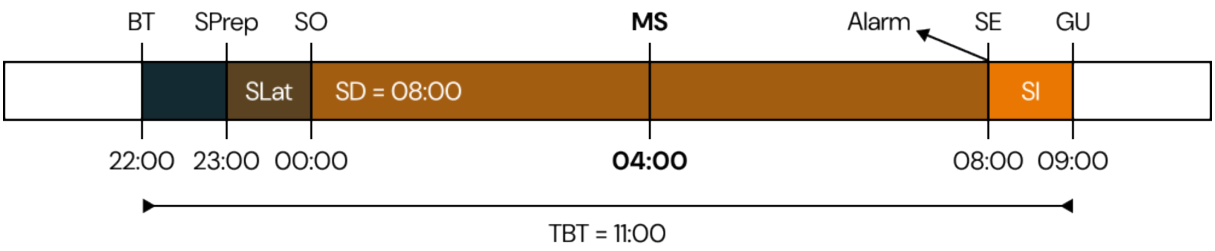


Source: Adapted by the author from Borbély (1982, Figure 4).

Building on this idea, Roenneberg et al. (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. From this information, the MCTQ derives the midpoint of sleep on work-free days, representing the average of sleep onset and offset times (Figure 4). If sleep deprivation is detected on workdays, the scale adjusts the measurement accordingly. This midpoint, reflecting sleep under minimal social constraints, is considered a closer approximation of the intrinsic circadian rhythm and, therefore, a useful proxy for estimating the circadian phenotype (the C process) (Leocadio-Miguel et al., 2014).

Figure 4 – Variables measured by the Munich Chronotype Questionnaire (MCTQ). 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 (Duration. 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 end. Alarm = Indicates whether the respondent uses an alarm clock. SI = "Sleep inertia" (Duration. 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: Created by the author.

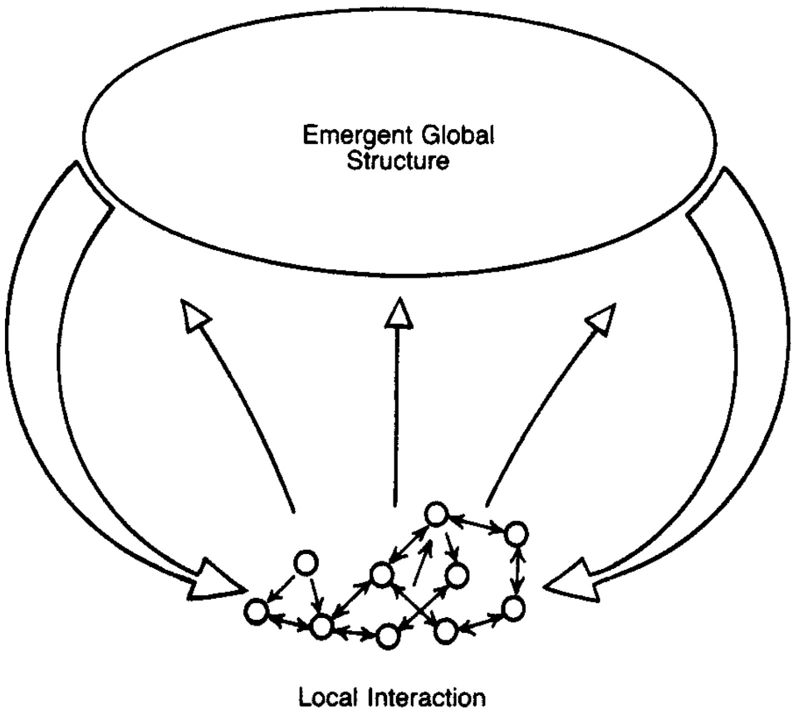
The MCTQ facilitates the evaluation of chronotype in population studies. This thesis employs the MCTQ to assess chronotype using data from a 2017 online survey conducted by the author, which includes responses from $65,824$ Brazilians and geographical information such as postal codes. This dataset enables the investigation of potential associations between chronotype and geographic factors.

3 ON COMPLEXITY SCIENCE

Complexity science is the science dedicated to understanding emergent phenomena (Krakauer & Wolpert, 2024). Like computer science and chronobiology, it began to take shape in the second half of the 20th century[1], by the convergence of several fields, such as systems theory, game theory, and nonlinear dynamics (Sayama, 2015).

At a fundamental level, emergence can be defined as stable macroscopic patterns arising from local interactions (Epstein, 1999). These patterns emerge from the collective actions of a system's parts, which cannot be attained by simply summing them up (Holland, 2014) (Figure 5). They may give rise to new properties in a system, which can only be studied by observing the interactions within it.

Figure 5 – An illustration of the reciprocal action between an emergent phenomenon derived by local interactions.



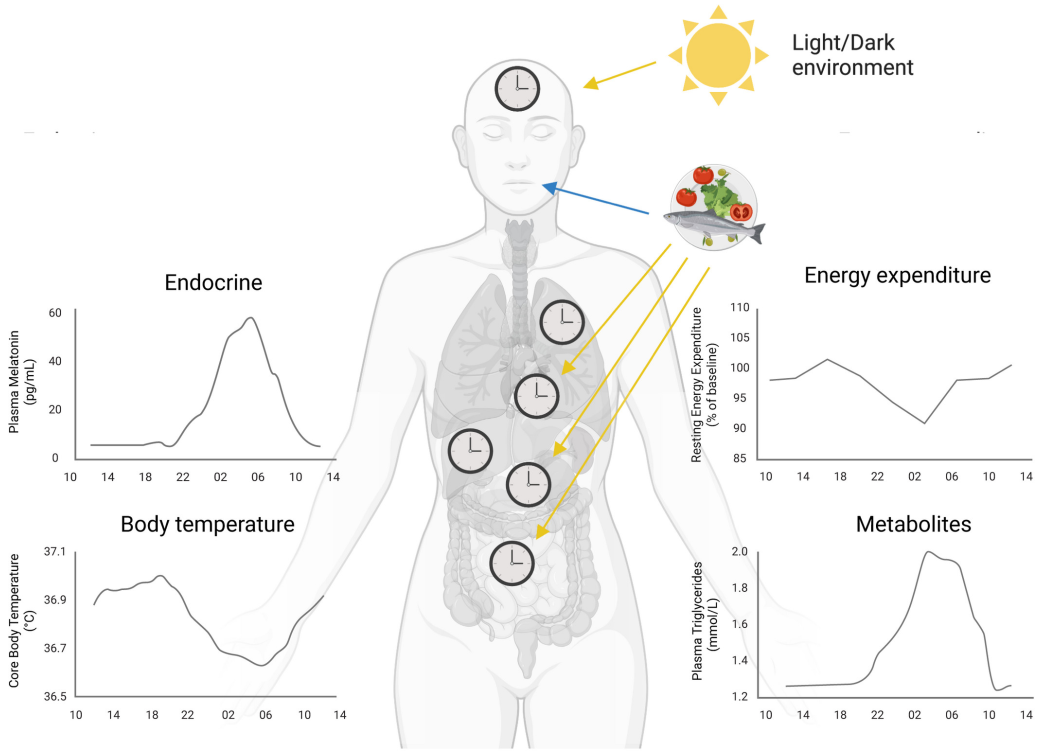
Source: Reproduced from Lewin (1993, Figure 1).

[1] Brian Castellani & Lasse Gerrits created a visual map to illustrate the different fields and components of complexity science. You can find it at https://www.art-sciencefactory.com/complexity-map_feb09.html.

Systems that exhibit emergent properties are considered complex systems (Holland, 2014; Mitchell, 2009). In a general sense, a system can be defined as a set of interacting parts that, through their interactions, produce a global behavior (von Bertalanffy, 1968). While both complicated and complex systems consist of many interacting parts, the defining characteristic of complex systems is that they cannot be fully understood by analyzing their components in isolation (Holland, 1992). This distinction poses significant challenges, as traditional methods for studying systems are often inadequate for capturing the intricate dynamics of complex systems (Holland, 2006).

Biological rhythms are an example of emergent properties produced by a complex system with multiple levels of interaction (Partch et al., 2014). Molecular oscillations are generated at the cellular level (Buhr & Takahashi, 2013; Merrow et al., 2005). These oscillations interact and couple with one another, forming a complex circadian network that coordinates rhythmic physiology and behavior (Foster, 2020; Raj & van Oudenaarden, 2008). Although science has not fully mapped all the pathways, it is understood that in this kaleidoscopic array of simultaneous interactions, a global rhythm emerges. Each rhythm, or clock, is itself an emergent phenomenon, interacting with others to produce a global behavior (Figure 6). As the parts generate these emergences, the emergent feedback to the parts, regulating and modulating functions at all levels (Roenneberg, Kuehnle, et al., 2007).

Figure 6 – An illustration depicting how the human circadian clock system regulate multiple aspects of metabolic physiology, such as: hormone secretion, core body temperature, resting metabolic rate, and plasma metabolite concentration.



Source: Reproduced from Flanagan et al. (2021, Figure 2).

The entrainment of these rhythms with environmental periodicities can involve different mechanisms. For the light/dark cycle, the main zeitgeber, this involves a network of photosensitive retinal ganglion cells (pRGCs) that send signals to the suprachiasmatic nucleus (SCN) in the hypothalamus (Brainard et al., 2001; Thapan et al., 2001). The SCN then sends signals to the pineal gland, which produces melatonin, a hormone that regulates sleep-wake cycles, among other functions (Foster, 2021).

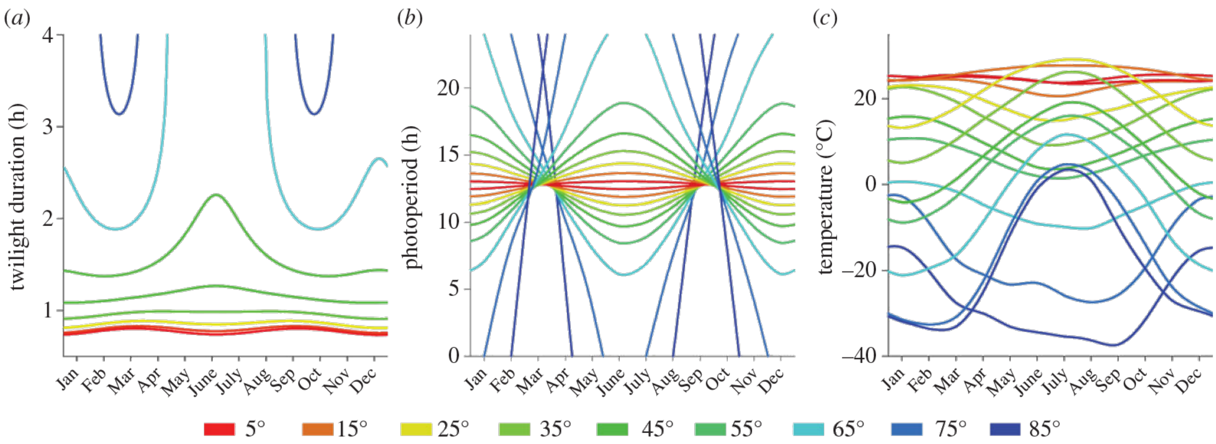
To model this phenomenon, one must understand how complex systems behave and can be studied. This thesis adopts a global approach to understanding the effect of light/dark cycle entrainment on circadian expressions of populations, considering potential interactions for proper system control. Given the thesis's aim to test the latitude hypothesis, a global approach is appropriate. Alternatively, a local approach could explore entrainment in populations by modeling individuals, each with their own circadian clock, and their interactions with the environment.

4 ON THE LATITUDE HYPOTHESIS

The first mention of this hypothesis regarding human populations dates back to at least 1973 (Bohlen & Simpson, 1973), with earlier hints of the idea coming 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[1].

The hypothesis, also called the environment hypothesis (Horzum et al., 2015), posits that regions closer to the poles receive, on average, less annual sunlight compared to regions near the equator (Figure 7). Consequently, regions around latitude $0°$ are thought to have a stronger solar zeitgeber. According to chronobiological theories, this stronger zeitgeber would enhance the entrainment of circadian rhythms with the light/dark cycle, resulting in lower variability of circadian phenotypes (Aschoff, 1960; Pittendrigh, 1960 Aschoff, 1981; Pittendrigh & Takamura, 1989; Pittendrigh et al., 1991). This reduced influence of individual endogenous periods is illustrated in Figure 8.

Figure 7 – Annual variations in (a) Twilight duration, (b) Photoperiod, and (c) Temperature across different latitudes. Each color represents a specific latitude.



Source: Reproduced from Hut et al. (2013, Figure 1).

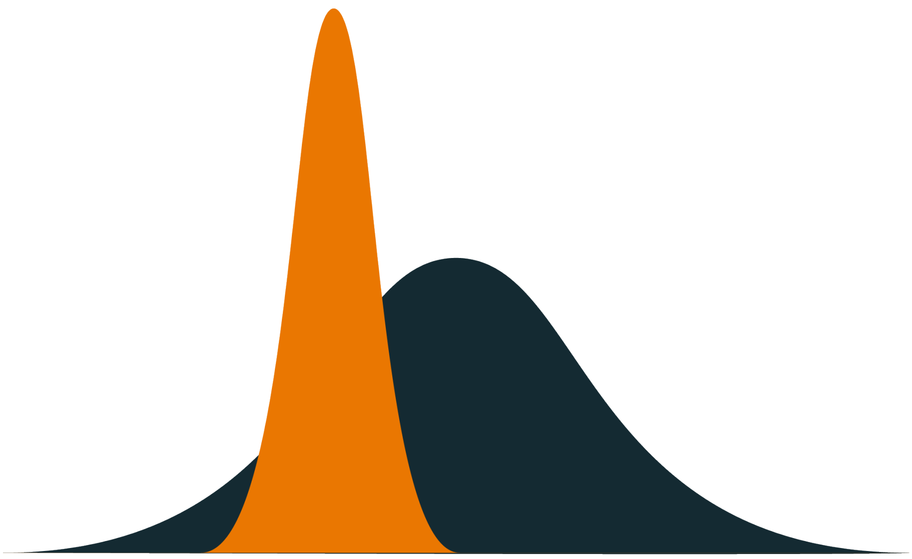
In contrast, populations near the poles would experience a weaker solar zeitgeber, leading to greater variability for the expression of circadian phenotypes. This disparity also would translate into differences in mean chronotype: Equatorial

[1] A systematic review on the subject is provided by Randler and Rahafar (2017).

populations would tend to exhibit a morningness orientation, while populations at higher and low latitudes would tend toward eveningness (Bohlen & Simpson, 1973; Roenneberg et al., 2003).

It's important to emphasize that the latitude hypothesis is grounded in underlying circadian rhythms, not in self-reported morningness-eveningness (ME) *preference*. Self-reported preference can be influenced by extraneous factors, such as social constraints. Reducing this hypothesis to individual preferences undermines its theoretical foundation and introduces unnecessary confounders. Therefore, chronotype scales focusing on the preference aspect of ME may be unsuitable for testing this hypothesis. This is illustrated by Leocadio-Miguel et al. (2014) when discussing differences between the Horne-Östberg (HO) ME questionnaire (Horne & Östberg, 1976), which treats chronotype as a psychological construct (Roenneberg, Pilz, et al., 2019), and the Munich Chronotype Questionnaire (Roenneberg et al., 2003), which addresses chronotype as a biological construct, in the context of the latitude hypothesis.

Figure 8 – Chronotype distributions under the influence of strong (orange) and weak (black) zeitgebers. This visualization reflects the effect proposed by the latitude hypothesis.



Source: Adapted by the author from Roenneberg et al. (2003, Figure 7F).

While there is some compelling evidence for this hypothesis in some insect species (Hut et al., 2013), the same cannot be said for this association in humans.

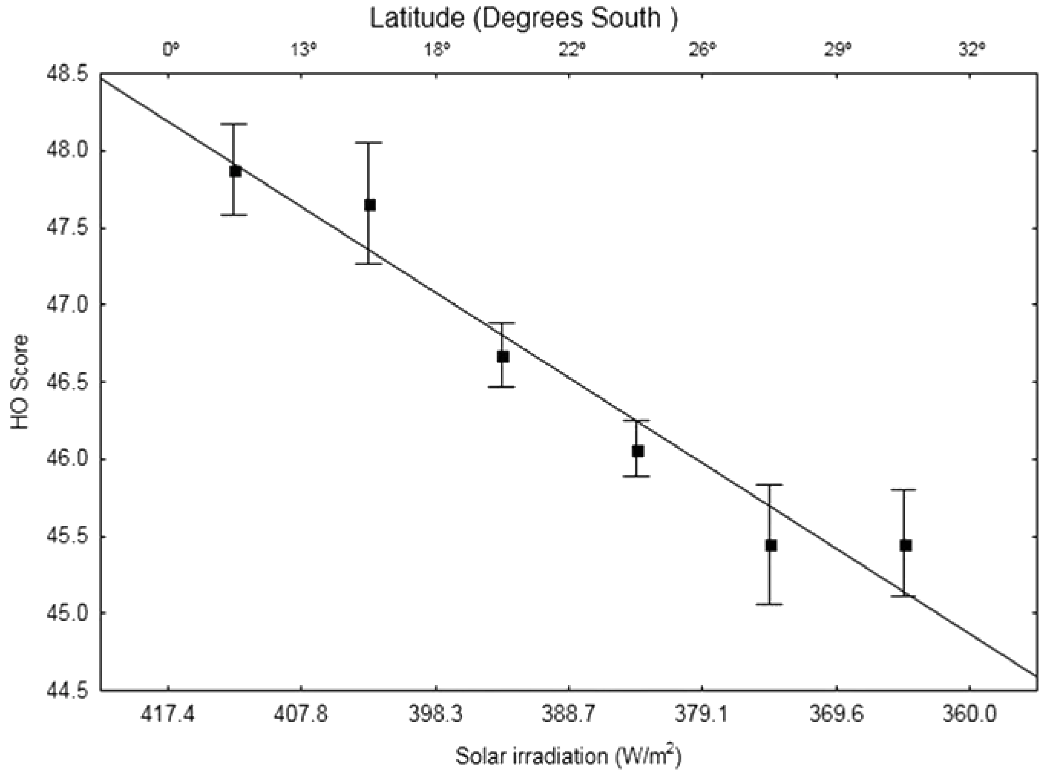
Some authors claim to found such an association (Randler, 2008; Leocadio-Miguel et al., 2014; Horzum et al., 2015; Leocadio-Miguel et al., 2017; Wang et al., 2023), but a closer look at the data reveals that the evidence is not as clear as it seems.

For example, Leocadio-Miguel et al. (2017) claimed to find a meaningful association between latitude and chronotype in a sample of $12,884$ Brazilian participants using the HO questionnaire. However, the reported effect size was too small to be considered practically significant (even by lenient standards), with latitude explaining only approximately 0.388% of the variance in chronotype (Cohen's $f^2 = 0.004143174$) (Figure 9) (See the Supplemental Materials for an in-depth analysis of this result). Considering the particular emphasis that the solar zeitgeber has on the entrainment of biological rhythms (as demonstrated by numerous studies), it is unreasonable to assume that the latitude hypothesis could be supported without at least a non-negligible effect size.

The results from the latitude hypothesis highlight common limitations of studies relying on Null Hypothesis Significance Testing (NHST). A *p*-value does not measure effect size; rather, it represents the conditional probability of observing the test statistic (or a more extreme value) given that the null hypothesis is true (Cohen, 1994; Wasserstein & Lazar, 2016). As Cohen (1988, p. 16) noted, the goal in NHST is not to test whether the population effect size is literally zero, but rather whether it is negligible or trivial.

Figure 9 – Mean scores (±SE) on the Horne & Östberg (HO) chronotype scale (Horne & Östberg, 1976) across a latitudinal gradient, along with 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 spans a range of only approximately 4 points, which overstate the perceived significance of the effect.



Source: Reproduced from Leocadio-Miguel et al. (2017, Figure 2).

Several factors may undermine this hypothesis, such as selective light exposure 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 and with robust statistical procedures. This study aims to address this gap.

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 entrain by zeitgebers—periodical environmental stimuli with the ability to regulate biological rhythmic expression, 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 a negligeble effect size of latitude on chronotype ($f^2 = 0.0031428, 95\%$ CI$[0, 0.012203]$), 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 exhibit a variety of observable traits, such as eye or hair color, which are referred to as phenotypes. These phenotypes also manifest in the way our bodies function.

A chronotype is a temporal phenotype (Ehret, 1974; Pittendrigh, 1993), typically used to refer to endogenous circadian rhythms—biological rhythms with periods close to 24 hours. Chronobiology, the science that studies biological rhythms, suggests that the evolution of these internal oscillators is closely linked to our environment, particularly the day/night cycle. This cycle, alongside human evolution, created environmental pressures that led to the development of temporal organization within organisms (Aschoff, 1989a; Paranjpe & Sharma, 2005; Pittendrigh, 1981). Such organization allowed organisms to predict events and better manage their needs, such as storing food for winter (Aschoff, 1989b).

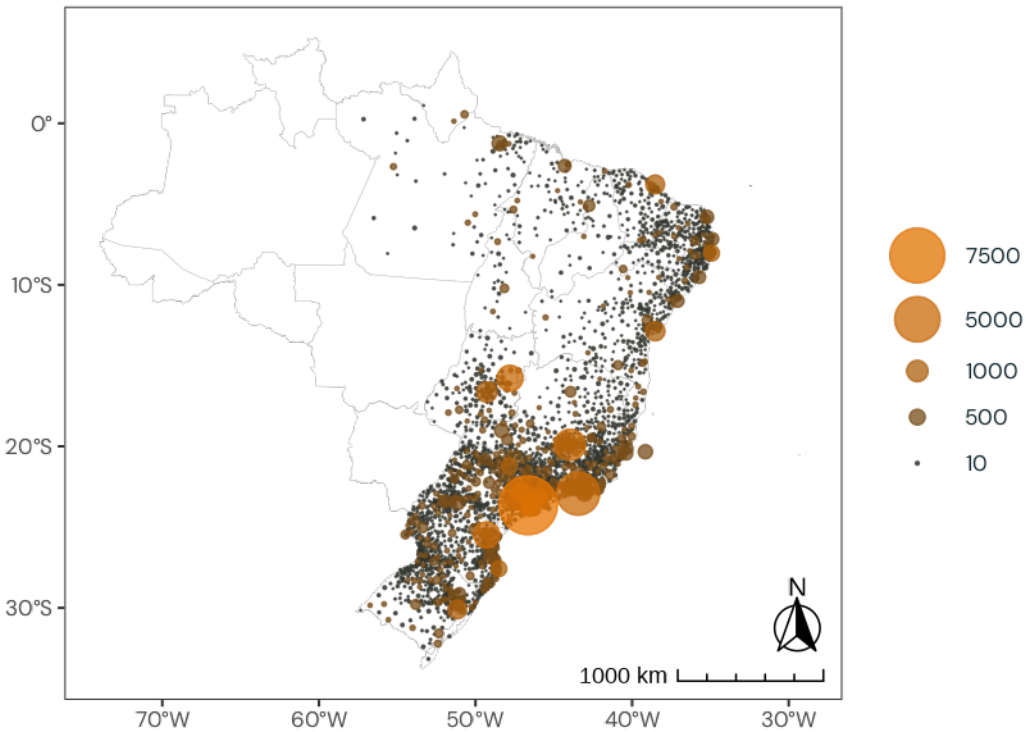
For a temporal system to be useful, it must be capable of adapting to environmental changes. Environmental signals capable of regulating biological rhythms are known as zeitgebers (from the German *zeit*, meaning time, and *geber*, meaning donor (Cambridge University Press, n.d.)). These zeitgebers provide inputs that can shift and synchronize biological rhythms in a process called entrainment (Khalsa et al., 2003; Minors et al., 1991).

The primary zeitgeber influencing biological rhythms is the light/dark cycle, or, simply, light exposure (Aschoff, 1960; Pittendrigh, 1960; Roenneberg & Merrow, 2016). Given its significant role in entraining the biological clock, several studies have hypothesized that the latitudinal shift of the sun, due to the Earth's axial tilt, might lead to different temporal traits in populations near the equator compared to those closer to the poles (Bohlen & Simpson, 1973; Horzum et al., 2015; Leocadio-Miguel et al., 2014, 2017; Randler, 2008). This is based on the idea that populations at low or higher latitudes experience greater fluctuations in sunlight and a weaker overall solar zeitgeber. This concept is known as the latitude hypothesis, or the environmental hypothesis of circadian rhythm regulation.

Several studies have claimed to find this association in humans, but the evidence they provide is of very low quality or is misleading (Horzum et al., 2015; Leocadio-Miguel et al., 2014, 2017; Randler, 2008; Wang et al., 2023). A notable attempt was made by Leocadio-Miguel et al. (2017), who measured the chronotype of $12,884$ Brazilian subjects across a wide latitudinal range using the Horne-Östberg (HO) Morningness–Eveningness questionnaire (Horne & Östberg, 1976). Although the authors concluded that there was a meaningful association between latitude and chronotype, their results were too small to be considered practically significant (even by lenient standards), with latitude explaining only approximately 0.388% of the variance in chronotype (Cohen's $f^2 = 0.004143174$). One possible explanation for this result is that the HO measures psychological traits rather than the biological states of circadian rhythms themselves (Roenneberg, Pilz, et al., 2019), suggesting it may not be the most suitable tool for testing the hypothesis (Leocadio-Miguel et al., 2014).

Building on Leocadio-Miguel et al. (2017), this study offers a novel attempt to test the latitude hypothesis by employing a biological approach through the Munich ChronoType Questionnaire (MCTQ) (Roenneberg et al., 2003) and an enhanced statistical methodology. Additionally, it utilizes the largest dataset on chronotype from a single country, as far as the existing literature suggests, comprising $65,824$ respondents, all residing within the same timezone in Brazil and completing the survey within a one-week window (Figure 10).

Figure 10 – 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 color intensity increasing with participant count. The sample includes Brazilian individuals aged 18 or older, residing in the UTC-3 timezone, who completed the survey between October 15th and 21st, 2017. The size and color scale are logarithmic ($\log_{10}$).



Source: Created by the author.

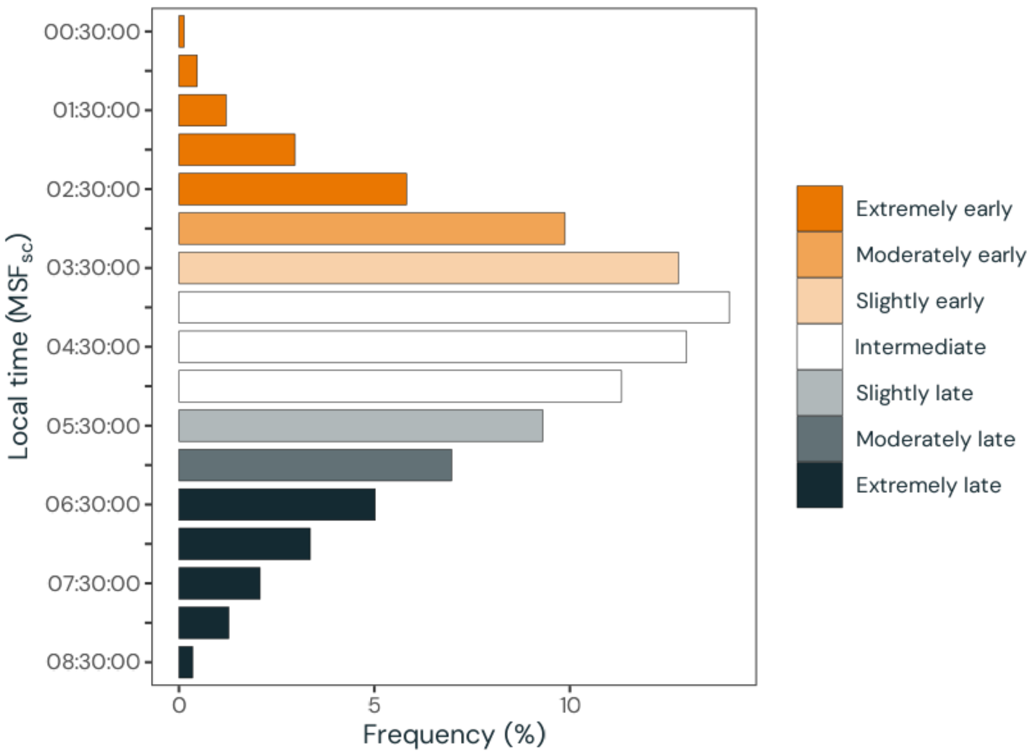
5.3 RESULTS

The Munich Chronotype Questionnaire (MCTQ) uses the midpoint between sleep onset (SO) and sleep end (SE) on work-free days (MSF_{sc}), with a sleep correction (sc) applied if sleep debt is detected, as a proxy for chronotype (Roenneberg et al., 2003). For example, if an individual sleeps from 00:00 to 08:00, the midpoint would be 04:00. This measure is based on the current understanding of sleep regulation, which comprises a homeostatic/sleep-dependent process (S process) and a circadian process (C process) (Borbély, 1982; Borbély et al., 2016). The midpoint of sleep on free days offers a way to observe unrestrained sleep behavior, thereby minimizing the influence of the S process and providing a better approximation of the circadian phenotype (i.e., the C process).

Our analysis revealed an overall mean MSF_{sc} of 04:28:41, with an standard deviation of 01:26:13. The distribution is shown in Figure 11.

Figure 11 – Observed distribution of the local time of 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 a data visualization from Roenneberg, Wirz-Justice, et al. (2019, Figure 1).

This represents the midsleep point for Brazilian subjects living in the UTC-3 timezone, with an intermediate or average chronotype. Considering the $7–9$ hours of sleep recommended for healthy adults by the American Academy of Sleep Medicine (AASM) (Watson et al., 2015), one might infer that this average individual, in the absence of social constraints, would typically wake up at approximately 08:28:41.

The study hypothesis was tested using nested multiple regressions, based on the design of the models presented in Leocadio-Miguel et al. (2017). The core idea of nested models is to evaluate the effect of including one or more predictors on the model's variance explanation (R^2) (Maxwell et al., 2018). This is achieved by comparing a restricted model (without the latitude) with a full model (with the latitude). Cell weights, based on sex, age group, and state of residence, were applied to account for sample imbalances.

To ensure practical significance, the hypothesis test incorporated a minimum effect size (MES) criterion, aligning with the original Neyman-Pearson framework for data testing (Neyman & Pearson, 1928a, 1928b; Perezgonzalez, 2015). The MES was set at a Cohen's f^2 of 0.02 (equivalent to an R^2 of 0.01960784), a lenient threshold (Cohen, 1988). Given the well-established influence of the solar zeitgeber on biological rhythm entrainment, it is unlikely that the latitude hypothesis could be meaningfully supported without demonstrating at least a non-trivial effect.

Two tests were conducted, both starting with the same restricted model, which included age, sex, longitude, and the average monthly Global Horizontal Irradiance (GHI) at the time of questionnaire completion as predictors related to the latitude/longitude of each respondent as predictors ($R^2_{adj} = $, $F(4, 65818) = 1531.808$, p-value $< 1e-05$). The first full model (**A**) added the average annual GHI and daylight duration for the nearest March equinox, as well as the June and December solstices, as proxies for latitude, following the methods of Leocadio-Miguel et al. (2017) ($R^2_{adj} = $, $F(8, 65814) = 794.12$, p-value $< 1e-05$). The second full model (**B**) added only latitude as a predictor ($R^2_{adj} = $, $F(5, 65817) = 1233.588$, p-value $< 1e-05$). All coefficients were statistically different from zero (p-value < 0.05). Assumption checking and residual diagnostics primarily relied on visual inspection, as formal assumption tests (e.g., Anderson-Darling) are often not recommended for large samples (Shatz, 2024). All validity assumptions were met, and no serious multicollinearity was found among the predictor variables.

Sunrise times for the nearest March and September equinoxes, as well as the June and December solstices, were excluded due to high multicollinearity. Daylight duration for the September equinox was excluded for its collinearity with daylight duration during the March equinox.

An ANOVA for nested models revealed a significant reduction in the residual sum of squares in both tests (**A** $F(4, 65814) = 51.71, p$-value $< 1e-05$) (**B** $F(1, 65817) = 37.325$, p-value $< 1e-05$). However, similarly to Leocadio-Miguel et al. (2017), when estimating Cohen's f^2 effect size, the results were below the MES (i.e., negligible) (**A** $f^2 = 0.0031428, 95\%$ CI$[0, 0.012203]$) (**B** $f^2 = 0.0005671, 95\%$ CI$[0, 0.0095426]$).

5.4 DISCUSSION

We emphasize that the assumption of a causal, linear relationship between latitude and chronotype constitutes an *a priori* hypothesis, which this study seeks to falsify.

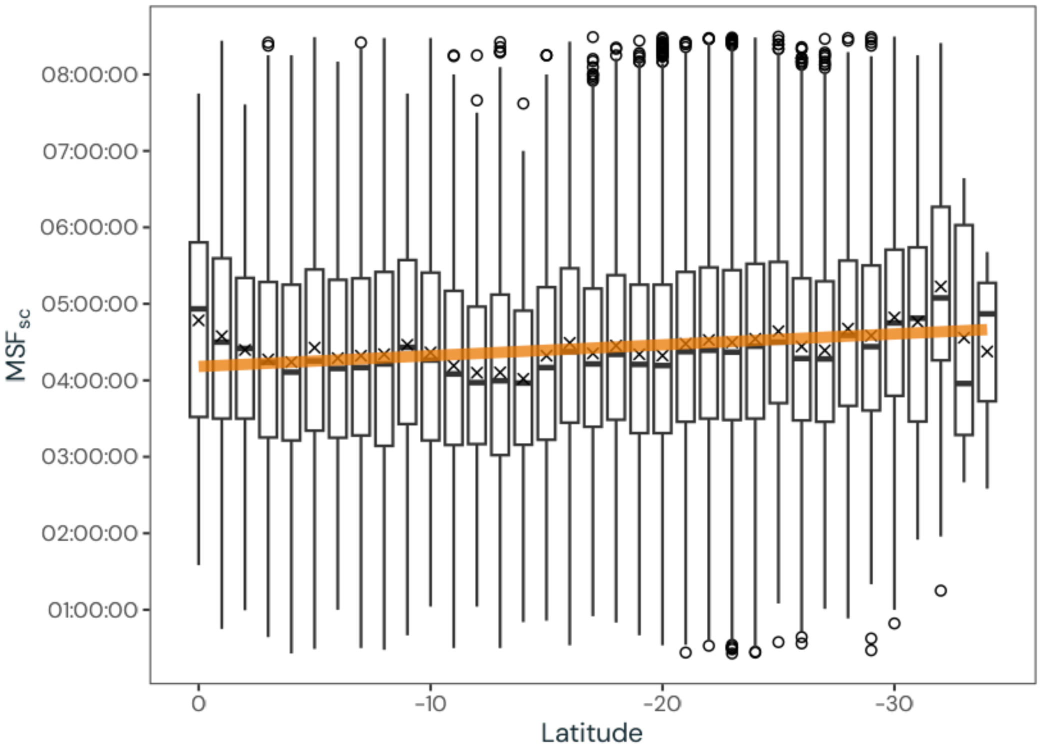
Despite a broad latitudinal range (33.85026 degrees) and a large, balanced sample, our results indicate that the effect of latitude on chronotype is negligible. Indeed, despite suggestions of a potential link in several studies, robust empirical evidence supporting this claim in humans is lacking.

Our results align with those of Leocadio-Miguel et al. (2017), who reported a similar effect size (Cohen's $f^2 = 0.004143174$). However, their analysis did not incorporate a minimum effect size criterion, leading to misleading interpretations.

The small and inconsistent nature of the latitude effect is illustrated in Figure 12, while Figure 13 displays the mean chronotype by Brazilian state. The distribution of chronotypes across latitudes is further illustrated in Figure 14.

Figure 12 – Boxplots of observed 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 orange line represents a linear regression. The differences in mean/median values across latitudes are minimal relative to the Munich ChronoType Questionnaire (MCTQ) scale.

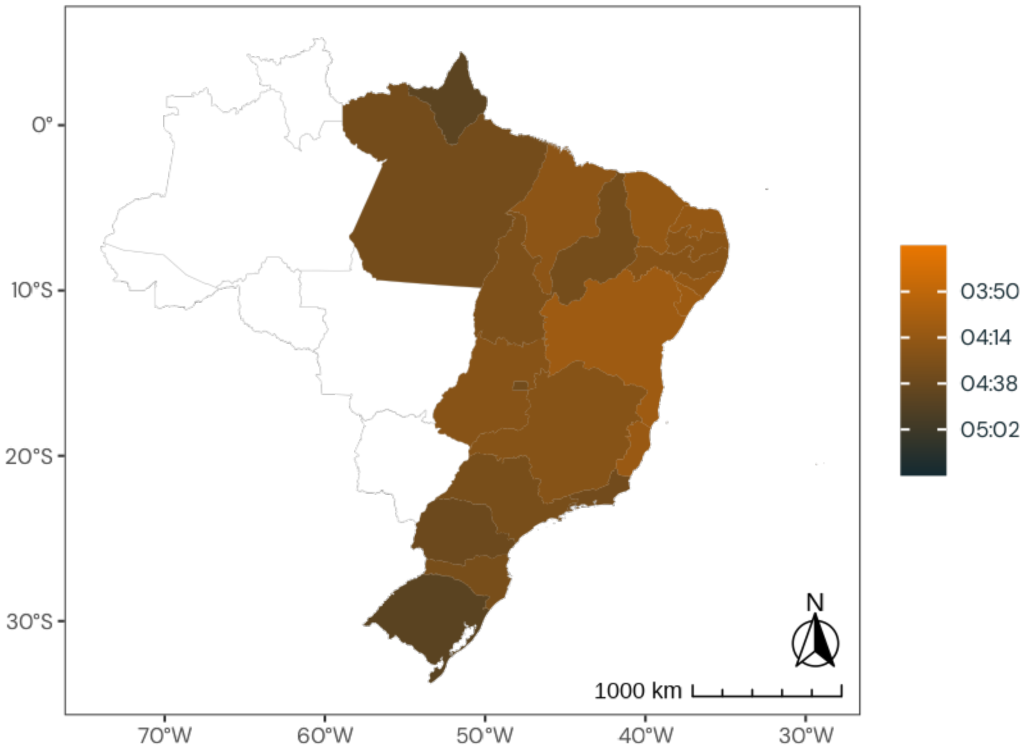


Source: Created by the author.

The absence of a clear relationship between latitude and chronotype can be attributed to multiple factors. As Jürgen Aschoff might have put it, this may reflect a lack of "ecological significance" (Aschoff et al., 1972). Even if latitude does influence circadian rhythms, the effect could be too minor to detect or might be overshadowed by other, more prominent factors like social behaviors, work hours, or the widespread use of artificial lighting. Furthermore, the variations in sunlight exposure between latitudes may not be substantial enough to meaningfully impact the circadian system, which is highly responsive to light. Given that even minor light fluctuations can lead to measurable physiological changes (Khalsa et al., 2003; Minors et al., 1991), latitude alone may not be a decisive factor in determining chronotype.

Figure 13 – Observed geographical distribution of MSF_{sc} values by Brazilian 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 is bounded by the first and third quartiles. 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.

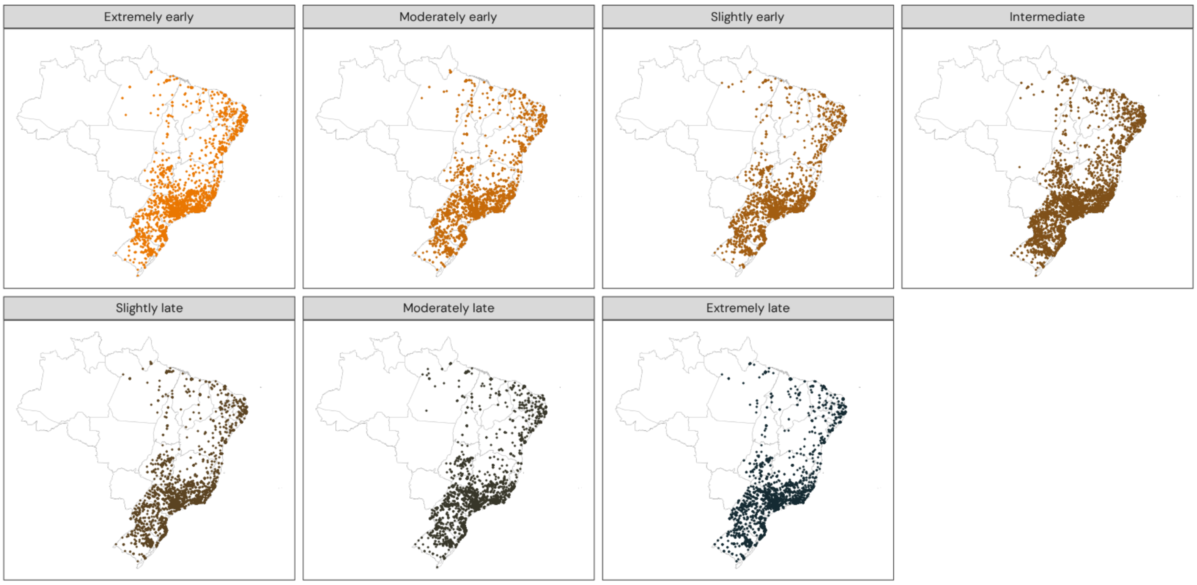


Source: Created by the author.

The results highlight the complex nature of the human chronotype and emphasize the importance of investigating alternative factors that may influence them. The perceived link between these variables may be a consequence of prioritizing statistical rituals over statistical thinking and a tendency toward confirmation bias, rather than rigorous and unbiased data analysis.

Figure 14 – Observed geographical distribution of MSF_{sc} values by a spectrum of extremely early and extremely late, 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. Chronotypes are categorized into quantiles, ranging from extremely early $(0| - 0.11)$ to extremely late $(0.88 - 1)$. No discernible pattern emerges from the distribution of chronotypes across latitudes.



Source: Created by the author.

While this study does not outright refute the hypothesis, the association between latitude and chronotype should remain an open scientific question rather than be treated as established knowledge until supported by robust evidence.

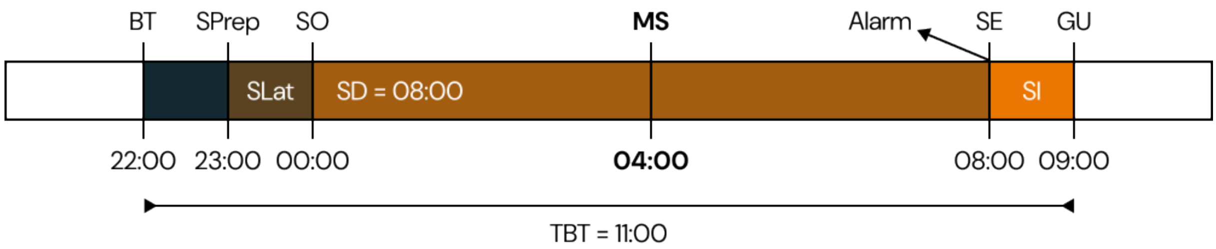
5.5 METHODS

5.5.1 Measurement Instrument

Chronotypes were assessed using a sleep log based on the standard version of the Munich ChronoType Questionnaire (MCTQ) (Roenneberg et al., 2003), a well-validated and widely used self-report tool for measuring sleep-wake behavior and determining chronotype (Roenneberg, Pilz, et al., 2019). The MCTQ derives chronotype from the sleep-corrected midpoint of sleep on free days (MSF_{sc}), which compensates for sleep debt incurred during the workweek (Roenneberg, 2012). Figure 15 illustrates the variables collected by the MCTQ.

Figure 15 – Variables measured by the Munich Chronotype Questionnaire (MCTQ). 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 (Duration. 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 end. Alarm = Indicates whether the respondent uses an alarm clock. SI = “Sleep inertia” (Duration. 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: Created by the author.

Participants completed an online questionnaire, which included the sleep log as well as sociodemographic (e.g., age, sex), geographic (e.g., full residential address), anthropometric (e.g., weight, height), and data on work and study routines. A version of the questionnaire, stored independently by the Internet Archive organization, can be viewed at https://web.archive.org/web/20171018043514/each.usp.br/gipso/mctq.

5.5.2 Geographic Parameters

We obtained latitude and longitude data by geocoding participants’ residential addresses using two main resources:

- QualoCEP (Qual o CEP, 2024): A dataset of Brazilian postal codes with integrated geocoding via the Google Geocoding API. This served as our primary source.
- Google Geocoding API: Used for addresses not included in QualoCEP. We employed the tidygeocoder R package (Cambon et al., 2021) to facilitate this process.

To ensure consistency, we randomly compared results from QualoCEP and Google Geocoding API. This can be seen in the supplementary materials.

5.5.3 Solar Irradiance Data

The solar irradiance data came from the 2017 Solar Energy Atlas of Brazil's National Institute for Space Research (INPE) (E. B. Pereira et al., 2017). We used the Global Horizontal Irradiance (GHI) data, representing the total amount of irradiance received from above by a surface horizontal to the ground.

5.5.4 Astronomical Calculations

The suntools R package (Bivand & Luque, n.d.) was employed to calculate sunrise, sunset times, and daylight duration for each participant's location. These calculations are based on equations provided by Meeus (1991) and the National Oceanic and Atmospheric Administration (NOAA).

The dates and times of equinoxes and solstices were acquired from the Time and Date AS service (Time and Date AS, n.d.). To verify accuracy, we compared this data with the equations from Meeus (1991) and the results from the National Aeronautics and Space Administration (NASA) ModelE AR5 Simulations (National Aeronautics and Space Administration & Goddard Institute for Space Studies, n.d.).

5.5.5 Sample Characteristics

The analysis dataset consisted of $65,824$ participants aged 18 or older residing in the UTC-3 timezone. These individuals completed the survey during a one-week period from October 15th to 21st, 2017, providing a snapshot of the population at that specific time.

The unfiltered valid sample included $115,166$ participants from all Brazilian states. The raw dataset contained $120,265$ individuals, with 98.173% of the responses collected between October 15th and 21st, 2017. This data collection period coincided with the promotion of the online questionnaire via a broadcast on a nationally televised Sunday show in Brazil (Rede Globo, 2017).

Based on 2017 data from the Brazilian Institute of Geography and Statistics's (IBGE) Continuous National Household Sample Survey (PNAD Contínua) (Instituto Brasileiro de Geografia e Estatística, n.d.), Brazil had 51.919% of females and 48.081% of males with an age equal to or greater than 18 years old. The sample is skewed for female subjects, with 66.433% of females and 33.567% of male subjects. The mean age was 32.109 (SD $= 9.258$), ranging from 18 to 58.95 years.

To balance the sample, weights were incorporated into the models. These weights were calculated through cell weighting, using sex, age group, and state of residence as references, based on population estimates from IBGE for the same year as the sample.

A survey conducted in 2019 by 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.

The sample latitudinal range is $33.85026°$ (Min. $= -33.52156°$, Max. $= 0.32869°$) with a longitudinal span of $22.74063°$ (Min. $= -57.5531°$, Max. $= -34.81247°$). For comparison, Brazil has a latitudinal range of $39.02299°$ (Min. $= -33.75115°$; Max. $= 5.27184°$) and a longitudinal span of $45.15451°$ (Min. $= -73.99045°$; Max. $= -28.83594°$), according to data from IBGE collected via the geobr R package (R. H. M. Pereira & Goncalves, n.d.).

Additional details about the sample are available in the supplementary materials.

5.5.6 Power Analysis

To assess the adequacy of the sample size for detecting effects reaching the Minimum Effect Size (MES) threshold ($f^2 = 0.02$), we conducted an *a posteriori* power analysis using the pwrss R package (Bulus, n.d.). This analysis revealed a minimum sample size of $1,895$ observations per variable to achieve a power ($1 - \beta$) of 0.99 with a significance level (α) of 0.01. Our sample size ($n = 65,824$) comfortably surpasses this threshold, ensuring adequate power.

5.5.7 Data Wrangling

Data wrangling and analysis followed the data science framework proposed by Hadley Wickham and Garrett Grolemund (Wickham et al., 2023). All processes were conducted using the R programming language (R Core Team, n.d.), the RStudio IDE (Posit Team, n.d.), and several R packages. The tidyverse and rOpenSci peer-reviewed package ecosystem and other R packages adherents of the tidy tools manifesto (Wickham, 2023) were prioritized.

The MCTQ data was analyzed using the mctq R package (Vartanian, n.d.), which is part of the rOpenSci peer-reviewed ecosystem. The data pipeline was built using the rOpenSci peer-reviewed targets R package (Landau, 2021), which provides a reproducible and efficient workflow for data analysis.

All processes were designed to ensure result reproducibility and adherence to the FAIR principles (Findability, Accessibility, Interoperability, and Reusability) (Wilkinson et al., 2016). All analyses are fully reproducible and were conducted using Quarto computational notebooks. The renv R package (Ushey & Wickham, n.d.) was employed to ensure that the R analysis environment can be reliably restored.

5.5.8 Hypothesis Test

To test the study hypothesis, nested multiple linear regression models were compared: a restricted model (excluding latitude) and a full model (including latitude). The restricted model included sex, age, longitude, and the average monthly Global Horizontal Irradiance (GHI) at the time of questionnaire completion related to the latitude/longitude of each respondent as predictors. Two full models were tested. The first one including the restricted predictors plus the average annual GHI (proxy for the latitude) and daylight duration for the nearest March equinox, as well as the June and December solstices—all related to the latitude/longitude of each respondent.The second one included the restricted model predictors with only the latitude decimal degrees as a predictor.

It is important to notice that the design of the models were based on Leocadio-Miguel et al. (2017) study. Cell weights were applied to account for sample imbalances.

The models were compared using an F-test for nested models, with a Type I error probability (α) of 0.05.

To ensure practical significance, a Minimum Effect Size (MES) criterion was applied, in line with the original Neyman-Pearson framework for data testing (Neyman & Pearson, 1928a, 1928b; Perezgonzalez, 2015). The MES was set at a Cohen's threshold for small effects ($f^2 = 0.02$, equivalent to $R^2 = 0.01960784$) ("just barely escaping triviality" (Cohen, 1988, p. 413)). Consequently, latitude was considered meaningful only if its inclusion explained at least 1.960784% of the variance in the dependent variable.

The hypothesis can be outlined as follows:

- **Null hypothesis** (H_0): Adding *latitude* does not meaningfully improve the model's fit, indicated by a negligible change in adjusted R^2 or a non-significant F-test (with a Type I error probability (α) of 0.05).
- **Alternative Hypothesis** (H_a): Adding *latitude* meaningfully improves the model's fit, indicated by an increase in adjusted R^2 exceeding the MES and a significant F-test (with $\alpha < 0.05$).

Formally:

$$\begin{cases} H_0 : \Delta \text{ Adjusted } R^2 \leq \text{MES} \quad \text{or} \quad \text{F-test is not significant } (\alpha \geq 0.05) \\ H_a : \Delta \text{ Adjusted } R^2 > \text{MES} \quad \text{and} \quad \text{F-test is significant } (\alpha < 0.05) \end{cases}$$

Where:

$$\Delta \text{ Adjusted } R^2 = \text{Adjusted } R^2_{\text{full}} - \text{Adjusted } R^2_{\text{restricted}}$$

5.6 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.

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.

5.7 ACKNOWLEDGMENTS

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.

5.8 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.

5.9 ADDITIONAL INFORMATION

See the supplementary material for more information.

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

5.10 RIGHTS AND PERMISSIONS

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6 CONCLUSION

> Every genuine test of a theory is an attempt to falsify it, or to refute it.
>
> — Popper (1963/2002)

> I suggest that it is the aim of science to find satisfactory explanations, of whatever strikes us as being in need of explanation.
>
> — Popper (1972/1979, p. 193)

The preceding chapters have presented a comprehensive examination of evidence and analyses pertaining to the latitude hypothesis in chronobiology. At this juncture, it is essential to return to the fundamental research question that motivated this thesis: *Is latitude associated with chronotype?*

This study, using what is arguably one of the largest datasets on chronotype, found no evidence supporting the latitude hypothesis in humans.

This study is not an outlier. Current evidence does not support the latitude hypothesis, and some claims made in its favor warrant further examination (see Supplemental Materials). Nevertheless, the hypothesis is often cited in chronobiology research as if it were well-established. While this study does not definitively refute it, the relationship between latitude and chronotype should remain an open scientific question until strong evidence substantiates it.

6.1 STRENGTHS

Testing any theory or hypothesis is inherently challenging and often sparks debate. In this context, it is important to acknowledge the significant strengths of this study:

- Large, focused sample: One of the largest chronotype datasets ever collected within a single time zone ($n = 65,824$).
- Minimal photoperiod variability: Data were collected over a single spring week as summer approached (October 15–21, 2017), effectively controlling for seasonal variations in daylight exposure across regions
- Broad latitudinal range: The sample spans a considerable range ($33.85026°$).

- Population balancing: The dataset was adjusted to reflect population proportions at the time of collection.
- Rigorous statistical analysis: The study accounted for confounders and incorporated a practical significance threshold.
- Full reproducibility: All analyses, from raw data processing through effect size estimation, follow transparent and reproducible protocols available for verification.

It is also worth noting that, despite methodological improvements, this study is grounded in the same core principles and variables that underpin the latitude hypothesis. Consequently, any critique of its methods should be considered in light of the methods used by studies that support the hypothesis.

6.2 LIMITATIONS

While this study provides valuable evidence, certain limitations should be acknowledged, as they may influence the interpretation of the findings.

The use of the Munich Chronotype Questionnaire (MCTQ), although a validated instrument, introduces potential recall and social desirability biases that are inherent in self-reported measures. The large sample size likely mitigates these biases, in line with the law of large numbers (DeGroot & Schervish, 2012, p. 352). Moreover, at the time of data collection, the MCTQ had not yet been officially validated in Portuguese (a process completed in 2020 by Reis et al., 2020), which may have introduced minor inconsistencies; however, the impact is expected to be minimal given its format as a sleep log.

The timing of data collection coincided with the onset of Daylight Saving Time (DST) in Brazil. On October 15th, 2017, the day data collection commenced (80.153% of the data used in this analysis were collected on this day), a significant portion of respondents adjusted their clocks forward by one hour. Although this adjustment could theoretically influence responses, the questions were designed to capture daily routines that were not directly affected by the DST shift. Furthermore, if DST had any effect, it would have been expected to bolster the latitude hypothesis; yet, this was not supported by the data.

The latitudinal range of $33.85026°$, while substantial, could be questioned as potentially insufficient to detect latitude effects on chronotype. However, the absence of a meaningful association within this range suggests that any such effect, if present, would be minimal.

While the analysis revealed a predominantly linear relationship between latitude and chronotype, non-linear modeling approaches might capture additional nuances, though at the cost of reduced interpretability. Similarly, while Cohen's effect size benchmarks have faced criticism, their foundation in psychological research—where complex phenomena often yield subtle effects—makes them particularly relevant for this study.

Although key confounders were controlled for, some variables remained unexamined (e.g., socioeconomic status, urbanization, social timing). While including these variables might enhance model precision, it could also introduce multicollinearity and overfitting concerns. In the context of the tested hypothesis, the exclusion of these additional predictors is unlikely to alter the overall conclusions.

The study used sleep as a proxy for measuring chronotype, leveraging its underlying circadian processes. However, sleep is not the only marker of circadian phenotypes. More precise methods, such as Dim Light Melatonin Onset (DLMO) (Ruiz et al., 2020), can provide a direct measure of circadian phase. Yet, these approaches are often more invasive and costly, limiting both sample size and the generalizability of findings.

Finally, the study's generalizability is limited to the Brazilian population. However, since the latitude hypothesis's underlying principles are not geographically constrained, these findings provide valuable insights for future research in other regions.

These limitations, while important to consider, do not undermine the study's findings; rather, they highlight areas where future research might further refine our understanding.

6.3 DIRECTIONS FOR FUTURE RESEARCH

This thesis proposed using a global modeling approach to investigate the latitude-chronotype relationship. As demonstrated by the results of this study and others, no meaningful 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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