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## Chapter 1

The University of São Paulo Twin Panel: fostering research on twin-related issues from a behavioral perspective

Emma Otta and Eloisa de Souza Fernandes

Twins provide us with a natural experimental design to conduct research from behavioral and health perspectives. They are currently helping scientists estimate the heritability of symptoms and better understand why Covid-19 makes some people sicker than others. Scientists are puzzled by asymptomatic coronavirus cases coexisting with seriously ill and death cases. Researchers are comparing monozygotic twins with their dizygotic counterparts (2,104 of the 2,633 twin pairs living apart) to understand how genetic and environmental factors influence the progression of the disease. Members of the Twins UK adult twin register reported the presence of symptoms using a smartphone-based application (the C-19 Covid Symptom Tracker app) during the pandemic, between March 25 and April 3, 2020. The predicted status of Covid-19 was determined by a combination of age, sex and symptoms of anosmia, severe persistent cough, fatigue and skipped meals. Analyses conducted to date have shown that the symptoms most predictive of SARS-CoV-2 infection are inheritable. Williams et al. (2020) reported 50% heritability. This means that 50% of the variability in the symptoms most predictive of SARS-CoV-2 infection in the population is due to genetic differences. Considering all the symptoms, a heritable component was found for delirium (49%), anosmia (47%), shortness of breath (43%), fever (41%), fatigue (32%), and diarrhea (34%), but not for hoarse voice, cough, skipped meals, chest pain, and abdominal pain.

Twins are also helping psychologists understand the factors involved in subjective well-being (SWB). In their review of three decades of research on subjective well-being, Diener et al. (1999) concluded that researchers have often been disappointed by the relatively small effect sizes of the sociodemographic variables (e.g., sex, age, years of education, family income, marital status) they investigated. These variables accounted for less than 20% of the variance in SWB.

On the other hand, studies conducted with twins showed that personality is one of the strongest predictors of SWB, explaining 40 to 55% of the variation in current SWB, and 80% of the long-term variation. Figure 1 shows intraclass correlations for scores on the Well-Being Scale of the Multidimensional Personality Questionnaire for middle-aged twins (N=1,380 pairs) reared together and apart in a study conducted by Lykken and Tellegen (1996). Statistically significant correlations were found for monozygotic twins (MZ) irrespective of whether they were brought up together (MZT) or apart (MZA). Statistically nonsignificant correlations were found for dizygotic twins (DZ) regardless of whether they were reared together (DZT) or apart (DZA).

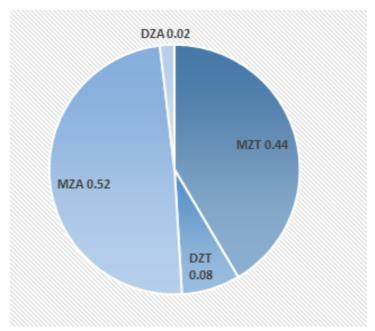


FIGURE 1.1 INTRACLASS CORRELATIONS ON THE WELL-BEING SCALE FOR TWINS REARED TOGETHER AND APART (MZT= 647 PAIRS, MZA=75 PAIRS, DZT=733 PAIRS, DZA=36 PAIRS) (BASED ON LYKKEN & TELLEGEN, 1996)

A team led by Robert Plomin carried out research in Great Britain to determine the psychological impact of a one-month coronavirus lockdown on young adult twins 21 to 24 years old (N=4,000), comparing their self-report in 2020 (17 April – 4 May 2020) and 2018 (Rimfeld et al., in prep.). They assessed 30 psychological and behavioral traits one month after the lockdown was imposed (T2), and compared

the responses to the same traits assessed in the pre-COVID-19 period (T1). In general, the negative impact of a one-month lockdown was small. The main negative changes were increased hyperactivity-inattention and reduced achievement motivation, regardless of the participant's sex, and increased anxiety, especially among women. The researchers concluded from their preliminary results that the COVID-19 crisis, which turned people's lives upside down, did not significantly change individuals psychologically, but genetic differences played a key role in shaping psychological and behavioral responses to the COVID-19 crisis.

# The continent of South America is heavily under-represented in twin studies

A group of researchers from the Center for Neurogenomics and Cognitive Research, Amsterdam (Polderman et al., 2015) conducted a comprehensive meta-analysis of the heritability of human traits based on fifty years of twin studies, published between 1958 and 2012, on a wide range of traits for 14,558,903 twin pairs from 39 countries. Most of the 2,748 studies examined were conducted in the US (34%), followed by the UK (14%) and Australia (9%). The underrepresentation of South America (0.5%), Africa (0.2%) and Asia (5%) is noteworthy. Only five studies (0.2%) headed by Brazilian researchers were included in this meta-analysis and none are from the area of psychology. The studies were from the areas of genetics (Jacques, Salzano & Penña, 1977, Rapaport, Colletto, Vainzof & Zatz, 1991), medicine (Custodio et al., 2007), and physical education (Reis et al., 2007, Machado et al., 2010). This warrants attention considering that psychological traits were highly represented among those investigated in the meta-analysis (e.g., temperament and personality, social interactions, cognitive functions).

The over-representation of some twin populations and underrepresentation of others affects the knowledge produced by research being carried out on a global scale. The genetic composition of the human population differs around the globe. The Brazilian population is characterized by a heterogeneous genetic composition, perhaps one of the most heterogeneous in the world. Its genetic background consists of three populations: European, African, and Indigenous (Souza, Resende, Sousa & Brito, 2019). There are regional differences in the country, with a greater African contribution in the Northeast, European in the South, and indigenous in the North.

Heritability is a statistical measure of a particular population and environment. Changing the population or the environment may alter heritability. In a study conducted with children in middle childhood, Turkheimer et al. (2003) found that socioeconomic status modifies the heritability of IQ. Among children reared in low-SES households, 60% of the variance in IQ was due to the shared environment and the contribution of genes was next to zero, whereas among children reared in affluent households, the result was diametrically opposite. Among adolescents, Rowe, Jacobson, and Van den Oord (1999) found that the contribution of the heritability of verbal IQ increased and that of shared environment decreased as parental education increased.

# The Painel USP de Gêmeos (The University of São Paulo Twin Panel)

We founded the Painel USP de Gêmeos [the University of São Paulo (USP) Twin Panell in 2017, at the Institute of Psychology, with the aim of fostering research on twin-related issues from a psychological perspective in Brazil and South America. It brings together twins interested in participating in research on basic psychological processes and behavior. Twins or their parents interested in participating in the Painel USP de Gêmeos can fill out a mobilefriendly registration form https://en.paineluspdegemeos.com.br/cadastro which takes only a followers few minutes. Our website has 1,729 (https://www.paineluspdegemeos.com.br), our Facebook community 3,754 (https://www.facebook.com/paineluspgemeos.perfil) and our 1899 Instagram account (https://www.instagram.com/paineluspdegemeos/) (Figure 2).



FIGURE 1.2. WE USE SOCIAL MEDIA TO STIMULATE COMMUNICATION WITH AND

AMONG TWINS

Annual Twin Festivals have been organized by the Painel USP de Gêmeos since 2016. In 2020, the 5th Twins' Meeting at USP took place over two days, the first for researchers and professionals on the topic

Twin Studies on Behavioral and Health Research: Current Status, Prospects and Applications, and the second with the aim of offering recreational activities and scientific dissemination talks for twins and their families.

The Painel USP de Gêmeos has 6,287 individuals currently registered: 75% from the Southeast, 15% from the South, 4% from the Northeast, 4% from the West, and 2% from the North (Figure 1.3). All the geopolitical macro regions of Brazil are represented, but with greater representation from the Southeast. Twins under 18 years old (4657 individuals: 74.1%) predominated over twins aged 18 years and older (1630 individuals: 25.9%).

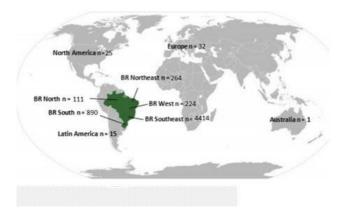


Figure 1.3. Distribution of Painel USP de Gêmeos twins by the macroregions of Brazil. Some Brazilians living abroad were also registered. (CC BY-SA 4.0)

#### Brief overview of the Painel USP de Gêmeos research projects

We present below a brief overview of the Painel USP de Gêmeos research projects, their current status and future directions: (i) population studies on twinning rates, (ii) validation of questionnaire-based zygosity assessment, (iii) self-report of twin relationship, (iv) non-verbal behavior. The chapter ends with a brief report on how we adapted our research and shifted to remote data collection due to the Covid-19 pandemic.

#### **Twinning Rates**

The first thing that caught our attention was the lack of basic data on the birth rate of twins in Brazil. The existing studies were limited to a single or a few hospitals and areas of a city. We started by collecting data on twinning rates in the city of São Paulo from the Brazilian Health Department's Sistema de Informações de Nascidos Vivos de São Paulo – SINASC database (Live Birth Information System of São

Paulo) (Otta et al., 2016). We found that from 2003 to 2014, there were 24,589 twin deliveries and 736 multiple deliveries out of a total of 2,056,016 deliveries registered in 140 hospitals. During that time period, the rate of twin births in the city rose from 10.19‰ to 13.33‰ of one thousand deliveries. The corresponding rates of multiple births were 0.44‰ and 0.31‰, respectively.

The next step was collecting data on twinning rates across the country. Figure 1.4 shows average twinning rates per Brazilian region (2003-2014). The data were drawn from an official public database. Comparing the five macro regions of the country, we found that the highest twinning rates were in the Southeast (10.34‰) and South (10.06 ‰), the lowest in the North (7.32‰), with the Midwest (9.05 ‰) and Northeast (8.68‰) holding intermediate positions (Varella et al., 2017, 2019). Maternal age exhibited a strong positive correlation with twin birth rates. In addition to higher maternal age, this regional discrepancy could also be explained by greater use of assisted reproduction technologies in the more developed regions of the country and the socioeconomic and ethnic composition of each population.



FIGURE 1.4. AVERAGE MATERNITY RATES OF TWINS PER BRAZILIAN REGION (2003-2014) (CC BY-SA 3.0)

#### **Zygosity Determination**

To the best of our knowledge, there are no DNA validated zygosity questionnaires in Brazil. Thus, we decided to conduct a validation study, comparing the results of a short zygosity questionnaire with the DNA in a Brazilian twin sample. We translated from English into Portuguese the 4-item questionnaire used by the Danish Twin Registry (Christiansen et al., 2003) for more than half a century (see Appendix 1.1). The sample consisted of 100 Brazilian same-sex twin pairs (27)

males and 73 females) with a mean age of 30.9 years (12 to 66 years). The questionnaire correctly established zygosity with 96.7% accuracy. This accuracy rate is virtually the same as the 96% reported by Christiansen et al. (2003). However, the accuracy of self-reported zygosity was 60.3%.

#### **Twin Relationship**

Annual Twin Festivals have been organized by the USP Twins Panel since 2016. We have now held 5 Twin Meetings, offering cultural and recreational activities for twins and their families and imparting evidence-based knowledge. Conversation circles (Figure 1.5) have attracted considerable interest over the years. Twin relationships are the main conversation topic among twins that give us a wealth of psychological insight. In a conversation circle, a young MZ man described how proud he was because his co-twin would obtain his engineering degree. However, his feelings were mixed. He was proud about his co-twin achieving a major life goal, but also sad because he would be starting a new life, moving from Belo Horizonte to Porto Alegre, located 1712 km away. The young man acknowledged that he would not miss other members of his family as intensely as he would miss his co-twin brother. Other MZ twins shared similar stories.



FIGURE 1.5. THIS PHOTO WAS TAKEN AT THE II TWINS FESTIVAL AT USP, IN 2017, DURING A CONVERSATION CIRCLE

To better understand the nature of the bond, we conducted research on attachment in adult twins and siblings of similar age from a psychological perspective (de Oliveira Landenberger et al., 2021). Our aims were: (i) to translate into Brazilian Portuguese the Attachment Features and Functions (AFF) Measure (Tancredy & Fraley, 2006); (ii) compare the relative rank of co-twins and parents in the attachment hierarchy of twins and non-twins with the Brazilian

version of the AFF; and (iii) test the hypothesis that MZ are more likely than DZ twins to use one another as attachment figures.

Below are examples of the 16-item Attachment Features and Functions (AFF) answered on a scale of 1 (strongly disagree) to 7 (strongly agree): My life would be severely disrupted if my \_\_\_\_\_ was no longer a part of it; It is important to me to see or talk with \_\_\_\_\_ regularly; When I am away from my \_\_\_\_\_, I feel down.

In this research, we found that DZ twins placed their mothers at the top of the attachment hierarchy, followed by siblings and, finally, by fathers. MZ twins, in turn, placed their siblings as high as mothers in the attachment hierarchy and fathers at the bottom. Finally, female non-twins showed a similar pattern as that of DZ: mothers > siblings > fathers. Male non-twins placed mothers at the top of the attachment hierarchy and siblings near fathers at the bottom. The relationship between siblings demonstrated that the attachment level to the MZ cotwin was greater than that of the DZ co-twin, which did not differ from female non-twins and were all larger than the average male non-twin attachment level (Figure 1.6).

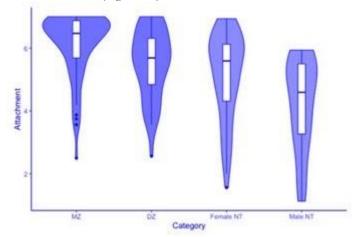


FIGURE 1.6. VIOLIN PLOTS SHOWING THE DISTRIBUTION OF THE ATTACHMENT SCORES TO SIBLINGS BY MONOZYGOTIC (MZ), DIZYGOTIC (DZ) AND NON-TWINS (NT)

We interpreted our findings based on evolutionary theories that focus on ultimate causation. Inclusive fitness theory states that natural selection favors characteristics that increase the chances of individual and related family members' survival or reproductive success (Hamilton, 1964; Mealey, 2001). As expected, MZ twins, who share 100% of their genes, reported higher levels of attachment to siblings that share on average 50% of their genes. We can also explain our

findings based on psychological theories that focus on proximate causation. Sharing life experiences that bring twins closer together could result in scores of (MZ = DZ) > NT. An integrated perspective such as that proposed by Tancredy and Fraley (2006) and Fraley and Tancredy (2012), can consider both kinds of factors and predict that: MZ > DZ > NT. The results of our study on sibling attachment are consistent with this prediction.

#### Nonverbal behavior

In addition to studying the relationship of twins through self-report instruments, we have been focusing on behavior. In our research on nonverbal behavior, we are investigating twin voice and facial expression signatures. These studies, each involving a small research team, are underway and we expect higher nonverbal behavior similarity in MZ than DZ twins.

We are studying acoustic similarities and differences in the voices of same-sex monozygotic (MZ) and dizygotic twins (DZ) (Figure 1.7). Acoustic analyses were carried out on vocal samples of 86 pairs of twins using the Praat Program (Boersma & Weenink, 2019; version 6.0.50). The voice of each individual was recorded at the Lab under three conditions: (i) saying "Oi, meu nome é \_\_\_\_\_" [Hi, my name is \_\_\_\_\_]; (ii) reciting the lyrics of "Happy birthday to you"; and (iii) singing "Happy birthday to you". We will analyze the following speech measures of each individual: values of the first four formants (F1-F4) in HZ, average value of the dominant frequency ("pitch"), "Jitter" and "Shimmer" values and duration (ms).

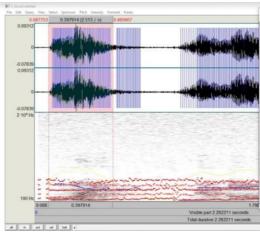


FIGURE 1.7. EXAMPLE OF A VOICE RECORDING ANALYZED WITH PRAAT (SOURCE: BRUNA CAMPOS DE PAULA)

In the study of facial expressions, the twins were recorded while they watched happiness-, disgust- and sadness-inducing films without seeing each other's reactions. We and the participants both considered of the videos (the amusing video) one hilarious (https://www.youtube.com/watch?v=y2004Xaz2HU). A 16-monthold baby was on his father's lap. His uncle, who arrived to visit them, was in front of them. It was the first time that the baby had met his father's identical twin brother. The confused baby had difficulty deciding which of the two men was his father. The sadness-inducing video clip was a short cartoon showing Ellie and Carl's relationship through life, ending with Ellie's death. The disgust-inducing film showed an individual frying cockroaches. Figure 1.8 shows two twin dyads watching the hilarious and disgust film. One pair demonstrates high expressiveness and the other low. There are individual differences that generate a facial expression "signature" for each person. What we want to know is if there is a unique twin facial expression signature.



FIGURE 1.8. TWO DYADS OF MZ TWINS WATCHING A HAPPINESS-INDUCING VIDEO CLIP (LEFT) AND DISGUST-INDUCING VIDEO CLIP (RIGHT) (PHOTOS BY TANIA K. LUCCI)

#### Reality Adapting our research to the New COVID-19 Reality

The Covid-19 pandemic has led us to shift from face-to-face to remote data collection. We had started collecting data on 10 pairs of 5-8 year-old twins observed during a joint task in our laboratory. They sat at a table next to one another and were filmed while they solved a puzzle (Figure 1.9). The films were analyzed using a list of behavioral categories based on Segal (1984), including: (i) time the siblings were mutually involved in the task, (ii) time during which only one sibling was involved in the activity, (iii) time the puzzle remained equidistant

between the siblings, (iv) time the puzzle was closer to each sibling. We hypothesized that the MZ twins would work more in concert with each other, while the DZ would work more individually.



FIGURE 1.9. TWINS SOLVING A PUZZLE AT OUR LAB (PHOTOS BY PAULA COELI)

We replaced the puzzle with a drawing made by the twins seated at a table next to one another at their house. Mothers answered the online Brazilian version of the Twin Relationship Questionnaire (Fortuna, Goldner & Knafo, 2010). Those who agreed to their children's participation in the drawing activity received a video with detailed instructions regarding the drawing task and how to film it (Almeida, 2020). We were able to successfully convert the obstacle imposed by the Covid-19 pandemic into an interesting and feasible research procedure.

Our research team has been discussing citizen science, the participation of the general public under the direction of professional researchers to build scientific knowledge (Riesch & Potter, 2014; Tauginienė et al, 2020). Well described research protocols will allow us to take research beyond the lab and draw our conclusions based on larger and more diversified samples than would be possible in standard laboratory settings.

#### In conclusion

The University of São Paulo Twin Panel (Painel USP de Gêmeos), based at the Department of Experimental Psychology of the Institute of Psychology of the University of São Paulo, officially started in 2017. Our registry is new, but in only three years we have compiled a volunteer sample of 6,287 individuals. This chapter has given a brief overview of the main research conducted with twins on psychological processes and behavior.

We are grateful for the privilege of being part of the Painel USP de Gêmeos and for hosting the online event "Twin Studies in Behavioral and Health Research: current status, prospects and applications" in the middle of the pandemic that inspired us to organize this book. We are looking forward to meeting everyone in person, at the Lab and at the Twin Festivals after the vaccines arrive.

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Appendix 1.1 – Brazilian version of the 4-item zygosity questionnaire (Danish Twin Registry)

Item	Original English	Brazilian Portuguese
	Version	Version
1	Do you and your twin look  — like two ordinary siblings?  — like two peas in a pod?  — not very much alike?	Sobre o quanto são parecidos(as), marque apenas uma alternativa. Você e seu(sua) irmão(ã) gêmeo(a): () são tão parecidos fisicamente como diz o ditado 'cara de um, focinho do outro'; () são tão parecidos fisicamente quanto dois irmãos biológicos não gêmeos; '() não são parecidos fisicamente, como vizinhos
2	In school, is/was it difficult for your teachers and friends to tell you apart?	Na escola, é/era difícil para seus professores e colegas distinguirem um(a) do(a) outro(a)?  () SIM  () NÃO
3	Is/was it difficult for your family and friends to tell you apart?	É/era difícil para sua família ou amigos diferenciarem um(a) do(a) outro(a)? () SIM () NÃO
4	In childhood, did you and your twin have both the same eye color and the same hair color?	Na infância, você e seu(sua) irmão(ã) gêmeo(a) tinham ambos(as) a mesma cor de olhos e a mesma cor de cabelo?  () SIM  () NÃO