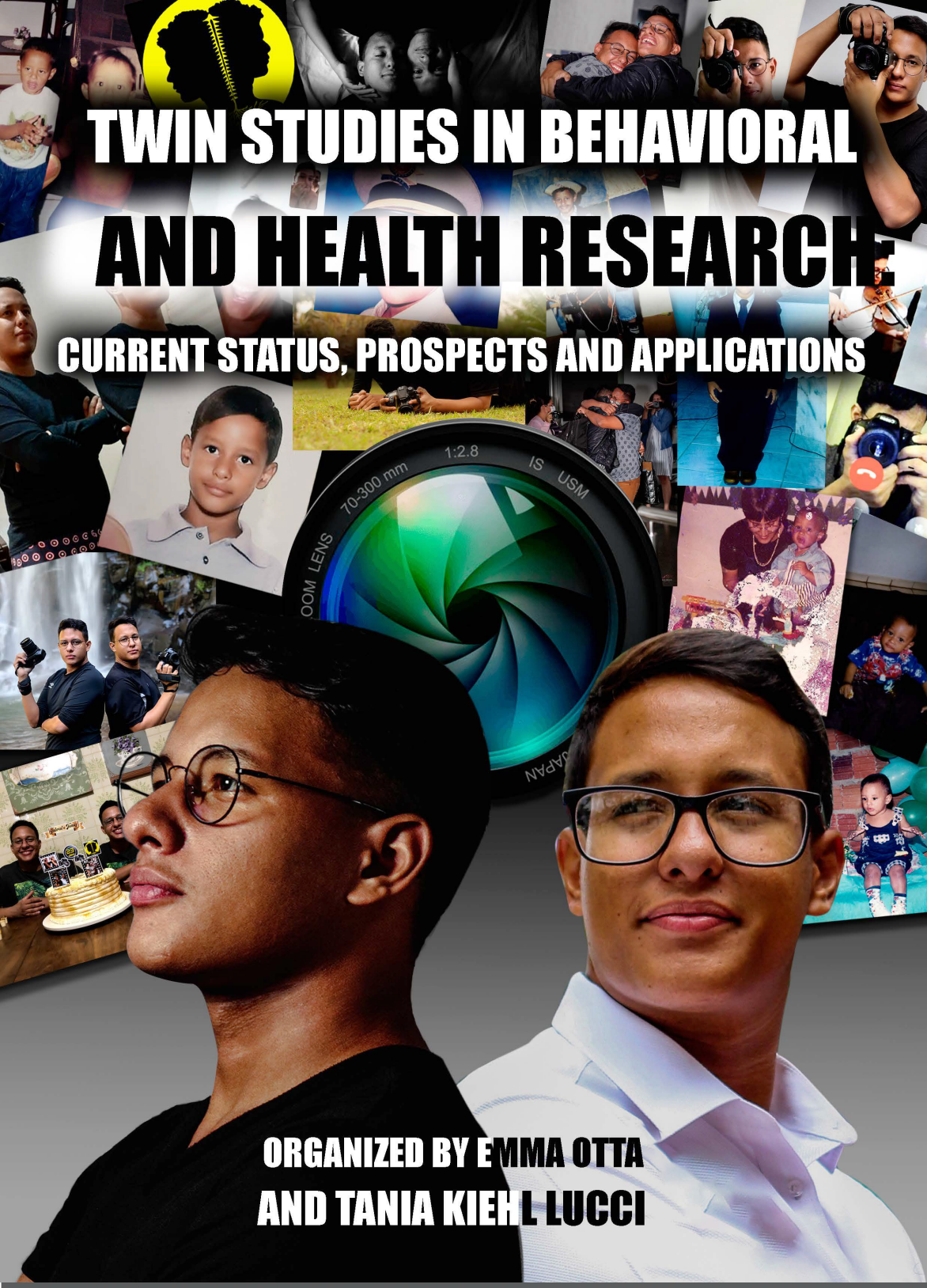

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Contents

Foreword

Nancy L. Segal

Twin research is expanding at an increasing rate, largely due to evidence of genetic influences on many human characteristics thought to be of mostly environmental origin, such as religiosity and political participation. Methodological advances and statistical refinements have reshaped the classic monozygotic (MZ) - dizygotic (DZ) twin comparison into one of the most informative investigative tools, without detracting from its simplicity and elegance.

First of all, I would like to revisit the idea behind the conception of this book. It was inspired by the online conference "Twin Studies in Behavioral and Health Research: Current Status, Prospects and Applications" organized by Emma Otta, Tania Kiehl Lucci and others from the University of São Paulo Twin Panel. The event was held on 11 December 2020 and brought together a group of international presenters and moderators who addressed a diversity of twin-related topics in the behavioral and health sciences.

It is a pleasure to introduce the distinguished authors and their chapters on twin studies**.** The book is divided into five sections that together provide a unifying framework for the different chapters. Part A. Laying the Foundation of a Research Program includes contributions from Emma Otta and Eloisa S. Fernandes of the University of São Paulo Twin Panel; and Sylvia Corte, Laura Szteren and Valentina Melo González of the Uruguayan Twins Project. Otta and Fernandes offer a comprehensive overview of research activities by the University of São Paulo Twin Panel, noting the underrepresentation of twin studies from South America. Corte and colleagues provide an overview of the new registry of Uruguayan twins, developed as part of the Uruguayan Twins Project. Part B. Statistical Analysis of Twin Data, provides an insightful chapter by Vinicius Frayze David of the Institute of Psychology of São Paulo. David defines the genetic components of heritability and describes analyses of twin data via intraclass correlations, mixed models and related procedures.

Part C. Subjective Well-Being and Individual Difference, includes chapters by Michael Pluess of the Queen Mary University of London, and Eric Turkheimer of the University of Virginia, Charlottesville. Pluess discusses the nature and mechanisms of environmental and vantage sensitivity in genetic perspective. Turkheimer explores relationships between behavior genetics and twin studies, asserting that behavior genetics is about using the structure of twin genetics to enable

inferences about causality in ways that cannot be inferred from ordinary data.

Part D. Psychological Perspective on Twinship Development, brought together Hila Segal and Ariel Knafo-Noam of the Hebrew University of Jerusalem, in Israel; Isabella França Ferreira, Tania Kiehl Lucci, Vinicius Frayze David and Emma Otta of the Institute of Psychology of São Paulo; Maria Elizabeth Barreto Tavares dos Reis from the State University of Londrina; Hellen Vivianni V. Corrêa, Luis C. P. Monteiro, Flávia I. B. Brandão, Nelson C. Medrado, Willian D. Ribeiro, Ana C. Miranda of the Federal University of Pará; and Nancy L. Segal from the Twin Studies Center at California State University, Fullerton.

H. Segal and Knafo-Noam present new directions in twin relationship research, defining specific dimensions of relatedness, while noting the relative lack of research in this area. Ferreira et al. focus on twin relationships in childhood, noting that MZ twins are closer and more dependent on one another than other pairs, and boys are less close and more conflictual regardless of zygosity. dos Reis examines twins' identity issues, in particular twins' early relations with and separation from their mother, and their complex sense of self as both part of and distinct from their twinship. Hellen Vivianni Veloso Corrêa and her colleagues examine mate selection and sexual orientation in MZ and DZ twins from the northern regions of Brazil. Segal surveys findings from past and present reared-apart twin research and presents outcomes from a study of doubly switched-at-birth Colombian twins.

Part E. The book concludes with Part E. Health and Well-Being. This section includes contributions from Fivia de Araújo Lopes, Luzia Elionaide Albuquerque Martins and Felipe Nalon Castro of the Federal University of Rio Grande do Norte; Maria de Lourdes Brizot of the University of São Paulo; Marina de Deus Moura de Lima of the Federal University of Piauí; Fausto Medeiros Mendes, Julia Gomes Freitas, and Laura Regina Antunes Pontes the University of São Paulo and Esperanza Angeles Martinez-Miller and Juan Sebastian Lara from the Indiana University School of Dentistry; and Maria Lívia Tourinho Moretto and Gustavo di Giorgi Ramos of the University of São Paulo.

Lopes, Martins and Castro compare resemblance in the food neophobia categories of twins and non-twins siblings, finding evidence for heritable effects, albeit less than estimates from previous research. Brizot documents challenges to multiple pregnancies, reviews the efficacy of different interventions and notes that the best method for preventing preterm twin birth in mothers with a short cervix has not been determined. de Lima reports findings from a cross-sectional twin

study of developmental enamel effects in which genetic influence on molar incisor hypomineralization was observed. Mendes and colleagues outline a study that will recruit young twins to assess variables related to oral health, determine if improvements in dental care affect quality of life similarly in MZ and DZ twins, identify twinning-related factors that may affect children's behavior during treatment and investigate whether these factors affect the success of dental treatment after a two-year period. Moretto and Ramos conclude with an overview of the clinical branch of the University of São Twin Panel, highlighting ongoing efforts by faculty and students to discuss and define the twin-related themes of identification, differentiation, affiliation and separation.

We trust that this book will stimulate further research efforts by twin researchers worldwide, and that we can look forward to future contributions to twin research from interested scholars and students.

Dedication

This book is dedicated to the memory of two great women who inspired us: Regina Célia Gomes de Sousa on the left, and Edila Aparecida de Souza on the right.

Regina passed away on July 22, 2020 from the complications of an aggressive form of myeloma diagnosed late. She chose to reassume her maiden name. Regina Brito and Regina Célia Gomes de Sousa are the same person: our Regina was Professor of the Programa de Pós-Graduação em Neurociências e Comportamento, at the Universidade Federal do Pará [Postgraduate Program of Neurosciences and Behavior, at the Federal University of Pará}. She was a member of our research network and helped found the Painel USP de Gêmeos [USP Twins Panel] in 2017. She also actively participated in the years prior to its formal creation, which were important in organizing the research network interested in studying the basic psychological processes and behavior of twins: subjective well-being and personality, the twin relationship as an attachment bond, sexual orientation, cooperation and competition. She was a passionate and dedicated research mentor. Some of the last dissertations on twins that she supervised were Apego adulto, estratégia de história de vida e vínculo afetivo em gêmeos [Adult attachment, life history strategy and affective bonding in twins] in 2016, Orientação sexual de gêmeos no Norte no Brasil [Sexual orientation of twins in Northern Brazil] in 2017; two graduate studies: Escolha de Parceiros(as) Românticos(as) Ideais em Gêmeos [Ideal Romantic Mate choice in twins] in 2018 and Preferências de

Atratividade Física na Escolha de Parceiros (as) românticos (as) em Gêmeos [Physical Attractiveness Preference in Romantic Mate choice in twins] in 2019. The researchers she inspired will continue her legacy. Some are authors of chapters in this book. We will move forward with our common research plans.

Edila Aparecida de Souza was a laboratory assistant in the Department of Experimental Psychology, at the Institute of Psychology of the University of São Paulo, where the Painel USP de Gêmeos is based. She worked for 23 years in the Ethology Lab. She was a motivated professional and gave her best to our University. Edila was one of thousands of people who have died with coronavirus. She passed away on June 3rd, 2020, at age 62. We remember her positive outlook on life, spontaneity, and willingness to work for a common goal and will continue working with this same attitude, in the face of enormous challenges. The Covid-19 pandemic has turned the world upside down. Vaccines are in development thanks to the efforts of scientists around the world. Science gives us hope for the future in our turbulent world.

Emma Otta and Tânia Kiehl Lucci

Acknowledges

The organizers of TWIN STUDIES IN BEHAVIORAL AND HEALTH RESEARCH: CURRENT STATUS, PROSPECTS AND APPLICATIONS are grateful to Professor Sylvio Canuto, Pro-Rector of Research of the University of São Paulo, Professor Ana Maria Loffredo, Director of the Institute of Psychology (IPUSP), and Professor Marcelo Fernandes da Costa, head of the Department of Experimental Psychology, for supporting the online event, which was the starting point for the book. We would also like to express our sincerest thanks to the Organizing Committee composed by Professor Claudio Possani, Professor Patricia Ferreira Monticelli, Ricardo Prist, Ph.D., and Vinicius Frayze David, doctoral student and to all the contributors. Special thanks are due to colleagues who contributed to the creation of the Painel USP de Gêmeos [the USP Twin Panel], since the original idea until its implementation in 2017, especially Professors Fernando Leite Ribeiro, Vera Silvia Raad Bussab, Jaroslava Varella Valentova, and Maria Livia Tourinho Moretto. We thank the IPUSP Publishing Center [Núcleo de Publicações do Instituto de Psicologia da USP] for their help in editing this book. The editors acknowledge grants no. 2014/50282-5 and 2017/10501-8 of São Paulo Research Foundation (FAPESP) and Natura Cosméticos S.A., no. 304740/2017-9 of Brazilian National Research Council (CNPq).

Emma Otta and Tânia Kiehl Lucci

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PART A - LAYING THE FOUNDATION FOR A RESEARCH PROGRAM

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).

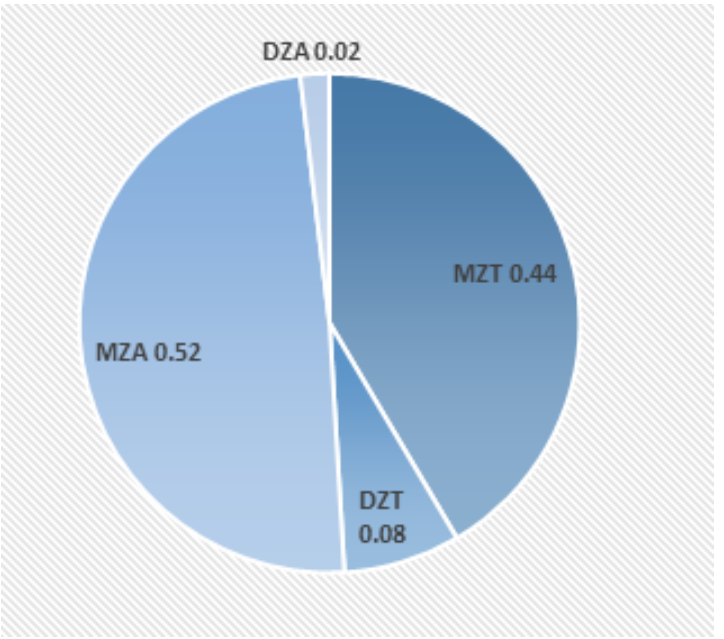

FIGURE 1.1 INTRACLASS CORRELATIONS ON THE WELL-BEING SCALE FOR TWINS REARED TOGETHER AND APART (MZT= 647 PAIRS, MZA=75 PAIRS, DZT=733 PAIRS, DZA=36 PAIRS) (BASED ON LYKKEN & TELLEGEN, 1996)

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 under-representation 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 under-representation 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 Panel] 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 mobile-friendly registration form at https://en.paineluspdegemeos.com.br/cadastro which takes only a few minutes. Our website has 1,729 followers (https://www.paineluspdegemeos.com.br), our Facebook community 3,754 (https://www.facebook.com/paineluspgemeos.perfil) and our Instagram account 1899 (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%).

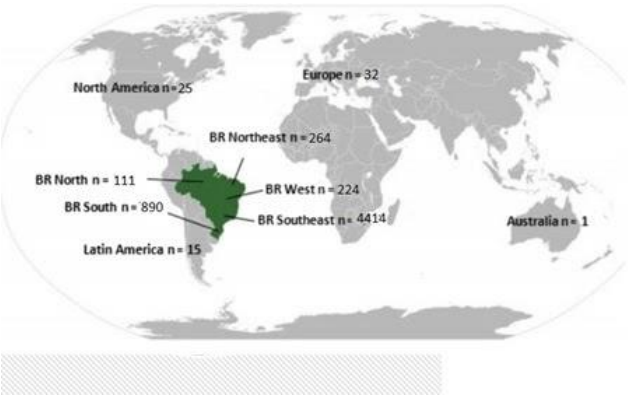

FIGURE 1.3. DISTRIBUTION OF PAINEL USP DE GÊMEOS TWINS BY THE MACROREGIONS OF BRAZIL. SOME BRAZILIANS LIVING ABROAD WERE ALSO REGISTERED. (CC BY-SA 4.0)

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)

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 co-twin 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).

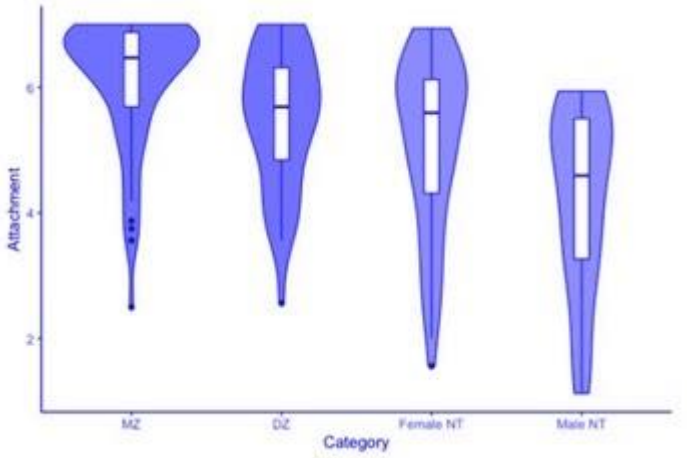

FIGURE 1.6. VIOLIN PLOTS SHOWING THE DISTRIBUTION OF THE ATTACHMENT SCORES TO SIBLINGS BY MONOZYGOTIC (MZ), DIZYGOTIC (DZ) AND NON-TWINS (NT)

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).

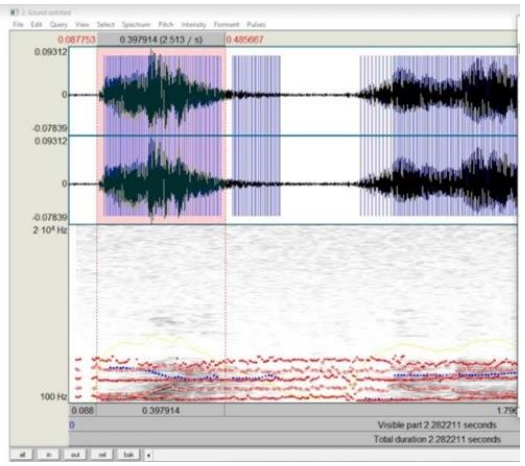

FIGURE 1.7. EXAMPLE OF A VOICE RECORDING ANALYZED WITH PRAAT (SOURCE: BRUNA CAMPOS DE PAULA)

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 one of the videos (the amusing video) hilarious (https://www.youtube.com/watch?v=y2004Xaz2HU). A 16-month-old 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 Version	Brazilian Portuguese 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

Chapter 2

The Uruguayan Twins Project: Foundation of a Registry of Uruguayan Twins

Sylvia Corte, Valentina Melo, Laura Szteren

In 2017, we began to liaise with the USP Twins Panel, and discovered the astonishing world of twin studies. We began a search of studies on twins in our country, but found only a book on generalities, and a psychoanalytic study of multiples, written by Dr. Cherro. Miguel Cherro has been a doctor for 53 years. He is a pediatrician and child and adolescent psychiatrist, now retired. In July 2019, he was appointed an honorary member of the National Academy of Medicine. We got in touch with him that year and he has been advising us ever since.

Since we had no other information regarding twinning in our country, Professor Emma Otta proposed that we carry out a study to contribute to knowledge of the subject in Latin America. To the best of our knowledge, this is the first study on twinning rates (TRs) in Uruguay.

The level of development of different countries determines the reliability of the available data on twinning rates and whether studies on twinning have been conducted or not (Smits & Monden, 2011). This issue is better known across the developed world, with analyses of representative national data (e.g., Pison et al., 2015).

We managed to find some accessible data from government institutions, such as the Ministry of Public Health of Uruguay. The focus was on describing and analyzing TRs between 1999 and 2015.

Twinning is not common among humans, although there is significant variability between populations (Smits & Monden, 2011; Monden, Pison & Smits, 2021). Some demographic and socioeconomic factors influence TR (Bortolus et al., 1999; Hoekstra et al., 2008; Lummaa et al., 1998). Studies carried out by the Twin Panel in São Paulo showed that the twin birth rate per thousand births increased from 10.19 to 13.33‰ between 2003 and 2014 (Otta et al, 2016).

Twin birth rates have changed around the world, increasing since 1980, probably due to the delayed pregnancy of mothers until they reached the age of 30 or 40 years, and the use of assisted reproductive

techniques (ART) (Bortolus et al., 1999). The Latin American Network of Assisted Reproduction (REDLARA) reported in 2013 that transferring three or more embryos increases the likelihood of multiple pregnancy (44.01% and 47.06% respectively) for heterologous embryo transfers. Furthermore, TR research in European countries determined that the most predictive factor for multiple births is maternal age (Bergh et al., 1999; Blondel et al., 2001; Blondel & Kaminski, 2002; 73 Guignon-Back, 1979; Sandra & Yip, 1995; Wood, 1997). Intriguingly, advanced maternal age has also been documented as a factor influencing the incidence of spontaneous dizygotic multiple pregnancies (Bortolus et al., 1999).

According to Monden, Pison and Smits (2021), since the first records were made, twinning rates have been changing with the tide of maternal age and family size. Higher maternal age and higher birth orders are associated with more twin births. However, they consider the effects of changes in age at birth and fertility small to modest compared to the regional differences found around the globe, with the highest twinning rates found in Africa, the lowest rates in Asia and moderate rates in North America and Europe (Bulmer,1970; Pison et al., 2015).

The TR is expected to remain well below singleton rates because giving birth to twins imposes high energetic costs on mothers during the gestation and lactation periods (Gomendio, 1995). Perinatal mortality is also higher for twin births (Olusanya, 2011). In Latin America, twin mortality is almost as high as in Africa, and influenced by socioeconomic variables, such as country developmental status and parent's educational level (Guo & Grummer-Strawn, 1993).

Our study, conducted from a human evolutionary perspective, intends to analyze different factors that could determine the TR in Uruguay and study its variation between 1999 and 2015. If Uruguay exhibits the same behavior as other countries, it would contribute to finding reliable predictors.

We analyzed TRs according to their geographic distribution within the country, by year, and compared the TR and socioeconomic status of the department (political division of the country) and mother's age and educational level.

We used data from the National Statistics Institute (INE) website (http://www.ine.gub.uy/) from censuses carried out in 1996 and 2011, and from the Ministry of Health (1995-2015) (http://colo1.msp.gub.uy/redbin/RpWebEngine.exe/Portal?lang=esp). The birth rate was calculated for every 1000 inhabitants in the department. The available data did not allow differentiating between MZ or DZ twins.

Monthly household income was defined by department, and the average for the region was calculated. The monthly per capita income was established for the urban area of each of the departments. The analyses were carried out using R software. A total of 18,297 twins were born out of 834.673 births, between 1995 and 2015, accounting for a TR of 8.51 to 13 ‰ for every 1000 inhabitants.

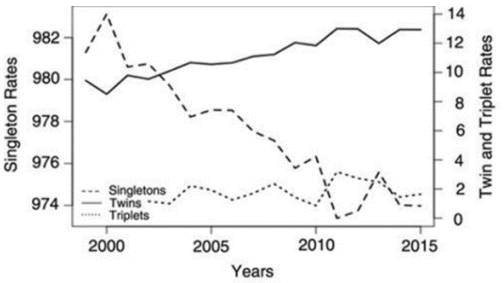

FIGURE 2.1 OVERVIEW OF BIRTH RATES IN URUGUAY BETWEEN 1999 AND 2015

The TR was calculated from the number of twin births according to the total deliveries per 1000, and the triplet rate per 10,000 (there were no data for triplets in the first 3 years).

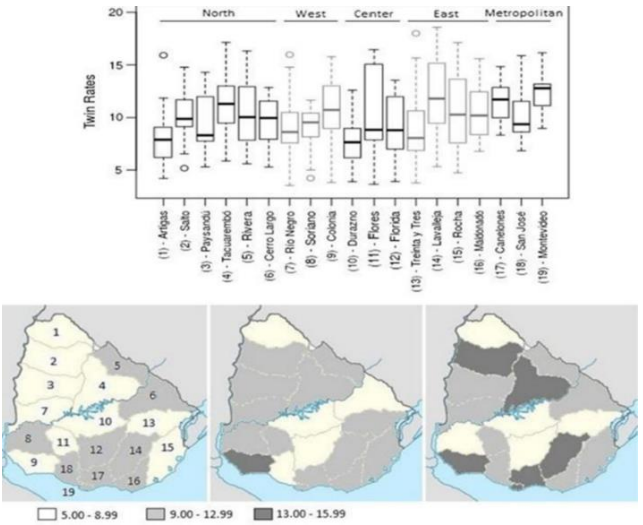

FIGURE 2.2. TWINNING RATES BY DEPARTMENT IN URUGUAY (1999-2015). THE THREE MAPS SHOW TRS IN THREE TIME PERIODS: 1999-2004 (LEFT), 2005-2010 (MIDDLE), 2011-2015 (RIGHT)

Figure 2.2 shows the evolution of TRs over time for each Uruguayan political department. Montevideo (the capital city) has the highest median and lowest variability when compared to other Uruguayan departments. Durazno and Flores (at the Center) have the lowest median and highest dispersion rate, respectively. It is important to underscore that 43.7% of all twins were born in Montevideo. The Metropolitan region had the highest TR, followed by the Eastern region. In the 2011 Census, the Metropolitan region had the highest average household income. The TRs are more or less constant in most departments, depending on their population. However, a marked increase is observed in the Metropolitan region and some departments of the Eastern and Northern region.

In all regions, as the educational level increases, the number of births decreases. In this study, TR rose as a function of mothers' educational level (X2 = 22.981 p-value (0.01) = 13.2767).

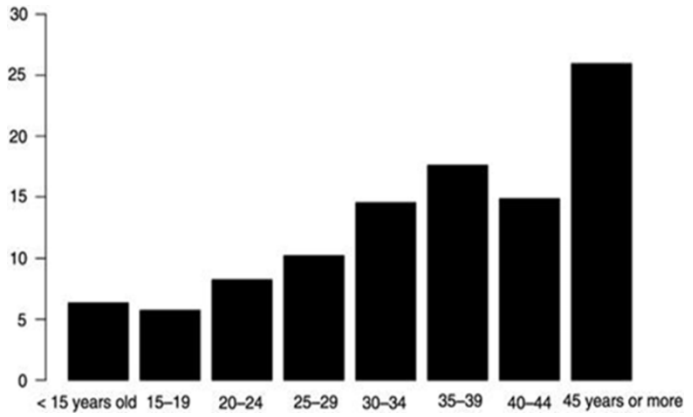

FIGURE 2.3 TWINNING RATES PER THOUSAND DELIVERIES AS A FUNCTION OF MATERNAL AGE

Figure 2.3 shows that the highest TR (28.94‰) was observed in women aged ≥ 45 years, which could be due to the low number of total births (0.17% for women of that age). Another possible explanation is the use of ARTs. Older women may have turned to ARTs as a last resource to have children. These techniques could be the cause of unnatural multiple births. Older mothers would have better educational levels and more income, favoring access to assisted reproduction.

In Uruguay during the study period, the average rate for twins and triplets was 10.9 ‰ and 1.5 per 10,000 births, respectively. There was

an increase in TR, as reported in other countries. The mother's age is a relevant variable in the TR, as well as certain factors related to her economic status, educational level and the use of ARTs. In conclusion, this study found social, economic and demographic factors significantly associated with the rate of twin births in Uruguay (Gómez et al, 2019). Data from national birth registries do not specify zygosity. The birth is registered individually and catalogued as multiple.

Thus, our goal is to establish a Uruguayan Twins Registry, which includes a zygosity questionnaire (Christiansen et al, 2003), already validated by the USP Twin Panel. Many countries have this type of registry, including Denmark, Germany, Israel, and Australia. These records have proven to be a source of research into the nature of twinning and other relevant aspects of the twins' life experience.

The Uruguayan Twins Registry will facilitate study recruitment. Dissemination will occur through social media and registration will be online (in line with the work carried out by Eloísa de Souza Fernández and Vinicius Frayze David, from the USP Twins Panel). The USP Twins Panel registry has been translated into Spanish, including the zygosity questionnaire. The questions on socio-demographic issues are being revised to adapt them to the Uruguayan reality (in collaboration with Professor Mariana Fernández Soto of the Faculty of Sociology, UdelaR).

The Uruguayan Twin Studies Group is being organized with a multidisciplinary perspective, addressing biology, ethology, sociology, psychology, statistics, and anthropology.

We are interested in conducting research on twin behavior from an ethological perspective, especially in babies and children, based on direct observations and inspired by the pioneering research of Dr. Nancy Segal, Director of the Twin Studies Center, at California State University, Fullerton (Segal, 1984).

We are currently collaborating with the USP Twins Panel on several projects, summarized below.

1. Cooperation and competition in twins. Study through drawings made by twins between 5 and 7 years old. Collaboration in the design of the investigation and the ethogram.

We use Citizen Science, which involves public participation and collaboration in scientific research to increase scientific knowledge. Through citizen science, people share and contribute to data monitoring and collection programs. Citizen science initiatives are a

new form of interaction between professional scientists and citizens, allowing them to participate in formal research activities, providing an opportunity for greater engagement with science. http://citizenscienceglobal.org

We also intend to carry out this research in Uruguay (the study is being conducted by Caroline Grecco de Almeida, an undergraduate psychology student, Universidade de São Paulo, and Timon Lebaron-Khérif, a master's student at Université Sorbonne Paris Nord during his internship at the Institute of Psychology, Universidade de São Paulo).

2. Attachment between adult twins (MZ and DZ), kin selection (Hamilton, 1964), satisfaction with life and perception of attachment with the co-twin.

Data collection using the Spanish version of the Attachment Features and Functions Measure (translated from Tancredy & Fraley, 2006) was carried out online in 2020, and emailed to people at our institutions (Faculty of Sciences). The questionnaire was also published on our Facebook page (https://www.facebook.com/gemelosuruguay/). Both the project and the informed consent form were approved by the ethics committee of the CLEMENTE ESTABLE Institute for Biological Research, Ministry of Culture of Uruguay. This research is being conducted by Valentina Melo-González, an advanced undergraduate student in Human Biology (Etología, Facultad de Ciencias. Universidad de la República, UdelaR), in collaboration with Dra. Laura Szteren (Facultad de Psicología, Universidad de la República, UdelaR). One hundred and thirty-five twins completed the questionnaire. The survey was disseminated on our social media accounts: Facebook, Instagram, and email.

Inspired by Bowlby's Attachment Theory (Bowlby, 1969), Tancredy and Fraley (2006) claim that twin relationships, such as infant–caregiver and many romantic adult relationships, are attachment relationships characterized by proximity seeking, separation distress, the use of the other as a safe haven during times of stress, and the use of the other as a secure base from which to explore the world. They found that twin siblings were more likely than their non-twin sibling counterparts to be attached to their siblings. They also found that monozygotic twins (MZ) were more likely to be attached to one another than dizygotic twins (DZ), as might be expected from an

inclusive fitness perspective. Cross-sectional analyses indicated that older people are less likely than younger people to use their sibling as an attachment figure and that married adults are less likely to do so than unmarried people.

Twin studies are the most widely used models in analyzing the relative contribution of genetic and environmental factors to behavior. Combining ethological methods with twin designs enables the testing of hypotheses regarding human behavior. In our research, we will compare the scores of MZ and DZ on the Attachment Features and Functions Measure. Our hypothesis is that attachment differs according to zygosity, with MZ twins being more attached to each other than their DZ counterparts. Twin zygosity was determined using the Spanish version of a four-item questionnaire created by Christiansen et al. (2003) (Appendix 2.1).

With a second questionnaire (the Spanish version of Neyer's questionnaire, 2002), the perception of the relationship with the twin brother or sister (MZ or DZ) was evaluated currently and retrospectively, at different stages of their lives (children, young adults, middle aged and elderly). To the best of our knowledge, this is the first time an investigation of this type will be carried out in Uruguay.

In conclusion

Accurate data on twinning rates are important in low- and middle-income countries, to contribute to a more complete overview of twinning distribution in the world, and to forsee demands for health services (Monden, Pison & Smits, 2021)

It is also important to delve into ethological questions about the nature of the sibling relationship helping parents and teachers regarding raising and educating their twins (Fortuna, Goldner & Knafo, 2010; Tancredy & Fraley, 2006).

Knowing more about twinning, attachment and delving into its causes and consequences will be a step forward in the well-being, health and quality of life of this very special group and of the Uruguayan population in general.

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Appendix 2.1 – Spanish version and original English version of the 4-item Zygosity Questionnaire (Christiansen et al., 2003)

SPANISH VERSION

Evaluación de la cigosidad en el Registro Uruguayo de Gemelos

1. Tu y tu hermano(a) gemelo(a) son…
   — como dos hermano(a)s comunes?
   — como dos gotas de agua?
   — no muy parecido(a)s?

2. En la escuela, es o fue difícil para tus maestros y/o amigos diferenciarlo(a)s?

3. Es o fue difícil diferenciarlo(a)s para sus familiares o amigos?

4. En la niñez, tu y tu hermano(a) gemelo(a) tenían el mismo color de ojos y el mismo color de pelo?

ORIGINAL ENGLISH VERSION

The Four Questions Used for Zygosity Assessment in the Danish Twin Registry

1. Do you and your twin look …
   — like two ordinary siblings?
   — like two peas in a pod?
   — not very much alike?

2. In school, is/was it difficult for your teachers and friends to tell you apart?

3. Is/was it difficult for your family and friends to tell you apart?

4. In childhood, did you and your twin have both the same eye color and the same hair color?

PART B – STATISTICAL ANALYSIS OF TWIN DATA

Chapter 3

Mixed models and statistical analysis of twin data

Vinicius Frayze David

Although I am a psychologist, I have been working with statistics for a number of years now. Usually, psychologists do not receive good statistics training in their undergraduate course, and I can say that, in the beginning, it is difficult to understand even the basics, but it is definitely worth the effort. Knowing statistics helps us better understand our data and other researcher`s studies, and think about new approaches to our research questions. My aim here is to address the overall aspects of using linear mixed models in twin designs. I will use almost no mathematics, because the aim is to show what these models can do more than how they work, and I will also show an example of how they can be applied using Stata software. More information on the mathematics involved can be found elsewhere (Wang et al, 2011). I hope that this chapter serves as an introduction for researchers who are not well versed in the issues surrounding twin data mixed models.

When I talk about statistics with other researchers, most of them view it according to its purpose: to teach people how to use a limited sample and make intelligent and accurate conclusions about a large population (Lammers & Badia, 2004). In this sense, statistics is interpreted as a tool and a means to an end. However, it is also a constantly changing field of knowledge, and we have to keep track of new developments that can help us in our studies. We should always be careful about statistics in any field of Experimental Psychology, but when we work with twin designs, even data from the most straightforward experimental design can be challenging to deal with.

What are the issues involving twin designs? One of the most uncomplicated designs is comparing the distribution of two groups with a particular observable trait. In this case, we have a "control group" and an "experimental group" with its participants and their measured trait (Figure 3.1a). This design is simple and allows us to compare different characteristics of intergroup trait distribution using means, standard deviations, medians, and frequencies, among others.

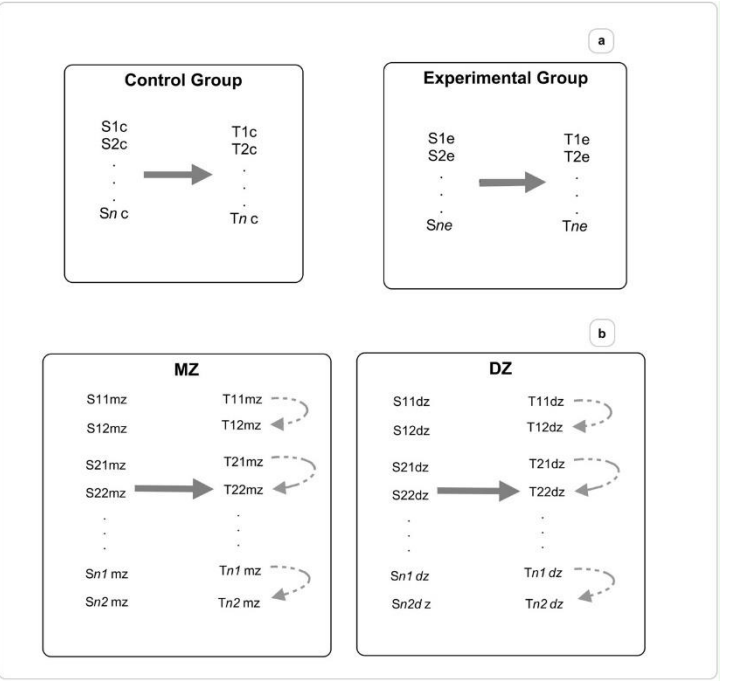

FIGURE 3.1. DATA STRUCTURE IN (A) TYPICAL DESIGNS, AND (B) TWIN DESIGNS

When we use the same design with twins, at first glance, it does not seem very different. We still have two groups of participants and their measured trait. Here, I am separating monozygotic (MZ) and dizygotic (DZ) participants because this is usually what we want to compare (Figure 3.1b). The problem is that these are not independent participants, as in the first case. In a twin design, we have pairs of participants: 11mz and 12mz, 21mz and 22mz, and so on. Since they are pairs, we expect some covariance between them in the measured trait and are interested in the value of this covariance. After all, if we find that MZ twins have a greater covariance than DZ twins, we can surmise that this observed trait involves some genetic influence.

For example, if we measure MZ and DZ pairs data, we may find that MZ pairs are much more similar than DZ, so there is probably a genetic influence on this trait (Figure 3.2).

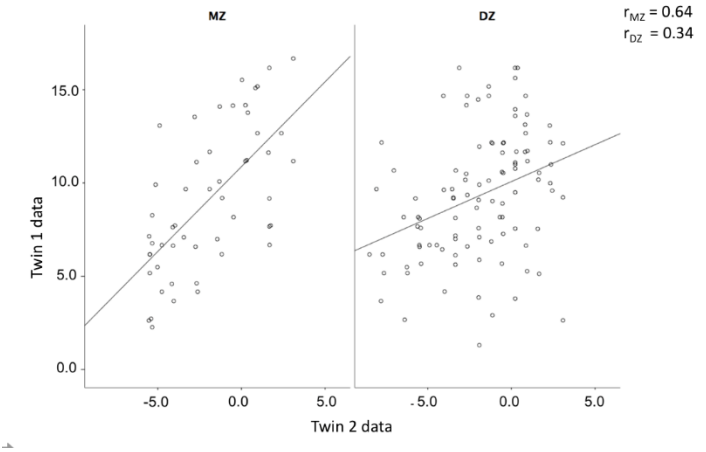

FIGURE 3.2. RELATIONSHIP BETWEEN HYPOTHETICAL DATA OF MZ AND DZ PAIRS

What do I mean when I say that there is a genetic influence? We know that heritability is defined as the portion of phenotype variability attributed to genetic variation. One of the most common approaches to calculate heritability is to use the ADCE model.

The ADCE model assumes that the variation of any individual trait is influenced by genetic and environmental variability, which can be divided into five different effects. The genetic effect is composed of the (1) Additive effect, (2) Dominance effect, and (3) Epistasis effect, while the environmental effect consists of the (4) Common environmental effect, and (5) Unique environmental effect.

Briefly, additive genetic effects (A) are those involving direct action of each allele of homologous chromosomes, so that each adds a direct value to the phenotype; dominant genetic effects (D) result from the joint action of homologous chromosomes; epistasis (I) is an effect resulting from the joint action of alleles on different loci. The common environment effect (C) is the result of the twins' common experiences, usually the family environment, parents, home, and others; and the individual environment effect (E) is the sum of the different experiences of each individual, along with errors of measurement, which are also individual.

Given that heritability is a relationship between the genetic and phenotypic variances, we can formulate heritability according to the ADCE model as:

$$H^2 = \frac{VarG}{VarF} = \frac{VarA + VarD + VarI}{VarA + VarD + VarI + VarC + VarE}$$

We can use variations of the ADCE model if we exclude some of the factors. One of the most common is the ACE model (Maes, 2005), in which we consider all genetic variation to be an additive effect. Mathematically, it is an easier model to work with because it assumes that the increase in observed trait differences is directly related to a difference in the genotype. In this case, we assume that the similarity in an observable trait due to genetic variation in MZ should be twice as large as in DZ.

We are interested in variances and covariances, so how can we calculate them? Two of the most widely used techniques are the intraclass correlation coefficient (ICC), and structural equation modeling (SEM) (Franic et al, 2012). The ICC quantifies the degree to which individuals with a fixed degree of relatedness resemble each other. It can be interpreted in the same way as a Pearson correlation, which varies from -1.0 to +1.0, where the closer to 1.0, the greater the similarity between the siblings (negative values are not typically expected). The most significant difference from a regular Pearson correlation is that the ICC uses the pooled mean of all the data and its standard deviation, whereas in the Pearson correlation, each variable is centered and scaled by its own mean and standard deviation. ICC is more accurate for twin designs because, when using it, the order of the pair is not important and there is usually no good reason to select a twin as number one or number two (which would be the variables in a Pearson correlation). There are different ICC models, but I will not discuss them here, and more information can be found in Koo and Li (2016).

Structural equation modeling (SEM) has been widely used in twin studies. It is a highly complex and versatile model, containing a set of methods that check hypotheses about the structure of the relationships between observed and non-observed (latent) variables (Kaplan, 2008), as defined by the researcher. It is typically represented as a path diagram, in which the paths constitute the set of model parameters. Covariances can be established or calculated for all paths as well as the variances, making it a very interesting model for twin designs. For example, the covariances of additive effects can be set at 1 for MZ, and 0.5 for DZ, and/or dominance effects at 1 for MZ, and 0.25 for DZ, and then calculate the other parameters. Several parameters can be obtained from the models, which also allows researchers to determine model goodness-of-fit and compare different models.

Although ICC and SEM are interesting approaches for studies, they have some limitations. The most notable limitation of ICC is that it compares only two sets of data, such as MZ or DZ siblings. If we are interested in studying other variables such as sex or age, several analyses must be conducted. For sex, we will have to calculate one coefficient for male MZ, another for female MZ, and then for male DZ and female DZ. This increases the likelihood of type I error and creates a need for larger sample sizes.

With respect to SEM, we know that most of the procedures that have been suggested involve non-standard and complex model specifications that are challenging for the average user and therefore susceptible to error, especially because some of the most promising models are not easily available in conventional SEM software (Tomarken & Waller, 2005). Moreover, convergence problems have been observed with some procedures, which may not work properly. Finally, it requires large sample sizes - some rules of thumb suggest at least 25 observations per parameter.

As such, we have mixed models as an alternative. The main difference between a linear mixed model (LMM) and a general linear model such as analysis of variance (ANOVA) is that an LMM includes both fixed and random effects (Baltagi, 2008). Random effects assume that the data come from a hierarchy of different populations and that the differences are related to this hierarchy. In other words, there is an assumption that individual traits are not related only to the independent (fixed) variables because non-random errors are present.

Mixed models are widely used in educational and health studies. One example is the comparison between the performance of male and female children on a test when we have data from more than one school in each group. The children's sex is our fixed factor, but we have to consider the school in our model because we expect to have some covariance in our data due to the school. Some schools may have better facilities and more qualified teachers than others, among several other differences. If we assume that test results can be influenced by the school, although not to the same extent as sex, we can include it in our model as a random factor. The idea of including schools as a random factor can serve both to control for this possible effect and calculate how large this effect can be. Mixed models can be used at several hierarchy levels, such as classrooms, schools and type of school (public or private), and can also include different effects for each level. However, for the purposes of this chapter, we will only discuss the inclusion of covariances between siblings in twin designs.

The logic of having a random effect has been adapted to twin designs. We are usually interested in some fixed factors and covariates

such as sex or age, but we also expect the errors of our participants' individual trait measures to be related to the error of their siblings. Thus, in twin designs, we use the pair of twins as our random factor, making it possible to calculate the covariance between them. Since we know that the total variance of any mixed model is the sum of the variance of the random factors and the residual variance, in our case the total variance will be the variance of the pairs plus the residual variance.

When dealing with twin data, we also have a second problem. The first problem I discussed was how to consider and calculate sibling covariances, and this is similar to many other studies, and not much different from the school example I used before. But when we use the school as a random factor, we can assume some form of regular distribution among schools, and use the school as a unique random factor. With twins, we want to calculate at least two different and very specific covariances to investigate the extent to which our trait can be considered heritable. So, what must we do? We need to separate DZ from MZ covariance in our model. This can be done using mixed models. Here, I used an adaptation of what can be found in Twins Research Australia (https://www.twins.org.au/). Covariance, which is a function of the twins, whether they are MZ or DZ, can be separated from the "extra" covariance because they are an MZ pair. In other words, we can examine the difference between the covariance of MZ and DZ pairs. Thus, our total variance will now be the sum of the variance of the pair, the extra variance of MZ pairs and the residual variance.

How can this be achieved? I will show you an example using Stata software. This analysis can also be carried out in R, SAS, or SPSS using the appropriate commands.

First, we need to organize our data set (Figure 3.3). Each participant must be in a different row and we need a variable to identify each pair. You can use any number, as long as it is the same for each pair and different pairs have different numbers. Then, the next columns can contain your variables of interest, such as zygosity, sex, or any other – the same as in any other analysis. The "trick" is to create three additional variables responsible for separating MZ covariation from DZ covariation. First, you have to identify your pair of participants as MZ or DZ twins, and the easiest way to do that is to create a column in which you assign 0s to DZ and 1s to MZ twins. Remember that you have to assign these values to each participant, even knowing that the sibling will have the same number. Next, you create two new variables that I call dz1 and dz2 in this example. You will have to assign each of the DZ twins from each pair to one of these

new columns, using 0s and 1s again. The first twin will be 1 and 0, and the second 0 and 1. It does not matter which one is which, as long as they are assigned differently. MZ will only have 0s here since they were already defined in the previous column.

	A	B	C	D	E	F	G	H
1	pairid	zygozity	male	age	mz	dz1	dz2	Closeness
2	1	1	0	3	1	0	0	1.32
3	1	1	0	3	1	0	0	0.26
4	2	1	1	2	1	0	0	0.85
5	2	1	1	2	1	0	0	0.16
6	3	0	1	6	0	1	0	1.32
7	3	0	1	6	0	0	1	1.34
8	4	1	0	2	1	0	0	0.81
9	4	1	0	2	1	0	0	3.32
10	5	0	0	6	0	1	0	1.32
11	5	0	0	6	0	0	1	0.81
12	6	0	0	5	0	1	0	3.36
13	6	0	0	5	0	0	0	1.34

FIGURE 3.3. ORGANIZATION OF THE DATASET FOR MIXED MODEL ANALYSES

Then we have our commands. Below are two examples that can be modified to fit different designs. First you declare that you are using a mixed model, then you have your observed trait; subsequently the fixed factors you are interested in – in the first command, I used only "male", the sex variable, and in the second, I declared a more complete model. The most important part is what comes next, when you need to declare your two random effects. The first is the pair random effect, irrespective of whether it is MZ or DZ, and it will calculate the covariance of the pair that is common to MZ and DZ twins. The other effect is only valid for MZ, and it will calculate the difference of covariance between MZ and DZ pairs. Then you can specify the structure for the covariance matrices of the twins. You can usually consider it to be identity.

The main difference between these two commands is that the first uses maximum likelihood estimation with a chi-square distribution, and the second a restrictive maximum likelihood estimation (reml) with a t distribution. As a rule of thumb, if you do not have reliable information to choose between them, and your sample is small, you should use the reml, and if it is large, you can use maximum likelihood, a more powerful model (less chance of type II error).

Commands:

- mixed Closeness male, || pairid: || pairid: mz dz1 dz2, covariance (identity) nocons

- mixed Closeness mz##male age || pairid: || pairid: mz dz1 dz2, reml cov(id) nocons dfmethod(residual)

Now we can look at our outputs. This first one is like any other general linear analysis: there are estimates, errors, statistical values, and p-values for each of the fixed factors and covariates. There was a significant effect of zygosity and age, but not sex (Figure 3.4a). It is important to underscore that these effects take into account the covariation between pairs.

a.

```
Mixed-effects REML regression                   Number of obs     =      1,300
Group variable: pairid                          Number of groups  =        650

                                                Obs per group:
                                                              min =          2
                                                              avg =        2.0
                                                              max =          2
DF method: Residual                             DF:           min =   1,295.00
                                                              avg =   1,295.00
                                                              max =   1,295.00

                                                F(4, 1295.00)     =       8.80
Log restricted-likelihood = -2668.199           Prob > F          =     0.0000
```

Closeness	Coef.	Std. Err.	t	P>|t|	[95% Conf.	Interval]
mz						
MZ	.5619628	.1813237	3.10	0.002	.2062424	.9176831
male						
Masculino	-.2294931	.1631751	-1.41	0.160	-.5496096	.0906235
mz#male						
MZ#Masculino	.2772218	.19641	1.41	0.158	-.1080949	.6625385
age	.096499	.0281407	3.43	0.001	.0412927	.1517054
_cons	-.6525122	.186734	-3.49	0.000	-1.018847	-.2861779

b.

Random-effects Parameters	Estimate	Std. Err.	[95% Conf.	Interval]
pairid: Identity				
var(_cons)	2.187427	.2297072	1.78052	2.687326
pairid: Identity				
var(mz dz1 dz2)	1.444014	.2060063	1.091785	1.909878
var(Residual)	1.12134	.0873257	.9626069	1.306248

LR test vs. linear model: chi2(2) = 327.14 Prob > chi2 = 0.0000

FIGURE 3.4. OUTPUTS OF MIXED MODEL ANALYSIS

The exciting part is in the other table, which contains the values of our variances (Figure 3.4b). The first, var(_cons), is the portion of the variance due to pair covariances, which shows how the pairs of siblings are related to each other, regardless of their zygosity. The second,

var(mz, dz1, dz2), is the increase of variance explained by the covariance being MZ and is only valid for MZ twins, and the third is residual variance, the portion not explained by the fact that they are siblings. Remembering the previous formula, total variance is the sum of pair variance, the extra variance of MZ and residual variance. Now we have the following:

$$VarTOTAL = VarPAIR + VarMZ + VarRESIDUAL = 2.19 + 1.44 + 1.12 = 4.75$$

What is the common variance of the pair, our first component? If we are using an ACE model, what is common for every pair? We have at least half of the genetic similarity (1/2A) and the common environment (C). The extra MZ variance is half of the genetic covariance that was missing (1/2 A), since MZ twins are expected to double their genetic similarity compared to DZ twins. Residual variance is what is explained by neither the additive effect, nor the common environment. In an ACE model, we can assume it is the portion of variance due to the unique environment effect (E). The sum of these three variances is the total variance in our sample. Putting this in numbers, we can conclude that, in this example, 15.6% of the trait variation is due to common environmental variations, 60.7% to additive effects, and the unique environmental variation is responsible for the remaining 23.6%.

It is important to consider that mixed models also have limitations. First, I showed you how to perform the analysis with an ACE model, and we know that this model can overestimate the genetic effects, so we need to keep this in mind. It is possible to include Dominance and Epistasis effects in the analysis, but it becomes much more complicated, resulting in the loss of the advantage of a simple model. More details can be found in Maes (2014).

Here, I only analyzed one independent variable at a time, but several independent variables could have been considered simultaneously. This is not difficult to do, since we just need to include another random factor: the participant. I also used a linear model, but could have used generalized mixed models, which consider different distributions, such as binary, ordinal, gamma, Poisson, and others. Finally, as far as I know, if you have both observed and latent independent variables, there is as yet no solution for mixed models

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PART C – SUBJECTIVE WELL-BEING AND INDIVIDUAL DIFFERENCES

Chapter 4

Environmental sensitivity and vantage sensitivity[1]

Michael Pluess

This chapter is about environmental sensitivity and vantage sensitivity. Let me start with defining what I mean with environmental sensitivity. Environmental sensitivity is a fundamental trait that we can observe in humans and also in many other species. It is defined as the basic ability to perceive and process information about the environment. We all function within a context, we live on the planet Earth, we live on a specific continent, in a specific setting with specific challenges and opportunities. Environmental sensitivity allows us to read the environment and perceive these differences in the environment, and then act upon that. Environmental sensitivity allows us to perceive threats, to perceive potential adverse situations and then respond. But it is not just helpful to perceive threats. It makes us also aware of opportunities that the environment provides. Environmental sensitivity helps us to perceive information about our surroundings, and process them in order to respond.

Sensitivity is important. But does everyone have the same degree of environmental sensitivity, or might people differ in their sensitivity? When we look at specific personality traits or at stress reactivity, we find that people differ in their sensitivity to the environment. Some people have a higher stress reactivity, and other have a lower stress reactivity. There are some who more affected by their experiences, and other who are less affected. Hence, the hypothesis is that some people are generally more sensitive to what they experience while other people are generally less sensitive. There are many theories that focused on that observation. Let me try to summarize them. Individual differences in sensitivity have often been framed within a *Diathesis-Stress* model. According to this model, individuals may not differ in the absence of a negative influence, but differences emerge in response to a negative influence. Some people are resilient, and are not really negatively affected by negative experiences, while some people are vulnerable,

[1] Talk from December 2020 which was transcribed by Vinicius Frayze David.

and show a decrease in their well-being or in any aspect of their functioning. Diathesis-stress usually focuses on the vulnerability in response to adversity.

Most researchers who studied individual differences used the *Diathesis-Stress* model. About 15 years ago, Differential Susceptibility has been proposed as an alternative model to look at individual differences. According to *Differential Susceptibility*, it is not so much that people are resilient or vulnerable, but maybe more that resilient people have a low degree of sensitivity and the vulnerable ones have a high degree of sensitivity. Sensitivity is not just restricted to the experience of adversity, but occurs also in response to positive experiences. In other words, if two individuals differ in sensitivity, we would expect that the individual with low sensitivity would also benefit less from a positive influence. The individual with high sensitivity, who is more negatively affected by adverse conditions, however, obtains also more benefits in response to positive influences. Therefore, factors that are associated with both a heightened response to negative experiences and a heightened response to positive experiences should be considered sensitivity factors rather than vulnerability factors.

So, basically, there are two sides to this story of Differential Susceptibility. We have individual differences in response to negative experiences, that is captured by the *Diathesis-Stress* model, with well-defined terminology and research providing evidence for that model. What was really new in the *Differential Susceptibility* model is the idea that people also differ in response to positive experiences, and we did not really have terminology to describe that. That is why we proposed the terminology of Vantage Sensitivity to describe this positive side to Differential Susceptibility. According to Vantage Sensitivity, two people may not necessarily differ in the absence of a negative influence. It is in response to a positive influence that differences emerge. Some individuals benefit from the positive influence; they show Vantage Sensitivity. Other individuals do not benefit; they show Vantage Resistance.

These different models are all models of Environmental Sensitivity but Vantage Sensitivity describes individual differences to exclusively positive experiences. Let me give an empirical example. In a study conducted with my colleague Francesca Leonetti we measured sensitivity in three-year-old children using an observer rating system for sensitivity (Lionetti, Aron, Aron, Klein, & Pluess, 2019). Importantly, sensitivity was coded with the Highly Sensitive Child-Rating System (HSC-RS) designed as an observational measure to assess sensitivity in children aged 3-5 years (Appendix 4.1). We had a sample of almost 300 children and measures of parenting in addition

to the HSC-RS sensitivity measure. In this particular example, we looked at the outcomes of social competence. We did have a measure of authoritative parenting at age 3, that was based on parent report, and a measure of social competence of the children age 3, also based on parent report. We found an interaction effect that fits best with Vantage Sensitivity rather than Differential Susceptibility or Diathesis-Stress. For the follow-up analysis we created extreme groups of high and low sensitivity (top and bottom 30%). Among the low sensitive children there was really no association between authoritative parenting, a positive parenting style, and social competence. But high sensitivity children had higher social competence at higher levels of authoritative parenting. These differences emerged particularly in a context of positive parenting, and that is why these findings are more consistent with Vantage Sensitivity.

Mechanisms of Environmental Sensitivity

Next, I will address the mechanisms of environmental sensitivity, why are some people, adults or children, more sensitive to what they experience? Most of the proponents of different theories about sensitivity suggest that sensitive individuals have a more sensitive central nervous system, in which experiences are registered more easily and more deeply. There are various factors that have been associated with sensitivity, such as genetic, physiological, and psychological factors, but all are really connected with each other and seem to reflect the contribution of this heightened sensitivity in the central nervous system. There are various brain regions that might play a role here, but one that seems of particular interest is the amygdala. The amygdala is a small but central part of the brain. It is part of the limbic system with a primary role in the processing of the memory of emotional reactions, not just negative but also positive ones. Interestingly, several of candidate genes that have been associated with sensitivity also seem to be associated with amygdala reactivity.

Let me just give you one example with the serotonin transporter polymorphism (5-HTTLPR). Some people carry a long version ("L") and some people have a short version ("S") of this gene (Caspi et al., 2003). A team of researchers found that people with the short version of the gene had a higher amygdala reactivity (von dem Hagen, Passamonti, Nutland, Sambrook, & Calder, 2011). That means that in this particular sample the amygdala was more responsive to negative emotional facial expressions. In a recent study I conducted with colleagues, we looked at brain structure (Pluess, De Brito, Bartoli, McCrory & Viding, 2020). We considered the size of the amygdala and also of the hippocampus. However, significant interactions only

emerged with the amygdala. Basically, we conducted this study with a subsample from the Twins Early Development Study (TEDS)[2] and we measured environmental quality (ranging from low to high), in the first nine years of life. We then collected measures of the amygdala volume between the ages of 10 and 12 for a sample of about 60 boys. We also had teacher reports of their behavior at age 12. We found that there was a significant interaction with the left amygdala volume, and we followed that up with simple slopes. To sum it up, in this study, we have environmental quality across childhood and total problems at age 12 for boys with small amygdala and with large amygdala. Among boys with a small amygdala, there was no significant association between the quality of the environment across the first nine years and total behavior problems at age 12, but a significant association was found among children with a large amygdala. Testing at what level of the environmental quality this association become significant (regions of significance analysis), we found that the interaction became significant at high quality of the environment. In other words, boys with a larger left amygdala benefited more from higher environmental quality than boys with a smaller left amygdala whilst not being more vulnerable to lower environmental quality, a finding that is more consistent again with the theoretical framework of *Vantage Sensitivity*. It may be important to say that the environmental quality scale used was capturing repeated measures of parental involvement, but also socioeconomic factors, the material environment of the child.

I would like to provide some more information on the genetics of sensitivity. Much of the studies that have been conducted in the last 15 years used a candidate gene approach. Researchers focused on individual genes and many of the gene environment interaction findings were consistent with different patterns of environmental sensitivity. However, more recently researchers came to understand that complex traits such as sensitivity are not likely the function of a few individual genes, but more likely the result of many different gene variants, and we are talking about thousands of different gene variants. Using genome-wide data, Robert Keers and associates (Keers et al., 2016) created a polygenic score for sensitivity using data from identical twins discordant for emotional problems. Using that score, they tested, in a separate sample of almost 1,500 children, if the children that have

[2] The Twins Early Development Study (TEDS) is one of the world's largest twin cohorts, investigating how genetic and environmental factors shape individual differences in cognitive and learning abilities, behaviour and emotions in the context of typical development (https://www.teds.ac.uk/).

more of those sensitivity genes are more affected by both negative and positive parenting, and that was indeed what they found. They found a significant interaction between their polygenic score of sensitivity that included more than 25,000 different gene variants and parenting in the prediction of emotional symptoms measured by the Strengths and Difficulties Questionnaire (SDQ). At the lower end of genetic sensitivity, the children did not differ regarding parenting and emotional symptoms. However, the more genetically sensitive children were, the more they were differentially affected by parenting quality: (i) those that experienced negative parenting had higher emotional symptoms and higher emotional problems, but (ii) those that experienced positive parenting had the lowest problems of all, a finding consistent with the differential susceptibility model.

All of these findings have been combined in the *Neurosensitivity* Hypothesis (Pluess, 2015, 2017) suggesting that yes, there is a genetic contribution associated with specific aspects of the neurocognitive mechanism underlying individual differences in environmental sensitivity, manifested in psychological/behavioral and physiological responsivity. Many different genes contribute to structures and functional aspects of the central nervous system, driving sensitivity to specific negative or positive environmental influences.

Self-report measures of sensitivity

But measuring the brain or genes is complicated and expensive. Furthermore, at this stage, it is also not possible to predict an individual's sensitivity based on genome-wide data. Hence, phenotypic measures of environmental sensitivity remain important and relevant. The sensitivity measure developed in the mid-90s by Elaine Aaron as part of her research on Sensory Processing Sensitivity (SPS) is an example. She suggested that SPS is a common personality trait. People with a heightened sensory sensitivity are more aware of subtleties in their surroundings. These people process experiences more deeply and are more easily overwhelmed in a highly stimulating environment. These are just a few of the aspects that describe the personality trait. Aron created the Highly Sensitive Person (HSP) scale. The original scale included 27 items and was designed for adults. Over the last few years, we created brief versions for children as well as for adults with 12 items (Pluess et al., 2018). Appendix 4.2 shows the scale that we use for children as young as 8 years old. Recently some additional items have been developed to strengthen the psychometric properties (e.g., Weyn et al. 2019), but we have used the 12 items-HSC scale now in many different studies and found fairly consistent findings. ´

In 2020 we published a new website https://sensitivityresearch.com/ where you will find information on frequently asked questions about sensitivity. You can link up with other researchers that conduct research in the area. Signing up as a member is free and provides access to all our measures. Once or twice a month we present new findings in a Blog format that is also suitable for the general public. When you click on sensitivity tests, you can choose between three different self-report tests: (a) for adults, (b) for children, and (c) for children based on parent report.

Recently, we have conducted and published a study on the heritability of environmental sensitivity using the Highly Sensitive Child scale (HSC) (Assary, Zavos, Krapohl, Keers & Pluess, 2020). We used a subsample of the Twin's Early Development Study, one of the largest twin studies in the UK and we have data from about almost 3,000 twin siblings. We conducted the study when they were 17 years of age. The results indicate that the heritability of sensitivity was 0.47, which means that genetic influences accounted for 47% of the variation in sensitivity. In other words, we found a substantial heritability of sensitivity, supporting the proposition that the phenotype of environmental sensitivity has a genetic basis. But the heritability index is fairly similar to other personality traits, what suggest that there is scope to explore the genetic components in more depth. Our findings encourage the use of environmental sensitivity as a proxy phenotype in further research applying genome-wide and polygenic approaches. For the genetic studies using genome-wide approaches we will need very large samples and that will be a bit of a challenge.

There is also quite a bit of work that has been done on sensitivity groups. A popular metaphor that has been picked up by many people is that people fall into two sensitivity groups: (a) the dandelions, a low sensitivity group, composed by the majority of people, about 80% of the population, and (b) the orchids, a highly sensitive group, composed by about 20% of the population.

However, whenever we use sensitivity scales for children or adults, we usually find a normal distribution. So, we decided to investigate this further. Even though the data fit a normal distribution we applied a latent class analysis. We did that now in many different samples and we often find that the normal distribution can be divided into three different groups: (a) 30% at the lower end, (b) 30% at the higher end and (c) 40% in the middle. These three groups were found among undergraduate students, among children, and among adolescents. We found them in samples in the UK in the US and other cultures as well. The proportions changed slightly, but on average we basically found

30% at the bottom, 40% in the middle and 30% at the high end. So, basically, we found evidence for dandelions that make up about 30% of the population, not 80%, and we found evidence for orchids again, who make up 30% rather than 20% of the population. And then we had the middle group. We had to find a flower for that group and called them "tulips". If you go on our website and complete the sensitivity test, it will give you the mean score of the sensitive scale, but it will also tell you whether you are a dandelion, a tulip, or an orchid.

Conclusion

Coming to the end of my chapter, I would like to conclude suggesting that people generally differ in their environmental sensitivity and this is an important trait for every single individual. People differ in their level of sensitivity with some being more and some being less sensitive. The level of environmental sensitivity is associated with different genetic, physiological, and psychological factors. There are underlying mechanisms for heightened sensitivity in the central nervous system and there might be specific brain regions involved. I mentioned the amygdala as one example, but there may be other brain regions that have to be identified. And all of this suggests what we should expect looking at the effects of positive experiences or negative experiences. More reactive individuals may be more sensitive to negative experiences, but also more sensitive to the beneficial effects of positive experiences.

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Appendix 4.1 – Abridged version of the Highly Sensitive Child-Rating System (HSC-RS) [extended version as Supplemental Material at Lionetti et al., 2019)

Episode	Description
Risk room	Phase 1 (5 min): The child is alone in a novel room with to explore novel and ambiguous stimuli (a large black box with eyes and teeth, a cloth tunnel, a Halloween mask, balance beam, and small staircase); Phase 2 (5 min): The experimenter returns and invites the child to play with novel and ambiguous objects.
Tower of patience	The child and the experimenter take turns in building a tower. In the successive turns, the experimenter takes longer and longer to place a block on the tower, making the child wait.

Stranger approach	Phase 1: The child is briefly left alone in an empty assessment room while the experimenter pretends to go out to look for toys. Phase 2: A male research assistant (the stranger) enters the room and spoke to the child in a neutral tone.
Exploring new objects	The child enters a room with a set of novel and ambiguous stimuli, (pretend mice in a cage, sticky water-filled gel balls, a mechanical bird, a mechanical spider, and a pretend skull covered under a blanket).
Pop-up snakes	The experimenter shows the child a pretended can of potato chips, containing instead coiled spring "snakes." The experimenter then encouraged the child to surprise his/her parent with the can of snakes.
Transparent box	Phase 1: The child is asked to select a toy among several toys, taken out of a box by the experimenter. Phase 2: Afterwards, the experimenter locks the toy chosen in a transparent box. The child is left in the room with a set of incorrect keys to use to open the box.

Appendix 4.2 –Highly Sensitive Child (HSC) scale. Each item is rated on a 7-point Likert scale ranging from "1 = strongly disagree", to "7 = strongly agree". (Source: Pluess et al., 2018)

	Item
1	I find it unpleasant to have a lot going on at once
2	Some music can make me really happy
3	I love nice tastes
4	Loud noises make me feel uncomfortable
5	I am annoyed when people try to get me to do too many things at once
6	I notice it when small things have changed in my environment
7	I get nervous when I have to do a lot in little time
8	I love nice smells
9	I don't like watching TV programs that have a lot of violence in them
10	I don't like loud noises
11	I don't like it when things change in my life
12	When someone observes me, I get nervous. This makes me perform worse than normal

Chapter 5

Genetically Informed Family Studies Are Social Science

Eric Turkheimer

Between and Within Families

Some time ago, Mortensen, Michaelsen, Sanders, and Reinisch (2002) addressed a difficult and important question: does breast feeding infants have an effect on the IQs of children as they grow up? Both the importance and the difficulty of the question should be appreciated. It is not a matter of the association between one arcane psychological self-report instrument and another; it is about the causal relationship between a profoundly important biological function and what is arguably the most important individual difference construct we know how to measure. If breast feeding causes IQ increases — more to the point, if bottle feeding causes IQ deficits — the societal implications would be profound. And in fact, Mortensen et al. report small but meaningful and significant IQ advantages in children who are breastfed longer.

At the same time, the basic scientific inference in question is in a fundamental sense impossible. If the question were about farm animals, it would be straightforward: randomly assign calves to different nursing protocols, confine them to carefully controlled rearing conditions, and take identical measures of behavior when they reach the appropriate age. In humans, obviously, none of this is remotely possible. Mothers who breast feed their children are different from mothers who do not, different genetically and environmentally, and mothers who breast feed raise their children differently than those who don't. The usual counter-measure, statistically "controlling" for covariates, is a worthwhile effort but is ultimately impotent against the hyper-complex tangle of confounds that surround the child rearing practices of human beings.

Is there anything that can be done? Family studies can offer some help. In the National Longitudinal Study of Youth (NLSY), for example, data are available on weeks of breast feeding and IQ. Moreover, data are available for siblings, i.e., more than one child per mother: the NLSY includes 3036 children born to 1849 mothers. Mothers do not breast feed all of the children for identical lengths of

time, and that creates a possibility: examine the relationship between breast feeding and IQ within and between mothers. The between portion of the analysis compares averages to averages — do mothers who breastfeed their children longer on average have children with higher IQs on average? The within portion compares siblings within a family — does the sibling who was breastfed longer have a higher IQ than the sibling who was breastfed less? Fortunately, modern hierarchical regression models were designed for exactly this kind of analysis.

There are two crucial insights about how this quasi-experimental design works. First, if duration of breast feeding is in fact a cause of IQ differences, that relationship should exist both within and between families: whatever causes one sibling in a family to be more intelligent than others ought to also cause families with high average values of the cause to produce children with high average intelligence. Second, most of the uncontrolled confounds of the true causal relations are likely to vary between families rather than within them. These between family confounds can be either genetic (the same parents are passing the relevant genes to all siblings) or environmental (family socioeconomic status affects all of the siblings). There are certainly possibilities for within-family confounds as well — parents might favor one child relative to the other, or one child might get lucky and be assigned to a star teacher — but the big potent confounds of genes and family environment mostly vary between families. Or, at least, one might be willing to assume as much, in the interest of permitting scientific inference under difficult non-experimental conditions.

Returning to the NLSY, the analysis between families shows a statistically significant association of about 1.5 IQ points per week of breastfeeding ($p < .05$), replicating Mortensen et al. (2002). Within families, however, the relation disappears: 0.2 IQ points per week of breastfeeding is a non-significant result. What does one conclude? Both associations share one component, the true causal effect of breast feeding on IQ. They each have their own confounds. The between portion includes socioeconomic status and family genetics, with within portion only those confounds that can differ among siblings reared together. Given that there are only substantial results for one of them, we conclude that it must be the confounds that are making the difference, and therefore that the relationship is unlikely to be causal.

Is the procedure foolproof? Of course not. Many potential confounds remain uncontrolled. Most obvious is the fathers of the children, who are providing both genes and environment to the children. The potential of within family confounds is not merely a formality: it is ultimately not possible to know about, measure and

control for all of the ways that breast fed siblings might differ from their bottle-fed siblings. The family design is just a way to control for some of uncontrolled confounds that contaminate non-experimental human science. It is a quasi-experimental method, in the classical tradition of Campbell and Stanley (1963). We honor that tradition by referring to the results produced by the design (when, unlike the current example, they actually suggest that something causal might be occurring) as "quasi-causal."

If the bad news is that quasi-experimental family methods can only recover a portion of the causal certainty afforded by random assignment to groups, the good news is that vast portions of utterly crucial human developmental psychology face the same difficulties as the breastfeeding problem, and can be substantially improved, if not completely redeemed, by the thoughtful application of family data. Our lab has been conducting studies of this kind for more than a decade; I summarize a few of them in what follows. Our studies introduce several new wrinkles to the design, starting with the use of identical and fraternal twins as a special variety of sibling. Twins do not change the fundamental inferential logic of the design. Instead, they offer a more refined way to think about the nature of the confounds of the purported causal relationship. In the breast-feeding study, the conclusion was that the association appeared to arise from between family confounds, without specifying whether the confounds might be genetic or environmental. Twin siblings allow us to make this distinction, using the familiar logic of the classical twin model. If the confound of the association appears to be stronger in fraternal twins compared to identical twins, it suggests that the confound is likely to be genetic in origin; in contrast, if the confound is equally strong in identical and fraternal twins, it suggests that the confound arises in the family environment.

Delinquency and Onset of Sexual Activity

Using the National Longitudinal Study of Adolescent Health (Add Health), Armour and Haney (2007) observed an association between age of sexual onset in adolescent girls and the likelihood of delinquent behavior several years later. Although the association was clearly correlational, not necessarily causal, the authors were not shy about touting its causal implications, concluding, that "the timing...of events such as sexual activity has profound consequences" (p. 149) and that "experiencing early or late sexual debut continues to have consequences for delinquent behavior occurring in young adulthood" (p. 150). Interestingly, the Add Health dataset includes twin pairs. These were simply a nuisance for Armour and Haney, because they

introduced non-independence among the participants, in fact, they allowed for a quasi-causal analysis of the association, and we undertook to conduct one (Harden et al., 2008). The results were striking. In the between pair portion of the analysis, we replicated the findings of Armour and Haynie (2007): twin pairs who on average initiated sex early were more likely to be delinquents later than twin pairs who initiated sex later. On the other hand, within pairs of twins, we not only did not observe this relationship, we found a significant association in the opposite direction, i.e., the member of a twin pair with earlier sexual initiation was less likely to be delinquent later, compared to her twin sister.

Step parenting and menarche

Early menarche is associated with a large number of mostly negative developmental outcomes, including depression and suicide attempts (Graber et al., 1997, Patton et al.,2008, Stice et al., 2001), substance use (Stice et al. 2001, Dick et al., 2000), poor body image, disordered eating patterns (Keel et al., 1997, Caufman and Steinberg, 1996), externalizing behavior (Caspi & Moffitt, 1991), breast cancer and obesity (Wellens, 1992), earlier first sexual intercourse, first pregnancy, and marriage (Newcomber & Udry, 1984, Udry, 1979).

An interesting but unexplained observation is that menarche occurs at an earlier age among girls raised in households in which there is a non-biological father present (Doughty & Rogers, 2000; Moffitt et al., 1992; Surbey 1990). Theories suggest that childhood stress predisposes girls to internalizing disorders that lower metabolism, thereby inciting a weight gain that accelerates menarche (Belsky, Steinberg & Draper, 1991). Unstable parental relationships lead to reproductive behavior focused on mating rather than parenting, initiating a cycle of limited parental investment in children.

A quasi-causal analysis of step-fathering and menarche requires another elaboration of the basic within and between design. Twin children are not genetically informative about the causal consequences of exposures related to parenting, because twins reared together, whether identical of fraternal, experience the same family exposures (Turkheimer, d'Onofrio, Maes & Eaves, 2005). Studies of parenting, therefore, must employ the children of twins design, in which it is the parents rather than the children who are twins. In this design, the twin parents provide differential exposures to their children, allowing the quasi-causal effects of parenting to be separated from the same sorts of non-experimental confounds that plague ordinary developmental studies (d'Onofrio et al., 2003).

In Mendle et al. (2006) we used the Australian National Health and Medical Research Council Twin Registry to identify twin mothers who may have introduced non-biological fathers into their daughter's homes. The most interesting comparison, of course, is between the age of menarche in the daughters of twin mothers who introduced a step-father, compared to the daughters of the cotwins who did not. The results are given in Table 1. In the between portion of the analysis, girls raised by stepfathers (N=112) had six-month earlier menarche (12.53 sd=1.39) than those not raised by stepfathers (N=1172; 13.06 sd=1.43). But when looking within the discordant families, that is families in which one mother has a stepfather and the other mother does not, there was no difference in menarche between their daughters (Table 1). The effect occurred between families and not within families. This suggest that it is a matter of some kind of confound rather than something specifically causal about step fathering that causes the observed association with early menarche.

TABLE 1. STEP-PARENTING AND MENARCHE

	N	Menarche	SD
Concordant no step	1111	13.09	1.43
Discordant no step	62	12.59	1.41
Discordant step	62	12.52	1.42
Concordant step	50	12.54	1.37

Conclusion

Too often, behavior genetics is thought of as a reductive methodology that assigns itself the task of showing that behavioral differences that had previously been thought of as psychological are, in fact, "genetic". The basis for that argument often takes the form of something called a "heritability coefficient," varying from zero to one. Heritability coefficients are ratios of genetic to phenotypic variability, developed by R. A. Fisher, Sewell Wright and Jay Lush in the 1920s. Heritability coefficients have useful applications within some restricted domains, mostly in plant and animal breeding, but are not usually adequate for the purpose to which they are usually put, that of asking "how genetic" some particular human trait may be. The goal of this brief chapter is not to present the full case against the application of heritability coefficients to human behavior (see Turkheimer & Downes, in press) but rather to make the simpler observation that the chapter has managed to discuss the relevance of behavior genetics to developmental psychology without mentioning heritability at all. Ultimately, behavior genetics, whether based on twins and families or more modern methods using DNA, is not or should not be focused

on the estimation of heritability coefficients, but rather on the judicious use of genetically informed quasi-experimental methods to help social scientists draw causal inferences where random assignment to condition is not possible.

Viewing behavior genetics in this way will serve to make it less revolutionary on the one hand, and less reductive on the other, ultimately integrating it with the broad domain of developmental social science. Using genetically informative designs makes it more difficult to claim causal relations in non-experimental data, but that is as it should be. When such designs cause researchers to question whether their observed correlational associations can be interpreted causally, it is not because psychology has turned out to be "genetic" but because the isolation of reliable causation is and always will be difficult in the human domain.

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PART D – PSYCHOLOGICAL PERSPECTIVE ON TWINSHIP DEVELOPMENT

Chapter 6

New Directions in Understanding Twin Relationships

Hila Segal and Ariel Knafo-Noam

Twinship and the unique relationships twins share have fascinated the human imagination and been the subject of many mythological stories (de Nooy, 2005; Schave & Ciriello, 1983; N. L. Segal, 2005). These mythological stories across various cultures demonstrate the complexity of twin relationships. Jacob and Esau, Castor and Pollox, and Romulus and Remus are all stories of twin relationships characterized by intense closeness, accompanied by feelings and behaviors of conflict and rivalry, indicating the special connection and common destiny between twins.

These special relationships are perhaps the most intense and longest that people have. Recent research has shown that the "twin bond" begins in the womb and continues throughout life (Castiello et al., 2010; Neyer, 2002; Schwarz et al., 2015). Especially during childhood, twins spend much of their time together, making the co-twin a significant person in the other's life. In some cases, the presence of the twin is comforting, and inspires confidence to explore the world together. In others, the twin may cause direct comparison and thereby evoke competition and rivalry (Ainslie, 1985; Fraley & Tancredy, 2012; Penninkilampi-Kerola et al., 2005).

Although multiple birth rates have grown dramatically in the past few decades (Bacon, 2018), research regarding twin relationships remains scarce. Since these relationships have a significant influence on the development of the twins themselves and their family life, many parents and teachers are concerned about their twins' relationships and wonder how to best raise them. For example, parents frequently have questions regarding separating twins at home and school, are troubled by their conflicts and curious about their mutual dependence (Gordon, 2015; Tourrette et al., 1989). Our research aimed to deepen understanding of the twin relationship and the developmental paths of its various dimensions. We were also interested in understanding the origins of these special relationships and giving parents and clinicians better tools to maximize their potential. We approached our aims by

investigating a large sample of twins in multiple measures from early to middle childhood, which were studied as part of the Longitudinal Israeli Study of Twins (LIST; Avinun & Knafo, 2013; Vertsberger et al., 2019).

We began by identifying the dimensions in twin relationships. Those of closeness, dependence, conflict, rivalry, and dominance are well-known factors in family relationships (Feinberg et al., 2003), specifically among siblings (Boer et al., 1997; Brody, 1998; Derkman et al., 2010; Furman & Buhrmester, 1985; Kramer & Baron, 1995; Stoneman & Brody, 1993). Thus, we expected these dimensions to also be present in twins, while taking into consideration the particular characteristics of twinship (Fortuna et al., 2010). While non-twin sibling relationships are unequal by nature because of age differences and different family history (stemming from possible changes in family structure and dynamics over the years), twin relationships have the potential to be more egalitarian and reciprocal (Smilansky, 1992). Indeed, previous studies found that twin relationships were characterized by more closeness than singleton-sibling relationships (Fortuna et al., 2010; Koch, 1966; Tancredy & Fraley, 2006), although the intensity of the closeness between the twins undergoes a significant change when they grow from childhood through adolescence, adulthood and old age (Neyer, 2002).

In our research, relationships between twins were assessed using the parent-reported Twin Relationship Questionnaire (TRQ; H. Segal & Knafo-Noam, 2019). Mothers and fathers reported separately on each twin in five measures throughout childhood (age 3, 5, 6.5, 7 and 8-9), covering five dimensions: closeness (e.g., "Likes to be with the other twin"), dependence (e.g., "Is upset when parted from the other twin"), conflict (e.g., "Fusses and argues with the other twin"), rivalry (e.g., "Is unhappy or jealous when you do things with the other twin"), and dominance (e.g., "Decides about games"). Indeed, the structure of the five dimensions was consistently found in each measurement point, in both mother and father reports, regarding each twin separately, reinforcing the existence of these five dimensions in the twins' relationships throughout childhood.

The various relationship dimensions indicated that closeness was the most common behavior reported by parents, while conflict behaviors were observed considerably less, with dependence, rivalry and dominance in between. These findings were observed throughout childhood, demonstrating the close social bond that twins share (H. Segal & Knafo-Noam, 2019).

We also found that mothers and fathers have shown substantial agreement (.30-.53) regarding their twins' relationships in

all measures and dimensions. However, the parents' assessments did not fully overlap, possibly indicating the different exposure of each parent to situations in which the twin relationships were expressed. Since mothers' and fathers' perception of their twins' relationship represented different perspectives, it is important that, whenever possible, future studies investigate these relationships by using the reports of both parents.

Another interesting finding was related to the association between the parents' assessments of their twins' relationship and the twins' prosocial behaviors toward one another, as measured in a laboratory experiment at the age of 6.5 years (Yirmiya et al., 2018). We found that prosocial behaviors were negatively associated with the twins' conflict (both parents' reports) and rivalry (fathers' reports), but were not significantly associated with their closeness, dependence or dominance. This interesting finding might imply that the motivation to "work for the co-twin" has more to do with lack of feelings of conflict and rivalry than to the closeness between them. Moreover, the parents' assessments of their twins' closeness throughout childhood predicted the twins' self-reports regarding their own closeness at the age of eleven. That is, the parents' perspective on their twins' closeness was a valid indication of how the twins would perceive their own closeness as they develop to late childhood (H. Segal & Knafo-Noam, 2019).

Given that the structure of twin relationships might be complex to grasp, we used Multidimensional Scaling Analysis (MDS) on the TRQ to graphically represent the dimensions in these relationships. MDS is a set of statistical techniques that allows a visual appreciation of the underlying relational structures of datasets (Hout et al., 2013). We conducted the MDS on mothers' and fathers' reports in all measures and found similar results. Figure 6.1 presents the MDS for mothers' reports of twins at age 9.

FIGURE 6.1: MULTIDIMENSIONAL SCALING OF TWIN RELATIONSHIP QUESTIONNAIRE ITEMS AT AGE 9, AS MEASURED BY 457 MOTHERS' REPORTS. ITEMS ARE ABBREVIATED.

Figure 6.1 shows that the MDS of the twin relationships displayed a quasi-circular structure. It seems that characteristics of conflict were opposite to those of closeness, representing one axis of relationship quality (negative vs. positive, respectively). The other axis seemed to depict the hierarchical (or not) nature of the twin relationship, contrasting dependency and dominance. The association between rivalry and conflict seems intuitive, since competition often leads to conflict (Deutsch, 2006; Johnson & Johnson, 1996), as does the association between closeness and dependence, which indicates the warmth in the twin relationship and their mutual need for one another. A less intuitive finding of the MDS is the association between dependence and rivalry and conflict, which was further supported by moderate correlations between dependence and the more negative aspects of the twin relationship (H. Segal & Knafo-Noam, 2019). These interesting associations may be due to the parents' difficulty in being available to both twins during their upbringing (Koch, 1966; Tourrette et al., 1989). On one hand, the parents' relative difficulty in being available increases twin interdependence, while on the other hand, the same lack of availability creates competition for their attention. This may lead to an association between twin dependence and competition (Rutter & Redshaw, 1991; H. Segal, & Knafo-Noam, 2018; Smilansky, 1992).

Our current on-going studies regarding twin relationships address the developmental trajectory of their various dimensions. Preliminary findings indicated that the positive aspects of twin relationships decreased throughout childhood while the negative aspects increased. Our findings further strengthen the idea that closeness and conflict are

not bi-polar opposites, while dependence and negative aspects of the relationships are associated both at their initial levels and developmental trajectories (H., Segal & Knafo-Noam, 2020b).

Our current study also addresses the impact of zygosity on relationships. Previous studies found that monozygotic twins (MZ, sharing virtually 100% of their genes) had higher levels of closeness than dizygotic twins (DZ, sharing an average of 50% of their genetic variation; (Bekkhus et al., 2011; de Oliveira Landenberger et al., 2021; Fortuna et al., 2010; Fraley & Tancredy, 2012; Kutschke et al., 2018; Neyer, 2002; Penninkilampi-Kerola et al., 2005; N. L. Segal, 2002). However, past research regarding the impact of zygosity on the negative aspects of the relationships yielded inconsistent results. Our current study supported the notion that the impact of zygosity on twin relationships should be qualified, since we repeatedly found associations between zygosity and closeness, but no significant associations between zygosity and the negative aspects of twin relationships during childhood (H., Segal & Knafo-Noam, 2020b).

Finally, our future research will investigate the origin of twin relationships. We are interested in deepening our understanding of the effect of the twins' own characteristics (zygosity and temperament) on their relationships, as well as potentially important environmental characteristics, such as the parenting they receive (H. Segal & Knafo-Noam, 2020a).

It is our hope that better understanding the complexities of twin relationships will help parents, educators and clinicians enhance the positive aspects of these relationships, giving their children a better opportunity to enjoy their unique relationships. Understanding that the development of the dimensions of relationships is dynamic and can change throughout childhood may ease parents' concerns and give clinicians a developmental perspective when treating twins. A better understanding of the twin relationship can also help shape informed decisions on the development of twins, such as decisions regarding separating them at school or helping them create their own individual identity within the twinship. Since the twins participating in the Longitudinal Israeli Study of Twins are entering adolescence, we will be able to investigate their relationship in the next significant stages of their lives.

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Chapter 7

Twin Relationships in Childhood: Effects of Zygosity, Age and Sex

Isabella França Ferreira, Tania Kiehl Lucci, Vinicius Frayze David, Emma Otta

The relationship between siblings is the most enduring in life, but has been less studied than that between parents and children (Dunn, 2014). Even less studied is the relationship between twin siblings. Twinship is a singular type of sibling relationship since children grow up with a constant presence of another individual of the same age and similar demands. A better knowledge of twin relationships by parents, teachers and counselors can promote healthier emotional development and improve decision making (Noble et al., 2017).

The classic twin design is considered an important methodological tool in understanding the genetic and environmental effects on human behavior (Knafo-Noam, Vertsbergez, & Israel, 2018). A comparison of monozygotic (MZ, virtually sharing all of their DNA sequence) and dizygotic twins (DZ, sharing an average of 50% of their genetic variability) is also informative for understanding the emergence of sibling relationships, considering that both types of twins develop in similar overlapping environments.

Twin studies have been inspired by developmental theories that emphasize proximal factors and evolutionary theories that focus on the ultimate causes of behavior (e.g., N. Segal, 1993, N. Segal et al., 2018, Vázquez et al., 2017). MZ twins are more altruistic (N. Segal et al., 1996), more cooperative, less competitive (N. Segal, 1984) and more likely to self-sacrifice for their co-twins (Tornero et al., 2018) than their DZ counterparts. Using a self-report scale, the Painel USP de Gêmeos [University of São Paulo Twin Panel] carried out a study on adult attachment between siblings, showing greater attachment between MZ than DZ twins and non-twins (Landenberger et al, 2021). From the perspective of ultimate causation (Hamilton, 1964), MZ twins, who are genetically identical, should be especially interested in supporting their co-twin and this perspective has been explored in several twin studies. From a proximate standpoint, MZ twins exhibit more similar psychological traits, which contribute to a more intense attachment

bond between them when compared to DZ twins. MZ twins share interests and abilities that contribute to establishing a closer relationship (Plomin et al., 2001; Tancredy & Fraley 2006; Landerberger et al. 2021).

Age is also a factor that influences the relationship between siblings. During childhood several prosocial skills are developed. Children become more aware of others' feelings and perspectives, understanding that these may differ from their own. These abilities are important elements for the development of interpersonal relationships (Eisenberg et al., 2015). Few studies have investigated the effect of age on twin relationships. Vandell et al. (1988) found that one-year-old same-sex twins interacted similarly with both their co-twins and peers. However, at three years old, they were more likely to interact with each other, compared to their peers.

Dyad sex-composition is another factor that may affect the quality of sibling relationships. Higher levels of negativity (physical aggression, arguing, and teasing) were reported by mothers of male twins when compared to female pairs and opposite-sex twins, but not by fathers (Mark et al., 2017). Fortuna et al. (2010) found that at age three, same-sex dizygotic (SS-DZ) girl dyads exhibited higher levels of closeness and lower levels of conflict than SS-DZ boy dyads. In opposite-sex twins (DZOP), girls were more dominant than boys, taking on a more active role in social relationships (Bryan, 1992). Based on adolescent twin self-reports, Penninkilampi-Kerola and Moilanen (2005) found that girls reported themselves as being more dependent on their co-twins than boys, regardless of the zygosity of the pair.

Although the relationship between twins can be assessed in several ways, there is a lack of validated instruments to evaluate twin relationships in their several dimensions. For a comprehensive overview, the Twin Relationship Questionnaire is an adequate approach, given that Closeness, Dependence, Conflict, Rivalry, and Dominance behaviors are assessed (Fortuna et al., 2010; H. Segal & Knafo-Noam, 2019). This instrument also allows intercultural comparisons. Since there are no studies on this perspective in Brazil, our goal is to examine the relationship between twins and compare it with a previous study (Fortuna et al., 2010).

Objectives and Hypotheses

Inspired by Fortuna et al. (2010), our aim was to investigate the influence of zygosity, age and dyad-sex composition on the dimensions of twin relationships (closeness, dependence, conflict, rivalry and dominance).

Our hypotheses were: a) monozygotic twins would be closer and more dependent than their dizygotic counterparts; b) male peers would be more conflictive, less close and less dependent than female peers; c) older children would be considered by their mothers to be closer than younger children; d) girls would be more dominant in opposite sex pairs than their brothers.

Methodology

Participants

The participants were 882 Brazilian mothers of one to twelve-year-old twins (N = 1764 twins). Mothers' mean age was 35.8 years (SD = 6.14). The majority of the sample lived in the Southeast (62.4%) or South of Brazil (24.2%), but there were respondents from all regions of the country (North=1.49%, Northeast=5.6%, Midwest=5.3%), and a small number of Brazilians living abroad (1%). Most respondents had high educational levels (High School = 35.4%, College = 23.5%, and Graduate Course = 35.1%).

The average age of the children was 4.56 years (SD = 2.72). According to the zygosity assessed by a questionnaire developed by Christiansen et al. (2003) and adapted for parental reports, 41.6 and 58.4% of the twins were MZ and DZ, respectively. With respect to MZ twin pairs, 49.9% were boys and 50.1% girls, while the DZ twin pairs consisted of 26.6% boys, 28.3% girls and 45.1% opposite-sex.

Instruments

Zygosity Questionnaire. To assess twin zygosity, we adapted for parental reports and translated the 4-item questionnaire (Christiansen et al., 2003) into Brazilian Portuguese, which has been used in the zygosity assessment of the Danish Twin Registry for more than half a century.

Twin Relationship Questionnaire. The original English version of the 22-item Twin Relationship Questionnaire was developed by Fortuna et al. (2010) and validated by H. Segal and Knafo-Noam (2019). The Brazilian Portuguese version is being validated by our group (Ferreira et al., in preparation). The twins' mothers rated the degree to which each item described each of their twins, using a 5-point Likert scale ranging from 1 = not characteristic at all to 5 = very characteristic.

Procedure

Respondents completed the Brazilian online version of the questionnaire, available on the Painel USP de Gêmeos website[3]. This study was approved by the Research Ethics Committee of the University of São Paulo's Institute of Psychology (CAAE: 79708517.8.0000.5561).

Data analysis

We performed data cleaning procedures with Excel. Initially, the dataset contained 937 mothers of twins, but 55 were excluded because their children were classified as unknown zygosity on Christiansen's Questionnaire, leaving 882 mothers and 1764 individual twins. Descriptive statistics of respondents' sociodemographic characteristics were calculated and the distribution of twin zygosity was examined.

The Linear Mixed Models (LMM) with restricted maximum likelihood (REML) was used to investigate the possible influence of zygosity, sex, and age on the latent variables of TRQ. Zygosity, sex and their interaction were included as fixed factors, age as a covariate and the responding mothers as a random factor. DZ and female were used as default. As in Fortuna et al. (2010), we started our analysis by comparing MZ and DZ same sex twins (DZSS) only, in order to unconfound the possible effects of sex composition. Because we were interested in dimensions with dyadic characteristics, we used dependence, closeness, conflict and rivalry as latent variables. Given that the dominance dimension is expected to have little to no inter-twin correlations (H. Segal & Knafo-Noam, 2019), it was not used in this model. For this dimension, we compared the dominance of females and males by applying Repeated Measures ANOVA on dizygotic opposite sex twins (DZOS). The analyses were conducted using Stata version 14.

Results

The values of each factor were calculated for the children using factor scores standardized as z-scores. For this analysis, we considered only MZ and DZSS for the dimensions Dependence, Closeness, Conflict and Rivalry. The LMM analysis of mothers' TRQ responses revealed a main effect of zygosity on Closeness, $t\ (1,1295) = 2.50$, $p = 0.012$, and Dependence, $t\ (1,1295) = 2.55$, $p = 0.011$, but not for Rivalry, $t\ (1,1295) = 0.18$, $p = 0.855$ or Conflict, $t\ (1,1295) = 1.62$, $p = 0.105$. LMM also revealed a significant main effect of age on Closeness,

[3] https://www.paineluspdegemeos.com.br/

t (1,1295) = 3.40, p = 0.001 and a marginally significant effect on Dependence, t (1,1295) = 1.91, p = 0.057. A main effect of sex was found for proximity, t (1,1295) = 2.00, p = 0.046 and conflict, t (1,1295) = 2.44, p = 0.015.

According to mothers' perception, MZ twins were closer than DZSS, β = 0.54 (CI: 0.12-0.96) more dependent on one another than DZSS, β = 0.48 (CI: 0.11-0.85). With respect to age, the older the children, the closer β = 0.10 (CI: 0.05-0.15) and more dependent β = 0.05 (CI: 0.01-0.055) they were perceived to be by their mothers. Mothers considered male twins to be less close, β = -0.44 (CI: 0.01-0.87) and more conflictive than their female counterparts, β = 0.54 (CI: 0.15-0.93). No interaction effects were found.

Although we did not analyze dominance in terms of zygosity for the reasons previously explained, a number of studies show that girls tend to be more dominant than their male twin in psychological aspects (Ebeling et al., 2003). Thus, we decided to analyze only the DZOP through a paired t-test. As expected, our results revealed that mothers considered girls to be more dominant than boys, t (1,231) = 11,508, p<0.001. The average difference was 2.23 SD (CI: 1.84-2.60).

Monozygotic twins exhibited greater covariance in all the dimensions, with the exception of dominance, demonstrating that they are more similar in the characteristics evaluated than dizygotic twins. The average increase in covariance of MZ twins was 30.8% for closeness, 22.7% for dependence, 22.4% for conflict, and 13.3% for rivalry.

Discussion

To encourage twin research from a psychological perspective in Brazil and throughout Latin America, we created the Painel USP de Gêmeos (The University of São Paulo Twin Panel) in 2017 at the USP Institute of Psychology (Otta et al., 2019).

In our study on twin relationships conducted with Brazilian mothers, MZ twin children were considered closer and more dependent than their DZ counterparts. These results are in accordance with previous studies (N. Segal, 2011; Fortuna et al., 2010). Differences in the relationships between MZ and DZ twin children can be explained by the Kin Selection Theory. This theory predicts that the greater the genetic similarity, the more altruistically an individual tends to behave toward another (i.e., close relatives), thereby contributing to increasing their inclusive fitness (Hamilton, 1964). This behavior is also associated with the similarity of psychological aspects, which contributes to a more intense attachment bond between MZ twins (Landenberger, 2021).

Additionally, MZ twin children showed greater covariance for proximity, dependence, rivalry and conflict than DZ twins. These results were expected since MZ share virtually 100% of their genes and therefore tend to be more similar in most temperamental and behavioral characteristics (Scott et al., 2016).

Our results showed that twins' proximity and dependence increased with age. Based on developmental patterns, it can be argued that children gradually develop abilities that are important for interpersonal relationships (Dumontheil et al., 2010), thereby improving their interpersonal skills from early childhood onwards. As in Vandell et al. (1988), our results showed that older siblings were considered closer by their mothers than their younger counterparts.

Considering same sex dyads, we found that boys were deemed less close and more conflictive than girls, regardless of their zygosity. We expected that girls would also be more dependent than boys, but this was not the case. This may have happened because our hypothesis was based on the results with adolescents (Penninkilampi-Kerola and Moilanen, 2005), and our study was with children. In addition, results regarding dominance in DZOP corroborate the findings of Ebeling et al. (2003), who explored the asymmetry of the twin relationship and found that girls were more dominant than boys in the psychological and verbal domains, before and during school age.

Conclusion

This was the first Brazilian study on twin relationships in childhood considering the five dimensions of TRQ. Similarly to studies conducted in other cultures, monozygotic twins were perceived by their mothers to be closer and more dependent than dizygotic twins, in line with the Kin Selection Theory. Our study also confirms that male peers were considered by their mothers to be more conflictive and less close than female peers. In addition, in opposite-sex pairs, the mothers reported that the girls were more dominant than the boys. In regard to age, our results showed that twins' proximity and dependence increased over time. The study of twin relationships is of great importance, especially in family and school contexts. Thus, understanding the peculiarities of the twin relationship dynamic will contribute to implementing better twin-related practices, as well as promoting policies that account for the specific needs of each pair and their well-being.

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Chapter 8

Building identity in twins: some reflections

Maria Elizabeth Barreto Tavares dos Reis

How does it feel to be a twin? How does it feel not being a twin?

These questions, at first, may seem naïve and even irrelevant, but they require some reflection.

Each human being is unique and should be considered as such from birth; however, with twins, experiences are sometimes different. From pregnancy onward, fathers and specifically, mothers are concerned with welcoming their twins in a similar manner.

Analyzing the comments of mothers in face-to-face situations or through social networks, we observed some shared circumstances in which they expressed concerns when their twins displayed differences in demands or pace of development. The preparation of the babies' bedroom and layette also caught our attention, since the similarities in clothes and objects were striking.

Another aspect to consider is the way mothers seek to relate to their twin babies and how much they worry when they realize that both are not relating to her in kind. For instance, some years ago, as a clinical psychologist, I was contacted by parents of one-year-old twins with a double concern: the boy was overly attached to his mother and tried to stay at her breast most of the day, while the girl refused to breastfeed (Reis, 2015). At the time of birth, the girl had to stay in hospital longer than her brother. The mother seemed to feel guilty and insisted on nursing her because she thought the girl had missed the benefits of early breastfeeding. At this point, it was possible to see the parents' difficulties in recognizing and respecting the differences and singularities of each baby. Thus, I helped the parents understand the different characteristics and needs of each twin.

Reflecting on how parents address their twins, I highlight some of the situations I witnessed when collecting data for my doctoral thesis (Reis, 2007), such as dressing them identically, giving them similar names or identical nicknames.

In this chapter, I invite readers to reflect on the circumstances experienced by twins and single-born children from birth on and how they interfere with the development of the person's identity.

As psychologists and psychoanalysts have reported for decades, we know that genetic, maturational, environmental, and affective-emotional factors contribute to the building of identity.

Identity is how people perceive themselves in their inner world, their way of being, and how they relate to the external world.

At the beginning of life, children feel so integrated with their mother that they do not even perceive themselves as being separated from her. As Winnicott (1979) states, in the eyes of the observer, there are two beings, but from the baby's point of view, there would only be one. In the process of physical, cognitive, and emotional maturation, individuation advances as children realize that they and their mother are two separate entities.

A recent integrative literature review (Gallo, Reis, & Cordeiro, 2020), found only 10 articles and five theses addressing individuation in twins. The other papers focused on socio-cultural aspects, educational practices in the family and at school, the mother's relationship with her twins, and the relationship between the twins themselves. We noticed the importance given to the mother's enabling each twin to be identified, perceived, and attended to according to their characteristics. At school, the authors disagree as to whether they should share the same classroom or not.

The few papers on the relationship between twins showed that they sometimes help each other, occasionally as the mother's substitute. However, to babies, the mother is the principal figure to offer care and comfort. The process of individuation, that is, the baby's need to develop and perceive himself/herself as separated from the other, is inherent to human beings. Twins face more challenging situations in the process because they need to separate themselves from both the maternal figure and their co-twin.

Observing how twins relate to each other, we noticed that they exchange glances and vocalizations from a very early age and, by 10/11 months, already play together, giving the impression that the affective bond between them is very intense and that the shared attention and playing somehow makes up for the mother's momentary absence.

Drawing upon these ideas, we need to reflect on the perception of the twins themselves regarding their relationship and the way they consider their individuation. Reis, Cordeiro and Simon (2018) interviewed 12 pairs of adult twins about their life histories and evaluated them using the Escala Diagnóstica Adaptativa Operacionalizada Revisada (EDAO-R) [Revised Operationalized Adaptive Diagnostic Scale]. They found that effectively-adapted twins had generally been identified by their parents since birth (even if they used artificial resources such as different clothes or adornments) and

in adolescence attempted to use these resources in order to differentiate themselves and facilitate recognition by other people.

One pair of twins that exhibited a mild adaptation crisis faced a number of difficulties in adulthood, likely because they were perceived and cared for in a very similar manner and wore the same clothes until the age of 15. "It seems that they need to maintain symbiosis to avoid chaos, as if the individualized experience could favor the success of the co-twin or even their own" (Reis, Simon, & Nogueira, 2018, p. 151). The study also addresses circumstances in which both twins had taken the same undergraduate course and worked for the same companies, giving the impression that the individuation process and the construction of one's own identity could be compromised.

The theoretical and empirical research we have conducted at the State University of Londrina, including observations of mother-baby relationships, interviews with mothers, educators, and adult twins, as well as psychological practice in clinical care and the supervision experience, lead us to believe that some factors may facilitate individuation in twins. Among the factors that facilitate this process are recognizing the physical and emotional characteristics of each twin from birth onward, using different adornments (bracelets, for instance) that enable identification, especially with identical twins, wearing different clothes, and avoiding similar names or nicknames.

Paraphrasing Winnicott (1979), I believe that if the individuation process occurs satisfactorily in twins, they can develop their own identity and respect their needs and desires, thereby favoring a healthier and more independent experience.

We underscore that the development of identity is a lifelong and continuous process of maturation and search for independence. We all depend on something or someone and are not totally independent. Nevertheless, those who manage to establish a personal identity are healthier and more independent to establish their place in the world, whether in affective, family, professional, or social relationships.

This chapter invites parents and educators to be attentive to the relationship dynamics created and foster the individual identity of twins. Differentiation is necessary for the healthy development of twins. Insight into these issues can improve parenting and educational practices that respect each individual's characteristics, aiming to promote their healthy social-emotional development and ability to adapt to new life circumstances. Twins should be encouraged to live their own life whenever possible, and share joys and sorrows with their co-twin without needing their presence to live a full life.

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Chapter 9

Mate Preference and Sexual Orientation in Twin Pairs

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This chapter presents the results of three research projects conducted on Brazilian twins at the Center for Behavioral Theory and Research of the Federal University of Pará. Two of these projects focus on human mate choice and the third on sexual orientation. Here, we present only preliminary results, since our data collection is still underway. We dedicate this chapter to our group leader, Professor Regina Brito (1951-2020), who left us too soon and unexpectedly.

Preference for Ideal Partners in Twins

Many studies in Evolutionary Psychology suggest that men and women show different mate choice criteria (Buss & Schmitt, 2016; Geary et al., 2004; Trivers, 1972). Although men's and women's criteria for long-term partners are similar, favoring traits such as kindness and intelligence (Altafim et al., 2017), women tend to prefer men who seem to be good resource providers, invest in affectional bonds, and are attractive (Buss, 1989; Buss & Kenrick, 1998; Buss & Schmitt, 1993; Geary et al., 2004; Kriegman, 1999), while men tend to have a higher preference for characteristics associated with physical attractiveness (Buss, 1989; Buss & Schmitt, 1993).

Ultimately, the sex differences in mate choice for long-term relationships probably arose from differential investment in offspring. According to Trivers (1972), the sex that invests more in offspring care tends to be more selective in mate choice, while the sex that invests less in offspring tends to show higher competition for mating partners. In our species, females invest more time, energy and resources in offspring (e.g., gestation, lactation and child care), and they tend to be more selective than males in the choice of a long-term mate (Buss, 2006; Trivers, 1972).

Some studies have investigated how human mate choice is affected by genetic and environmental factors. Environmental factors that influence human mate choice have been widely investigated (for a review, see Buss & Schmitt 2019). On the other hand, several studies comparing monozygotic and dizygotic twins suggest a genetic influence on human mate choice. When compared to dizygotic twins, monozygotic twins have greater similarities in the criteria for choosing long-term partners (Verweij et al., 2014) and in the desirability of altruistic behaviors in a potential mate (Phillips et al., 2010), as well as a higher tendency to choose partners more similar to those of their co-twins (Rushton & Bons 2005). Assortative mating, or homogamy, may be explained by the genetic similarity theory as an extension of inclusive fitness theory (Hamilton, 1964) to incorporate mates. Russell, Wells and Rushton (1985) proposed that humans are capable of detecting genetic similarity and prefer others who are similar to them. Mating with partners who are genetically moderately similar to themselves may assure people that their own segment of the gene pool will be transmitted to future generations. In fact, humans tend to favor traits similar to their own when choosing mates (such as social aspects, socioeconomic status, personality, education, and physical characteristics), and it is suggested that this can decrease the likelihood of conflict between the couple, thus having an adaptive potential (Dijkstra & Barelds, 2008; Furnham, 2009; Lucas et al., 2004). However, this does not mean that humans choose extreme similarities between their partners' characteristics and themselves, and in fact, for the sexual selection of some traits, such as body scent, it has been shown that 100% is not an optimal amount of similarity (Jacob, et al., 2002). Odor preferences for dissimilarity regarding the major histocompatibility complex (MHC) maybe involved in achieving an optimal level of genetic variability (Havlicek & Roberts, 2009). Compared with disassortative odor preferences, facial preferences appear to be assortative (Roberts et al., 2005).

As far as we know, there are no studies on the preference of Brazilian twins for psychological characteristics and the somatotypes of romantic partners. The goal of our two ongoing human mate choice projects is to investigate the preference for ideal physical and psychological characteristics in romantic partners in Brazilian female monozygotic and dizygotic twins.

Sexual Orientation in twins

Sexual orientation has been defined as the individual's development of sexual behavior and fantasies towards other individuals (Balthazart, 2012). The American Psychological

Association (APA, 2013) adopts the following multidimensional definition:

"Sexual orientation refers to an enduring pattern of emotional, romantic, and/or sexual attraction to men, women, or both sexes. Sexual orientation also refers to a person's sense of identity based on these attractions, related behaviors, and membership in a community of others who share those attractions. Research over several decades has demonstrated that sexual orientation ranges along a continuum, from exclusive attraction to the other sex to exclusive attraction to the same sex."

The Klein Sexual Orientation Grid (Klein, 2014; Klein et al., 1985; Appendix 1) assesses different dimensions of sexuality – (a) Sexual Attraction, (b) Sexual Behavior, (c) Sexual Fantasies, (d) Emotional Preference, (e) Social Preference, (f) Self-Identification and (g) Heterosexual/homosexual Lifestyle – that compose a person's sexual orientation in distinct periods of life (past, present and ideal). Considering these three time periods, the Klein Sexual Orientation Grid takes into account that sexual orientation may not be fixed. According to Klein (2014), sexual orientation is fluid for many people, who move across the spectrum, move part way or go back and forth.

If monozygotic twins share the same genetic code, they may exhibit some degree of agreement for sexual orientation, demonstrating the influence of hereditary biological variables that may contribute to the expression of sexual orientation. A study with female twins found a heritability of 40% for characteristics involving the sexuality of participants, gender nonconformity in childhood, number of lifetime sexual partners and sexual orientation (Burri et al., 2015). Another study investigating the number and frequency of sexual practices with same-sex partners among Swedish twins found a heritability of 39% for a lifetime same-sex partner and of 34% for the total number of same-sex partners in men, and of 18 and 19% in women, respectively (Långström et al., 2010). Kirk et al. (2000) investigated sexual orientation among Australian twins and found heritability estimates for homosexuality between 50 and 60% in women, but significantly lower in men (about 30%). In a sample of 161 pairs of male siblings, Bailey and Pillard (1992) found 52% agreement for sexual orientation in monozygotic twins, 22% in dizygotic twins and 11% in adoptive siblings. In general, the scientific literature has shown higher agreement regarding sexual orientation in monozygotic than in dizygotic twins (Whitam et al., 1993). The existence of genetic effects does not exclude the importance of environmental effects — in fact, the low-to-intermediate heritability values for sexual orientation strongly suggest

that environmental factors also play an important role in sexual orientation (Dawood et al., 2009).

As far as we know, there are no studies on the sexual orientation of Brazilian twins using the Klein Sexual Orientation Grid. With its seven dimensions and three time periods, the Klein Sexual Orientation Grid captures various aspects of sexual orientation, not limiting individuals to the three usual labels: 'heterosexual', 'homosexual' and 'bisexual'. Although it may be somewhat more burdensome than the usual categorization, it is our conviction that twin research would greatly benefit from assessing the multiple dimensions and temporal heterogeneity of sexual orientation. The goal of our ongoing sexual orientation project is to investigate the degree of agreement in sexual orientation domains in monozygotic and dizygotic Brazilian twins using the Klein Sexual Orientation Grid.

Methods

Ethical considerations

This research was approved by the Comissão Nacional de Ética em Pesquisa (Brazilian Research Ethics Committee, protocol number 1.879.610). All participants gave their written informed consent to participate in this study.

Participants

Choice for Ideal Partners in Twins. We show data from 23 pairs of heterosexual female twins (18 monozygotic and 5 dizygotic pairs). Participants were between 18 and 49 years old (24.2 ± 5.39 yrs).

Sexual orientation in twins. To date, we collected data from 60 pairs of monozygotic twins (14 male pairs and 28 female pairs) and 18 pairs of dizygotic twins (1 male pair, 6 female pairs and 11 opposite-sex pairs). Participants were between 18 and 49 years old (24.4 ± 5.54 yrs).

Materials and Procedure

Twin Registration Form: built and managed by the Grupo de Estudos Avançados em Psicologia Evolucionista (Group of Advanced Studies in Evolutionary Psychology) at the Federal University of Pará, it aims to recruit twins for behavioral research.

Zygosity Inventory: this instrument (Hora, 2011) contains a set of questions about physical similarity and the ability of family members, friends and teachers from childhood to the present to tell the twins apart. It was used to determine the twin pair's zygosity, and was completed by the pair themselves.

Desirable Attributes of Ideal Partner Scale: this instrument, developed and validated by Gouveia et al. (2014), evaluates the desirable psychological characteristics of ideal partners in a long-term relationship. It consists of 20 attributes evaluated on a five-point scale, in terms of their importance to the respondent (1 = not important at all to 5 = extremely important). The attributes can be grouped into five components: Affectionate (loving, good nature, kind and companion), Athletic (muscular, in good shape, sexy, handsome), Sociable (attentive, determined, tolerant, gentle), Traditional (sensitive, homely, from a good and supportive family) and Accomplished (studious, cultured, successful and determined).

Male somatotypes figures: women were asked to rate the attractiveness of outline drawings of male figures (Dixson et al., 2003). In this instrument, nine illustrations of back-facing males with different somatotypes were presented (Figure 9.1): ectomorph (ECTO), endomorph (ENDO) and mesomorph (MESO). Participants used a 6-point Likert scale to evaluate attractiveness (0 = unattractive, 1 = slightly attractive, 2 = mildly attractive, 3 = moderately attractive, 4 = very attractive and 5 = extremely attractive).

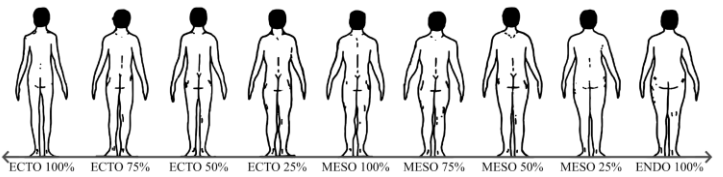

FIGURE 9.1. MALE SOMATOTYPES FIGURES (ADAPTED FROM DIXSON ET AL. 2003).

Klein Sexual Orientation Grid: this instrument (see Appendix 9.1) assesses seven different domains of sexual orientation (sexual attraction, sexual behavior, sexual fantasies, emotional preference, social preference, life preference and sexual identity), in three different periods: past, present and ideal (Klein, 2014).

Procedure

Invitations to participate in the surveys were sent via email, social media, electronic newspapers, radio and television. When twins accepted our invitation, an initial contact was made through social media or by phone and they completed the twin registration form. All the data regarding the partner selection projects were collected online. The data from the sexual orientation project was collected both in

person (21 pairs) and online (39 pairs). We considered that the differences in the procedure did not influence the data, since statistical analysis did not reveal significant differences between the in-person and online groups. Google Forms was used for online data collection. In-person data collection was carried out at the Laboratório de Estudos avançados em Psicologia Evolucionista (LEAPE - Laboratory of Advanced Studies in Evolutionary Psychology) at the Federal University of Pará.

Statistical Analysis

We used intraclass correlation tests to assess agreement between the pairs of twins regarding the components of the Desirable Attributes of Ideal Partner Scale, the preferences for male somatotypes figures, and the different domains and the three periods of the Klein Sexual Orientation Grid.

Results

Choice for Ideal Partners in Twins

Figure 9.2 shows the average preference of our female participants regarding the Desirable Attributes of the Ideal Partner Scale (Figure 9.2A) and the male somatotypes (Figure 9.2B). The results reveal that the non-physical attributes most valued by the participants were "affectionate" and "sociable", and the most valued physical somatotype was the 100% mesomorphic.

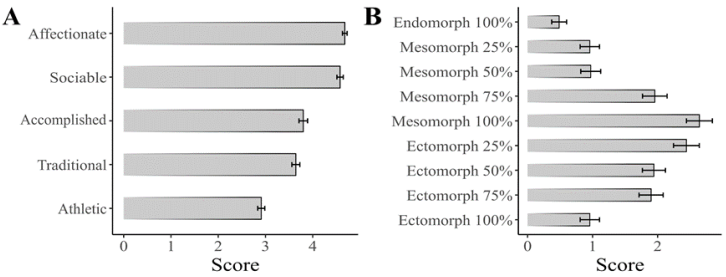

FIGURE 9.2. MEAN ± SE SCORE GIVEN BY THE 23 FEMALE TWIN PAIRS FOR THE (A) DESIRABLE ATTRIBUTES OF THE IDEAL PARTNER SCALE AND (B) MALE SOMATOTYPE FIGURES.

Figures 9.3 and 9.4 show the results of intraclass correlation tests for monozygotic and dizygotic twins for both the Desirable Attributes of the Ideal Partner Scale (Figure 9.3) and male somatotypes (Figure 9.4). Intraclass correlation tests for the Desirable Attributes of the Ideal Partner Scale (Figure 9.3) revealed a greater number of significant intra-class correlations for the 18 monozygotic pairs (for the

"affectionate", ICC = 0.40, $p < 0.05$; "sociable", ICC = 0.58, $p < 0.0$; and "traditional" components, ICC = 0.52, $p < 0.05$) than for the 5 dizygotic pairs (only the "traditional" component, ICC = 0.65, $p < 0.05$). Intraclass correlation tests for the preference for male somatotypes (Figure 9.4) revealed several significant intraclass correlations for the 18 monozygotic twins (in Ectomorph 50%, ICC = 0.48, $p < 0.05$; Ectomorph 75%, ICC = 0.42, $p < 0.05$; Ectomorph 100%, ICC = 0.51, $p < 0.01$; and Mesomorph 25%, ICC = 0.43, $p < 0.05$), but never for the 5 dizygotic twins.

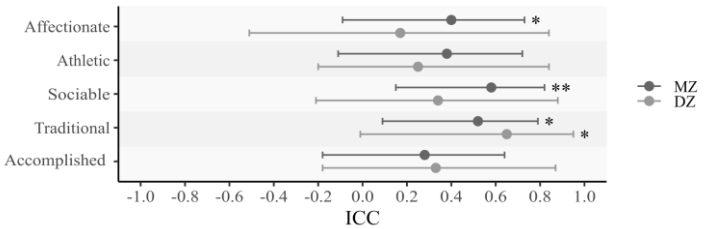

FIGURE 9.3. INTRACLASS CORRELATION COEFFICIENTS (ICC) FOR EACH COMPONENT OF THE DESIRABLE ATTRIBUTES OF IDEAL PARTNER SCALE FOR MONOZYGOTIC (MZ, 18 PAIRS) AND DIZYGOTIC (DZ, 5 PAIRS) FEMALE TWINS. DOTS REPRESENT THE ICC AND BARS THE 95% CONFIDENCE INTERVALS. *P < 0.05; **P < 0.01.

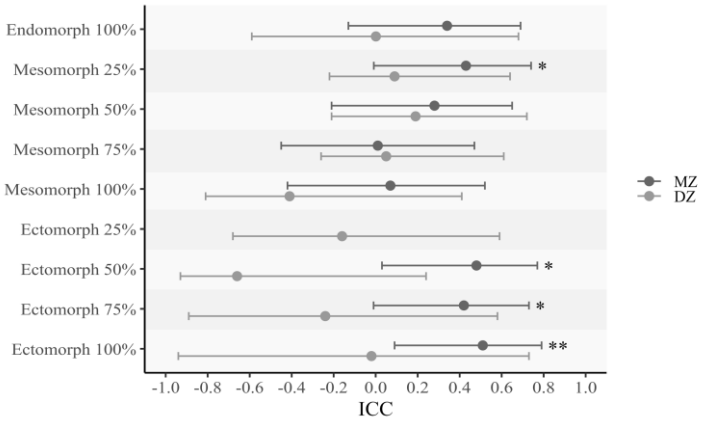

FIGURE 9.4. INTRACLASS CORRELATION COEFFICIENTS (ICC) OF THE SCORE FOR EACH MALE SOMATOTYPE GIVEN BY MONOZYGOTIC (MZ, 18 PAIRS) AND DIZYGOTIC (DZ, 5 PAIRS) FEMALE TWINS. DOTS REPRESENT THE ICC AND BARS THE 95% CONFIDENCE INTERVALS. *P < 0.05; **P < 0.01.

Sexual Orientation in twins

Figure 9.5 shows the results of the intraclass correlation for the domains of sexual attraction, sexual behavior and sexual identity

assessed by the Klein Sexual Orientation Grid. For the 42 pairs of monozygotic twins, the sexual attraction, sexual behavior and sexual identity domains showed significant intraclass correlation coefficients for all three periods (past, present and ideal), with values between 0.28 and 0.43. We found no significant intraclass correlation coefficients among the 18 pairs of dizygotic twins, with most of the intraclass correlation estimates quite low.

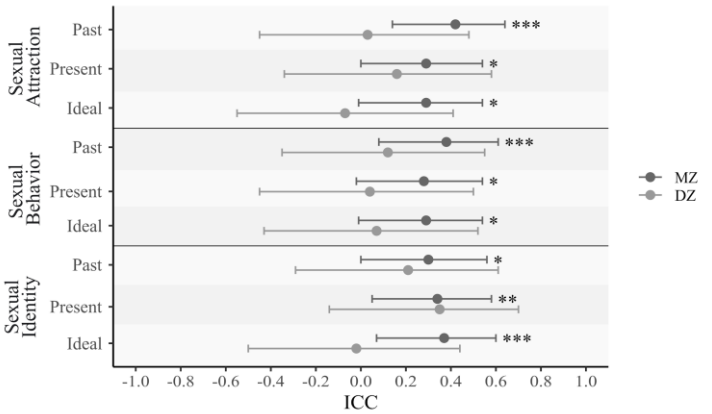

FIGURE 9.5. INTRACLASS CORRELATION COEFFICIENTS (ICC) FOR SEXUAL ATTRACTION, SEXUAL BEHAVIOR AND SEXUAL IDENTITY FOR MONOZYGOTIC (MZ, 42 PAIRS) AND DIZYGOTIC (DZ, 18 PAIRS) TWINS. ERROR BARS INDICATE 95% CONFIDENCE INTERVALS. *P < 0.05; **P < 0.01; ***P < 0.001.

Discussion

Preference for Ideal Partners in Twins

With regard to male somatotypes, female twins preferred the mesomorphic (muscular) male somatotypes, while the 100% endomorph somatotype (with higher percentage of body fat and little muscle definition) was considered less desirable by all female twins (Figure 9.2B). This result is in agreement with the reported preference for mesomorphic male somatotypes in heterosexual females (Dixson et al., 2003). Bearing in mind that mesomorphic males perform best in physical fitness tests (Sugiyama, 2005), the women's preference for these men might reflect an evolutionary strategy of using male somatotype as a cue of a potential mate's health.

The results of the Desirable Attributes of Ideal Partner Scale can be interpreted from the perspective of evolutionary theories of long-term romantic partner preferences, at least with respect to two of the analyzed components. The affectionate component, which includes attributes related to emotional investment (loving, good natured, kind

and companion), was the most relevant for the 23 women who participated in this study (Figure 9.2A). Paternal investment is unusually high in humans compared to most mammals and primates. The huge costs of female reproduction and the high parental care requirements of dependent infants suggest that paternal support may have provided a selective advantage to offspring survival rate and reproductive success (Bribiescas, Ellison & Gray, 2012). The attributes that make up the Affectionate component may function as cues of a prospective partner's willingness to invest in children.

In the Desirable Attributes of Ideal Partner Scale, the 18 monozygotic twin pairs showed significant agreement in the Affectionate, Sociable and Traditional domains, while the 5 dizygotic twin pairs only had significant agreement in the Traditional domain (Figure 9.3). This preliminary result is in line with other studies. Rushton and Bons (2005) found that monozygotic twins, who share ~100% of their genes, are approximately twice as similar in personality and social attitudes as dizygotic twins, who share ~50% of their genes. They also found that monozygotic twins chose spouses more similar to their co-twins' friends and spouses in socioemotional and personality characteristics than do dizygotic twins. However, there are also contradictory results. In a large community-based sample of twins and their partners, Zietsch et al. (2011) found near-zero genetic influences on mate choice. However, they found evidence of assortative mating, interpreting it as a result of social homogamy, where individuals pair according to social or environmental background similarities. According to Robinson et al. (2017), the causes and genetic consequences of assortative mating remain unresolved because partner similarity can arise from different mechanisms: phenotypic assortment based on mate choice, partner interaction and convergence in phenotype over time, or social homogamy, where individuals pair according to social or environmental background.

Similarly, in intraclass correlation tests for the somatotype scores of the female twins, the 18 monozygotic twin pairs showed higher agreement than the 5 dizygotic twin pairs. In fact, while the monozygotic pairs showed significant agreement in four of the nine somatotypes presented, the dizygotic pairs did not reveal significant agreement in any of the somatotypes (Figure 9.4). But these are preliminary results and any conclusion depends on enlarging sample size. We are increasing the sample and will compare our results with those of other studies on human mate preferences for general and sexually dimorphic physical traits (e.g., height, hair color, breast size) (Mitchem et al., 2013; Verweij et al., 2012).

Sexual Orientation in twins

Our preliminary results for sexual orientation (assessed by Klein's Sexual Orientation Grid) suggest higher agreement rates in the 42 monozygotic twin pairs when compared to the 18 dizygotic twin pairs. This could indicate a genetic basis for sexual orientation, corroborating other twin studies that suggest a heritable component of sexual orientation (Kirk et al., 2000, Bailey and Pillard 1992) in twins. An alternative explanation is that similarities in prenatal androgen secretion, or even in receptor sensitivity to prenatal androgens (Collaer & Hines, 1995, Berenbaum et al., 2011), could be behind the higher agreement in sexual orientation in monozygotic twins when compared to their dizygotic counterparts. In fact, about one-third of monozygotic twin fetuses develop in separate placentas, a factor that may differentially regulate the hormone level transferred from the mother to the fetus (Watts, Holmes, Raines, Orbell & Rieger, 2018).

Genetic and Environmental Effects

Although we found greater agreement in mate choice and sexual orientation in monozygotic twins, suggestive of genetic effects, it is important to underscore that this does not exclude the presence of important environmental effects. The importance of environmental effects has been previously suggested in several studies (e.g., Kirk et al. 2000). In addition, the generally intermediate values of intraclass correlation coefficients that we obtained for monozygotic twins (and that can be considered an upper bound heritability estimate) both for mate choice and sexual orientation, suggest that environmental effects also play an important role in the expression of these traits. However, it remains unclear which environmental variables alter the expression of mate choice and sexual orientation.

General Limitations

Our results are preliminary, and their limitations should be taken into account. Voluntary participation in research is frequently subject to some bias (e.g., Prescott, & Kendler, 1995). For example, it is known that both monozygotic twins and women are more prone to participating in voluntary research actions, when compared to dizygotic twins and men, respectively (Boomsma et al., 2002; Polderman et al., 2015). As expected, this bias also affects our ongoing research. For the mate preference projects, we cannot present the results for men due to their lower participation rate, and our female sample comprises 18 monozygotic and 5 dizygotic twin pairs. For the sexual orientation project, the same biases were present (14 pairs of

MZ male twins, 28 pairs of MZ female twins, 11 pairs of DZ opposite sex twins, 6 pairs of DZ same-sex females, 1 pair of DZ same-sex males). We intend to balance our final samples both in terms of sex and zygosity. However, at present, we cannot exclude the possibility that the general lack of significance of the intraclass correlations in the group of female dizygotic twins might be due to the smaller sample sizes of this group.

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Appendix 9.1. Brazilian version of the Klein Sexual Orientation Grid (or KSOG) - Grade de Orientação Sexual de Klein[4]

Para responder os itens a seguir, considere o PASSADO (a vida toda até um ano atrás), o PRESENTE (até 12 meses atrás) e a situação IDEAL (como você gostaria que fosse). Responda usando a escala a seguir de 1 a 7.

Grade de Orientação Sexual de Klein

Variáveis	Passado	Presente	Ideal
A. Atração Sexual			
B. Comportamento Sexual			
C. Fantasias Sexuais			
D. Preferência Emocional (de quem você gosta e ama)			
E. Preferência Social (homens vs. mulheres)			
F. Estilo de vida (orientação sexual das pessoas com as quais você passa seu tempo)			
G. Auto-Identificação			

[4] Source: Weinrich, J. D., Klein, F., McCutchan, J. A., Grant, I., & the HNRC Group (2014). Cluster Analysis of the Klein Sexual Orientation Grid in Clinical and Nonclinical Samples: When Bisexuality Is Not Bisexuality. Journal of Bisexuality, 14(3-4), 349–372.

Descrição dos itens
A) Atração Sexual: Por pessoas de quais sexos você se sente sexualmente atraído? **B) Comportamento Sexual:** Com pessoas de quais sexos você tem relações sexuais? **C) Fantasias Sexuais:** Sobre pessoas de quais sexos são suas fantasias sexuais? (Podem ocorrer quando você se masturba, sonha ou simplesmente imagina) **D) Preferência Emocional:** Você se relaciona amorosamente (namoro, casamento, etc.) com pessoas do mesmo sexo, do sexo oposto ou de ambos os sexos? **E) Preferência Social:** Com pessoas de quais sexos você socializa? Seu círculo social é formado por pessoas de quais sexos? **F) Estilo de Vida:** Qual a identidade sexual das pessoas com quem você socializa? (qual a orientação sexual das pessoas com quem você socializa?) **G) Auto-Identificação:** Como você se identifica? (qual a sua orientação sexual?)

Para responder os ítens A a E:		**Para responder os ítens F e G:**
1=Somente o outro sexo		1=Somente heterossexual
2=Geralmente o outro sexo		2=Geralmente heterossexual
3=Mais ou outro sexo		3=Mais heterossexual
4=Igualmente ambos os sexos		4=Igualmente hetero/homossexual
5=Mais o mesmo sexo		5=Mais homossexual
6=Geralmente o mesmo sexo		6=Geralmente homossexual
7=Somente o mesmo sexo		7=Somente homossexual

English version of the Klein Sexual Orientation Grid (or KSOG)

Use the following choices to answer questions about the Past (Your life up to 12 months ago), Present (The most recent 12 months) and Ideal (What do you think you would eventually like?).

Variable	Past	Present	Ideal
A. Sexual Atraction			
B. Sexual Behavior			
C. Sexual Fantasies			
D. Preferência Emocional (whom you love and like)			
E. Socialize with (men vs. women)			
F. Lifestyle (sexual orientation of people with whoom you spend time)			
G. Self-Identification			

Definitions of rating scale values

Scale for A - E:		Scale for F e G:
1=Other sex only		1=Heterosexual only
2=Other sex mostly		2= Heterosexual mostly
3=Other sex somewhat more		3=Heterosexual somewhat more
4=Both sexes equally		4=Heterosexual/homossexual equally
5=Same sex somewhat more		5=Homosexual somewhat more
6=Same sex mostly		6=Homosexual mostly
7=Same sex only		7=Homosexual only

Chapter 10

Twins Reared Apart: History, Findings, Unique Cases and Implications

Nancy L. Segal

Reared-apart twins are relatively rare, but enormously informative when it comes to studying and understanding human development. Classic twin studies contrast the level of trait resemblance between monozygotic (MZ or identical) and dizygotic (DZ or fraternal) twin pairs. The logic behind these undertakings is simple, yet elegant. MZ twins share 100% of their genes, whereas their DZ counterparts share 50%, on average, by descent. Greater MZ than DZ twin resemblance is indicative, but not proof of genetic influence on the characteristics under study (Knopik, Neiderhiser, DeFries, & Plomin, 2016). This is because twin studies must satisfy the equal environment assumption or EEA, namely that the environments of MZ twins purposefully align more closely than those of DZ twins, increasing their similarity. However, most inquiries have produced little evidence that the EEA was violated (Segal, 2012).

Studying the relatively rare occurrence of MZ and DZ twins reared apart from birth (MZA and DZA pairs, respectively) is a way to manage possible environmental bias. Genetic factors underlie MZA twin similarities because they grew up apart. Including DZA twins in the study offers opportunities to examine various gene x environment interactions, in addition to providing a crucial comparison group. MZ and MZA twin resemblance is less impressive than their resemblance to other kinship groups, especially virtual twins (VTs). I will discuss this in more detail later.

This chapter presents a brief chronology of reared-apart twin studies. A unique case involving two doubly switched-at-birth MZ male twins from Colombia will also be described. This is followed by a discussion of current and future directions in twin research.

Historical Perspectives: Twins Raised Apart

The first formal study of reared-apart twins was conducted by Popenoe (1922) in his case study of MZA twins, Bessie and Jessie.

These twins were studied again by Muller (1925). The first formal study of reared-apart twins took place in Chicago, IL and was reported by Newman, Freeman and Holzinger (1937). Their book presents findings on a range of psychological and physical measures for 19 MZA, 50 MZT (MZT; reared together) and 50 DZ twin pairs (DZT). Significant findings were MZA IQ correlations of r_i = 0.64-0.68, within-pair correlations between IQ and educational differences of r_i = 0.55-0.79 and within-pair correlations between IQ and social differences of r_i = 0.51-0.53. The correlations involving IQ and the environment are sometimes misconstrued. Critics suggested that the findings cancelled out the genetic effects on IQ, reducing the role played by parenting. However, the IQ correlation showing that co-twins are more like each other than they are to the members of other pairs supports genetic effects.

Next, British investigator James Shields from England, studied 44 MZA twin pairs and provided brief data for 11 DZA twin pairs (Shields, 1962). He also included comparative data on 44 MZT and 32 DZT twin pairs. Several of his personality results are striking: MZA twins were more similar than their MZT counterparts in extraversion (r_i = 0.61 and 0.42, respectively) and neuroticism (r_i = 0.53 and 0.38, respectively). Shields suggested that separated twins express their genetically-based characteristics more freely when they are not in a relationship with their twin. Other investigators have reported similar results when comparing similarity in divergent thinking and extraversion between MZT twins living together and apart (Wilde, 1964; Claridge, Hume & Canter, 1973). By contrast, this result was not upheld for psychosomatic complaints (Wilde, 1964). Recent reared-apart studies have failed to find these effects. Instead, they have reported that MZA and MZT twin pairs show similar levels of personality resemblance (Tellegen, Lykken, Bouchard, Wilcox, Segal, & Rich, 1988). The reasons for this discrepancy are unknown, but could be associated partly with the different personality inventories administered. Anecdotally, my colleagues and I have found that when MZ twins are together, one tends to dominate the conversation and that they appear to interact more similarly with others than when they are apart.

Niels Juel-Nielsen published his study of 12 separated Danish twins several years later (Juel-Nielsen, 1965), with a follow-up in 1980. Juel-Nielsen's pairs were the entire population of reared-apart twins born between 1870 and 1910. He had access to a registry at the Institute of Human Genetics, of the University of Copenhagen, although some pairs born after 1910 were obtained from other sources. The author found few co-twin differences, generally health-related;

similarities and differences in somatic disorders; differences in verbal areas, intellectual differences associated with differences in educational levels, personality similarities associated with genetic factors; and personality differences in areas associated with interpersonal contact. Childhood psychiatric symptoms were infrequent among these 12 pairs.

The Minnesota Study of Twins Reared Apart, directed by Dr. Thomas J, Bouchard, Jr., at the University of Minnesota, in Minneapolis, was launched in 1979 and conducted until 1999 (Bouchard, Lykken, McGue, Segal, & Tellegen,1990; Segal, 2012). This is the only study that included both MZA and DZA twins. This methodological feature avoided possible exclusion of dissimilar MZA pairs.

Minnesota Study of Twins Reared Apart

Twins spent an entire week at the University of Minnesota undergoing extensive medical and psychological examinations. The IQ correlation for 48 MZA twin pairs was $r_i = 0.69$ (primary test) and 0.79 (mean of three tests) (Bouchard et al., 1990). In addition, the Minnesota twins' IQ scores were not associated with their parents' education, home facilities, family achievement or intellectual motivation. The weighted average IQ intraclass correlation, based on 162 MZA twin pairs from the four studies plus one study from Sweden (Pedersen, Plomin, Nesselroade, & McClearn, 1992), was $r_i = 0.75$ (see Segal, 2012). The five correlations across studies show little variation. This is noteworthy because the studies were conducted by different investigators using different tests, and working in different countries. The Minnesota study later reported a heritability estimate of 0.77 for general intelligence (Johnson, Bouchard, McGue, Segal et al., 2007). However, intelligence is also influenced by environmental factors. Turkheimer, Haley, Waldron, d'Onofrio, and Gottesman (2003) found that the IQ scores of young twins from low socioeconomic status families showed reduced heritability.

MZA twins were as similar as MZT twins across eleven personality traits (median $r_i = 0.48$ and 0.52, respectively; see Tellegen et al., 1988). This indicates that personality similarities between biological relatives living together are explained by their common genes, not by their common environment. Heritability estimates were also approximately 0.50 for religiosity, social attitudes and periodontal characteristics. It had generally been assumed that these traits were shaped mostly by environmental factors. For example, prior research had shown that young MZ and DZ twins were equally alike in religiosity—however, studies of adult twins showed that MZ twins were more alike than their

DZ counterparts. It appears that as people age and are more able to make their own lifestyle choices, their genetic inclinations are more freely expressed.

Other reared-apart twin studies have been carried out or are ongoing in Japan, China, Sweden, Finland and the United States (see Segal, 2012). I will briefly discuss the Fullerton Study of Chinese Twins Reared Apart.

Fullerton Study of Chinese Twins Reared Apart

I have launched the first prospective study of separated twins. The twins are mostly females who were separated indirectly because of China's One-Child Policy between 1979 and 2015 (Evans, 2008). Rural families were entitled to two children, while urban families were limited to one. The policy was responsible for the abandonment of hundreds of thousands of female infants, including twins. My current sample includes 22 pairs (15 MZA and 7 DZA). Two pairs are male, one pair is opposite-sex and several sets are from Taiwan and Vietnam.

All twins are given a general mental ability test, and parents complete a series of inventories and questionnaires about their child's background, health history, educational history, personality traits, behavioral problems and creative tendencies. A parallel study of 50 pairs of Chinese twins adopted together is ongoing. Two papers from the project have been published. The first found that twins older than 18 months expressed greater emotion at the first meeting than younger twins (Segal, Stohs, & Evans, 2011). In the second study, genetic effects were found for all developmental measures (Developmental Delays at Adoption, Crying/Clinging, Initial Adaptation to Adoption, and Refusal/Avoidance), with shared environmental variance also affecting the first two measures (Segal, Tan, & Graham, 2015). A paper comparing the IQ resemblance of the Chinese twins reared apart to that of those reared together and to virtual twins (same-age unrelated children raised together since birth) is in progress (Segal Niculae, Becker & Shih, 2021, in press).

Twins Switched at Birth

A special class of reared-apart twins are those who are reared apart because one twin was switched with an unrelated infant soon after birth. This mistake appears to reflect hospital staff negligence. The first case occurred in 1941 and the last in 1999. These cases come from different countries around the world. In two cases there was a double switch, meaning that one twin in one pair was exchanged with one twin in another pair. Case chronology is presented in Table 10.1. I will then discuss the Colombian twins, shown in Figure 10.1.

TABLE 10.1. SWITCHED-AT-BIRTH TWINS

Location	Date of Birth	Gender	Age at Meeting (Years)
Switzerland	1941	male	5.0
Canada	1971	male	20.0
Poland	1983	female	16.0
Puerto Rico[a]	1985	female	1.5
Canary Islands	1973	female	28.0
Canary Islands	--	male	--
Colombia[a]	1988	male	25.0
Kenya	1999	female	19.0

[a]Doubly switched twins. Source: Segal & Montova (2018).

FIGURE 10.1. DOUBLY SWITCHED COLOMBIAN TWINS (PHOTO CREDIT: DR. NANCY L. SEGAL)

The Colombian twins include two MZA and two virtual twin (VT) pairs (same-age unrelated siblings raised together); in this case, the VTs were raised as DZ twins. There are also two "replicas," or unrelated siblings who were not reared together, but who genetically repeat the unrelated reared-together pairs. Comparing replicas with VTs provides an index of shared environmental effects. One pair of unrelated twins lived in a rural area far from any city and did not go beyond the fifth grade. (One of the twins later completed a high school equivalency course.) The other pair lived in the capital city of Bogotá and attended

college. Selected findings are presented below; further information is available in Segal and Montoya (2018) and in the papers cited below.

Both reared-apart pairs showed similarities and differences in general mental abilities, as did the VTs, although the profiles of the reared-apart twins appeared somewhat more alike. One set of replicas exhibited marked differences, while the other set appeared similar. The twins raised in Bogotá either performed similarly or outperformed the twins raised in the country. This is expected, due to the extreme differences in their education. The twins also completed the Raven APM, Set II. Both twins from Bogotá obtained higher scores than their co-twins and scored closer to each other than to their reared-apart co-twin. The biggest difference was between one of the replicas (Segal, Montoya, & Becker, 2018).

In terms of physical conditions, the origins of myopia (nearsightedness) are of timely interest. My research team examined the hypothesis that spending more time outdoors is associated with normal vision and refractive status, relative to spending more time indoors (Segal, Montoya, Pena, Burgos, & Katz, 2019). We found that uncorrected visual acuities were 20/160 and 20/200 for the city-raised twins and 20/20 and 20/30 for their country-raised counterparts. These differences could not be explained by premature birth, low birth weight, reading time or computer use. Time spent outdoors appears to be a critical factor in developing myopia.

Twin Study Controversy

Twin research has yielded numerous psychological and medical findings that have improved lives and enhanced understanding of human development. However, a few studies depart from conventional standards and their methods disturb our sensibilities. One such study took place in the 1960s and 1970s in New York City. Twins were adopted apart, then investigators examined them from birth to age twelve without informing their parents that they were raising a singleton twin. Two recent documentary films, *The Twinning Reaction* (Shinseki, 2017) and *Three Identical Strangers* (Wardle, 2018), helped bring needed attention to this case, but cannot capture the details that a book can provide. Therefore, I have written *Deliberately Divided: Inside the Controversial Study of Twins and Triplets Adopted Apart*, which will be available in spring 2021. It is dedicated to the twins.

Closing Quote

I will leave readers with this statement from Dr. Thomas J. Bouchard, Jr., the Director of the Minnesota Study of Twins Reared Apart:

"Twin studies . . . refute both biological and environmental determinism. They do not negate the effect of the environment on behavior, nor do they overglorify the role of genes. They account for the uniqueness of each of us."

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PART E – HEALTH AND WELL-BEING

Chapter 11

Evaluation of food neophobia in twins

Fívia de Araújo Lopes, Luzia Elionaide Albuquerque Martins, Felipe Nalon Castro

Obtaining energy through a nutritionally balanced diet is essential for the proper functioning of any organism, including humans. Feeding is part of everyone's daily life and involves factors external to the organism, such as social context and physical environment, food accessibility and availability, costs and structure for its preparation, as well as individual factors such as physiological characteristics and food preferences (Deliens et al., 2014).

In the modulation of individual experience related to food acquisition and eating, some aspects may be essential: sensory properties inherent to food, physiological state such as hunger or satiety, as well as psychological and cognitive factors (Fernandez et al., 2013; Rolls, 2012). Food neophobia is an individual's resistance to taste, or the avoidance of new foods, thereby exerting a significant influence on food choices (Birch & Fischer, 1998). This behavior has an evolutionary function as a defense mechanism for omnivores against poisoning associated with unfamiliar foods, and can prevent expansion of the diet (Knaapila et al., 2011).

Among humans, there is great variability in the expression of neophobic behavior throughout life. It is normally low after birth until the first year (breastfeeding period), rises from the age of 2 years onwards, peaks between 2 and 4.5 years, when children begin to gain autonomy in choosing their own food (Kral, 2018; Moding & Stifter, 2016), decreases during adolescence and stabilizes in adulthood, rising again slightly over time (Kral, 2018; Meiselman et al., 2010). Several other factors may be involved in neophobia, such as education, sex, income, workplace, where you live (urban or rural area), as well as the degree of nutritional information about food (Ferreira et al., 2017; Flight et al., 2003; Lopes et al., 2006; Meiselman et al., 2010). The influence of these factors on food neophobia can be assessed using instruments such as the Food Neophobia Scale (FNS) (Pliner & Hobden, 1992), used in adults.

Genetics is also a factor related to food neophobia. Genes allow organisms to respond to and use what is around them in their environment. But how? Heredity is the sum of the qualities and potentialities genetically derived from ancestors, or the transmission of these qualities (Baker, 2004). In turn, heritability is the proportion of phenotypic variation in a population that is due to genetic variation (Baker, 2004).

The twin study design is a valuable tool widely used by different research areas in order to investigate the relationship between environmental and genetic factors, since it offers the possibility of natural control of essential variables in this discussion: genetic similarity and shared environment. Zygosity can be reliably assessed by investigating the agreement between twins in relation to genetic markers including DNA polymorphisms and classic genetic polymorphisms, through fingerprints and based on physical biometric characteristics such as eye and hair color, nose and mouth shape, height, weight, among others, as well as investigating this similarity from the twins' self-perception and that of their relatives through self-report questionnaires (Beiguelman, 2008; Ooki & Asaka, 2004).

Investigating genetic influence on the expression of neophobic behavior shows the results of studies carried out with twins, whose findings suggest heritability between 72-78% for food neophobia, including in childhood (Cooke et al., 2007; Faith et al., 2013). Similar prospects were reported in a study with Finnish adult twins, which found a 69% heritability index in the expression of this behavior (Knaapila et al., 2015).

The present chapter aims to evaluate food neophobia and its association with zygosity in adult siblings. The main hypothesis of the research reported here is that the expression of neophobic behavior differs between pairs of monozygotic, dizygotic and non-twin siblings. Our expectation is that differences in the expression of this behavior will gradually increase as genetic similarity decreases.

Methods

Ethical aspects

The study protocol was approved by the Research Ethics Committee of the Federal University of Rio Grande do Norte (Protocol no. 2.401.159). Individuals were selected from the Physical Activity and Health Research Group database, social networks and the community. Study objectives and procedures were explained and those who agreed to participate signed two copies of the Informed Consent Form (ICF).

Design and Participants

The cross-sectional study was carried out with adults and siblings of both sexes, divided into three groups: monozygotic twins (MZ) (35 pairs/70 individuals), dizygotic twins (DZ) (8 pairs/16 individuals) and non-twins with an age difference of up to three years (NT) (26 pairs/52 individual), totaling 138 individuals. For twin siblings, zygosity was determined according to the final Zygosity Questionnaire score (Ooki & Asaka, 2004). Data were collected in the Biodynamics Laboratory of the Department of Physical Education of the Federal University of Rio Grande do Norte (UFRN), Natal, Brazil, from January 2018 to January 2019.

Instruments and Measures

Twin zygosity was determined using a questionnaire validated by Ooki and Asaka (2004), based on identification of similarity and confusion of identity. Appendix 11.1 shows the Brazilian version of the Zygosity Questionnaire used for adults. Both twins were classified according to the total score of the pair. Their individual scores were computed for each question: 1-3, 1-3, and 1-4 points were assigned to the answers of each of the questions. The total individual score and total score of the pair were then computed. The latter scores ranged from 6 to 20, with a score between 6 and 13 indicating monozygosity and 14 and 20 dizygosity.

To assess the level of food neophobia, participants responded to the Brazilian version of the Food Neophobia Scale (FNS) (Pliner & Robden, 1992), which addresses attitudes related to acceptance and consumption of unknown foods, comprising 10 statements evaluated on a seven-point Likert scale ranging from "strongly disagree" (1) to "strongly agree" (7). Appendix 11.2 shows the Brazilian version of the FNS. Total scores were calculated to determine the food neophobia score (ranging from 10 to 70 points), as described by the authors of the original scale. Based on the food neophobia score, participants were categorized according to Olabi et al. (2009): $\leq$ 30 points = neophilic; 31 to 49 points = neutral; and $\geq$ 50 points = neophobic.

Heritability was estimated using the equation $h^2 = (S^2\,DZ - S^2\,MZ) / S^2\,DZ$, where S^2 represents the variance of each series of differences, DZ refers to dizygotic and MZ to monozygotic twins. When h^2 is equal to 1, the variance of a trait is attributed exclusively to genetic causes since MZ twins are concordant ($s^2 = 0$) and the trait has a constant expression in each pair. When h^2 is equal to 0, the variation of the trait is entirely attributed to environmental factors. For both cases, we

assume that measurement errors are random and tend to cancel out (Beiguelman et al., 1996; Clark, 1956).

Statistical analysis

A descriptive analysis was carried out to characterize the sample, according to the sociodemographic data. The Kolmogorov-Smirnov and Levene tests were used to analyze assumptions of normality in sample distribution and homogeneity of variances for the means of the compared individuals, respectively.

To analyze the similarity or difference in the neophobic response, we subtracted the FNS scores of each pair of siblings (A and B) in each group (MZ, DZ, NT), using this difference as a variable. The Kruskal-Wallis test was applied to analyze the difference in responses between siblings for the final FNS score in each group. The heritability index was calculated as previously described, using the variance of the neophobia score, extracted from the FNS. The results were categorized into quartiles, with the lowest quartile below 25% labeled as low heritability, 26 to 50% moderately low, 51 to 75% moderately high, and >75 % high (Oliveira, 2013).

The analyses were performed with the SPSS 25 Program and significance was set at $p < 0.05$.

Results

According to the information obtained from the socioeconomic questionnaire, the sample was predominantly female (MZ: 63%; DZ: 75%; NT: 60%), single (MZ: 79%; DZ: 75%. NT: 83%), self-declared pardo (mixed ethnicity) (MZ: 46%; DZ: 31%; NT: 37%) or white (MZ: 41%; DZ: 50%; NT: 60%), and belonging to class B2 (upper-middle) (MZ: 37%; DZ: 31%; NT: 25%) or A (upper) (MZ: 19%; DZ: 31%; NT: 36%). Average age was 26.5±6.0 years.

With respect to food neophobia classification, according to the FNS, most MZ and NT individuals were classified as neutral, with the DZ group exhibiting a higher incidence of neophilic participants. Similar percentages of neophobic participants were observed between the MZ and NT groups, and these were lower than those of the DZ group (Table 11.1).

TABLE 11.1 – CATEGORIZATION OF PARTICIPANTS ACCORDING TO THE FINAL FNS SCORE AND DISTRIBUTED BY GROUPS.

Groups	MZ (70)		DZ (16)		NT (52)	
(N=138)	n	%	n	%	n	%
1. Neophilics	18	25.71	9	56.25	21	40.39
2. Neutral	45	64.29	6	37.50	25	48.07
3. Neophobics	7	10.00	1	6.25	6	11.54

When comparing the classification of each individual within each pair of siblings, we observed greater similarity in the categorization between MZ than NT siblings. The DZ twins obtained intermediate results (Figure 11.1).

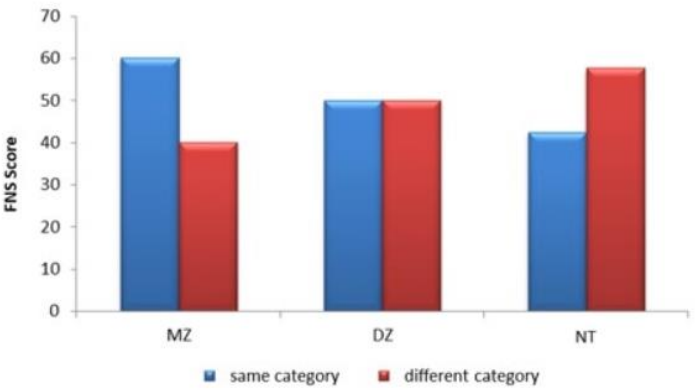

FIGURE 11.1. DIFFERENCE IN FINAL FNS SCORE WITHIN EACH GROUP.

The difference in final FNS score was calculated between siblings A and B and transformed into positive values through the absolute value of the Z-score. Although we did not observe a significant intergroup difference (χ^2 (2) = 1.857; p = 0.395), the tendency of MZ twins to show less difference in neophobia is noteworthy, with an increase for DZ twins and the greatest difference between NT siblings (Figure 11.2).

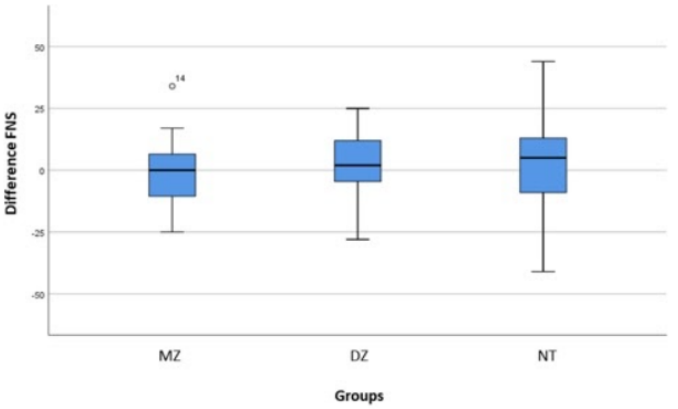

FIGURE 11.2. INTRAPAIR DIFFERENCE IN THE FNS SCORES FOR THE THREE GROUPS (MZ, DZ, AND NT)

Finally, the heritability index obtained through the variance in final FNS score between twin siblings was 29%, classified according to Oliveira (2013) as moderately low heritability.

Discussion

The present study sought to evaluate the similarity in the expression of food neophobia between pairs of monozygotic and dizygotic twins and non-twin siblings. The results showed a tendency to differentiate between the groups, but it was not statistically significant. Although we have not corroborated our hypothesis, the trend seems to follow the expectations of the study, whereby the monozygotic twins showed less difference in the expression of neophobia, followed by dizygotic twins, increasing slightly among the non-twins. We found no similar studies in the literature for comparison purposes, emphasizing the scarcity of research in this area.

The heritability index was 29%, classified as mild for the neophobic trait, and different from the results presented in the literature for both adults and children. Knaapila et al. (2011) evaluated, among other factors, food neophobia in young adults, and found 61% heritability in women, corresponding to a moderately high level, according to Oliveira (2013). A similar result was reported by Knaapila et al. (2007), who observed 69% heritability in the expression of food neophobia in Finnish adults. Faith et al. (2013) studied children and found 72% heritability for neophobia, suggesting, based on other studies that investigated heritability in both children and adults, that the magnitude of the genetic influence on neophobic behavior seems to be constant

across the entire development spectrum (Cooke et al., 2007; Knaapila et al., 2007).

With respect to study limitations, despite referring to the effects of family ties on the expression of food neophobia, the results could be attributed to the influence of a shared environment, especially when we consider same-sex monozygotic twins, given that we identified moderately low heritability indexes. In addition, our sample of dizygotic twins was very small, which may have compromised the analyses.

In conclusion, taking into account the 29% heritability for the neophobic response in our sample as corresponding to genetic effects, the environment could be responsible in greater proportion for the expression of this trait. This may be a good indicator, from a clinical point of view, of a context in which strategies are thought to reduce the effects of food neophobia, providing better diet quality and the possibility of including new items, favoring its expansion and emphasizing the social factor as a good modulator of food neophobia.

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Appendix 11.1 - Brazilian version of the Zygosity Assessment Questionnaire (Adapted from Ooki & Asaka, 2004)

As seguintes questões se referem a semelhança entre você e seu(sua) gêmeo(a) na infância.

1. Quando crianças, você e seu(sua) gêmeo(a) eram "iguaizinhos" e difíceis de diferenciar? Favor escolher SOMENTE UMA das opções:

a) "Iguaizinhos" e difícil de diferenciar
b) Como irmãos normais
c) Muito diferentes

2. Você e seu(sua) gêmeo(a) eram confundidos quando crianças? Favor escolher SOMENTE UMA das opções:

a) Sim, frequentemente ("toda hora")
b) De vez em quando
c) Nunca

3. Quem confundia vocês quando crianças? Favor escolher TODAS opções que achar apropriadas:

a) Pais
b) Professores
c) Outros: _____
d) Ninguém

Appendix 11.2 – Brazilian version of the Food Neophobia Scale (Adaptado de Pliner & Robden, 1992)

1. Eu sempre como alimentos novos e diferentes.	Discordo totalmente	1	2	3	4	5	6	7	Concordo totalmente
2. Eu desconfio de alimentos novos.	Discordo totalmente	1	2	3	4	5	6	7	Concordo totalmente
3. Experimento um alimento só quando sei do que ele é feito	Discordo totalmente	1	2	3	4	5	6	7	Concordo totalmente
4. Eu gosto de comidas estrangeiras.	Discordo totalmente	1	2	3	4	5	6	7	Concordo totalmente
5. Comidas estrangeiras são diferentes demais para comer.	Discordo totalmente	1	2	3	4	5	6	7	Concordo totalmente
6. Em festas eu experimento alimentos novos.	Discordo totalmente	1	2	3	4	5	6	7	Concordo totalmente
7. Eu tenho receio de comer coisas que eu nunca experimentei antes.	Discordo totalmente	1	2	3	4	5	6	7	Concordo totalmente
8. Com relação a comida, sou difícil de agradar.	Discordo totalmente	1	2	3	4	5	6	7	Concordo totalmente
9. Eu como de tudo.	Discordo totalmente	1	2	3	4	5	6	7	Concordo totalmente
10. Eu gosto de experimentar comidas estrangeiras em restaurantes.	Discordo totalmente	1	2	3	4	5	6	7	Concordo totalmente

Chapter 12

Twin pregnancy: challenges to reduce preterm births

Maria de Lourdes Brizot

Twin gestations are at increased risk of maternal perinatal and infant morbidity and mortality, as well as long-term neurodevelopmental disability in the children. We know that the main cause of these adverse outcomes in children is premature delivery, which is eight to nine times higher in twin pregnancies than their singleton counterparts. Twin pregnancies account for one to three percent of all pregnancies in Europe and in the United States; however, they represent approximately 20% of all premature deliveries. Thus, reducing premature birth in twin pregnancies has been a big challenge in recent decades.

Premature delivery has multifactorial causes and a specific cause cannot usually be determined (Stock & Norman, 2010). The main factors involved in the risk of preterm birth are previous history of preterm birth and cervical length (To, Fonseca, Molina, Cacho & Nicolaides, 2006). We know that the shorter the uterine cervix, the higher the rate of preterm birth. Cervical length shorter than or equal to 25 millimeters between 20-24 weeks of gestation is found in 54% of deliveries prior to 32 weeks and 40% of those before 34 weeks (Conde-Agudelo, Romero, Hassan & Yeo, 2010). We chose this gestational age because deliveries before 32 weeks are known as extreme prematurity.

Several treatments and interventions have been investigated for the prevention of preterm delivery in twin pregnancies, but none have proven to be effective. As such, research on the use of (i) progesterone, (ii) cervical pessary and (iii) cerclage is still underway. All of these interventions are effective for singleton pregnancy, but as yet not for twin pregnancy.

The majority of the studies investigating intervention procedures to prevent preterm birth in twin pregnancies initiated with non-selected twin pregnancies. They included all twin pregnancies, irrespective of cervical length. At the Hospital das Clinicas of the University of São Paulo (USP), we conducted a double-blind randomized placebo-controlled study involving 380 patients (Brizot et al., 2015). We found no difference between the placebo and

progesterone groups in the premature birth rates at any of the gestational ages examined. In addition, there were no differences in perinatal outcomes between the placebo and progesterone treatment groups. We concluded that in non-selected twin pregnancies, vaginal progesterone administration does not prevent preterm delivery or reduce neonatal morbidity and mortality.

Similar studies have also been conducted in twin pregnancies using other interventions. However, none have shown the benefits of progesterone, pessary or cerclage in preventing preterm birth in unselected twin pregnancies (Norman et al, 2009; Rode et al, 2011; Cetingoz et al 2011; Aboulghar et al 2012; Serra et al 2012; Wood et al, 2012; Brizot et al, 2015; Liem et al, 2013; Nicolaides et al, 2016). As such, these interventions are not recommended for unselected twin gestations.

Later, it was investigated whether vaginal progesterone produced benefits for a selected group of twin pregnancies with short cervical length (< 25 millimeters). A meta-analysis conducted by Romero et al. (2017), which also included our study, showed a 31% reduction in preterm births in the progesterone group. It also demonstrated a positive effect on the risk of adverse perinatal outcomes, with a 39% decline in composite neonatal morbidity and mortality. As such, vaginal progesterone may be potentially useful in treating twin pregnancies with short cervical length. However, it should be noted that the conclusion of this meta-analysis was that further research is required to definitively recommend vaginal progesterone treatment for women with twin pregnancies with a short cervix. The same can be said about cervical pessary.

Goya et al. (2016) investigated the effectiveness of cervical pessary in preventing preterm birth in twin pregnancies with a short cervix. The study showed a 59% reduction in preterm births before 34 weeks, and a 28% decline in birthweights below 2500 g. However, a meta-analysis (Saccone et al, 2017) including data from Goya et al. (2016) and Nicolaides et al. (2016) showed no intergroup difference in prematurity rates with the use or not of pessary. Therefore, more studies are needed to investigate the benefits of cervical pessary in women with a short cervix.

A retrospective study investigating the use of cervical pessary and vaginal progesterone in patients with twin gestation and short cervix showed that the use of a cervical pessary combined with vaginal progesterone was associated with prolonged pregnancy and reduced risk of adverse neonatal outcomes (Fox et al, 2016). The authors suggested that a large randomized trial be conducted to confirm the results of this retrospective study.

In 2017, we designed a study to investigate the effectiveness of a combined treatment of progesterone and cervical pessary in twin pregnancies to prevent preterm birth. The study was registered at Clinical Trials and aimed to determine whether cervical pessary plus vaginal progesterone would reduce preterm birth at <34 weeks and improve perinatal outcomes. The estimated sample size for this study is 312 asymptomatic twin pregnancies with a short cervix between 16 and 27 weeks of gestation. The patients will be randomized into four groups: (1) pessary, (2) pessary plus vaginal progesterone, (3) vaginal progesterone and (4) placebo.

We will also investigate women's preferred treatment method to prevent preterm birth. In addition, the study will evaluate treatment adherence and tolerability.

A questionnaire specifically developed for this study will be applied three times to the patients: at study entry, halfway through the study and after delivery. The State-Trait Anxiety Inventory and Quality of Life Questionnaire will also be applied to the patients. We hope that this study will help us define the preferred methods from the patients' perspective and improve the quality of care provided.

In conclusion, the best intervention for the prevention of preterm birth in twin pregnancy with a short cervix has yet to be established. Thus, we must wait for the results of several studies currently underway throughout the world to determine the best prevention of preterm birth in twin pregnancies.

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Chapter 13

Genetic influence on developmental enamel defects

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Teeth are composed of three mineralized tissues: enamel, dentin, and cementum. Enamel is the most mineralized tissue of the human body, covering the clinically visible surfaces of the tooth crown (Taji et al., 2011; Chavez et al., 2020).

Amelogenesis is enamel formation characterized by an extremely intricate process that progresses from early embryonic development throughout an individual's lifespan. During the so-called secretion phase, an organic matrix is secreted, which later undergoes mineralization during the maturation phase. In the course of the evolution from the earlier to the later stage of enamel maturation, a dynamic and complex process occurs (Seow, 2014).

The developing enamel undergoes cellular, biochemical, genetic, and epigenetic changes while amelogenesis takes place. It is known that a high incidence of mineral acquisition, associated fluctuations in extracellular pH, and resorption of extracellular enamel proteins occur during this process (Lacruz et al., 2017).

Ameloblasts, a group of cells recognized by their significant sensitivity, are responsible for enamel development. During maturation, ameloblasts undergo altered organization from a tall, thin, and highly polarized arrangement, to a low columnar and extended morphology. These results demonstrate that ameloblasts experience extensive molecular modifications during the maturation stage of amelogenesis (Lacruz et al., 2017; Seow, 2014).

Disturbances that occur during amelogenesis due to ameloblast sensitivity can cause developmental enamel defects (DED), one of the most common oral conditions in childhood. DED are a series of anomalies in both the quality and amount of enamel and can affect tooth formation, hardness and coloring (Figure 13.1). The reported prevalence of DED ranges from 5.3 to 78.9% (Andrade et al., 2019; Oliveira et al., 2006; Wada et al., 2017; Wagner, 2016).

FIGURE 13.1 – DIFFERENT CASES OF DEVELOPMENTAL ENAMEL DEFECTS. (PHOTO CREDIT: DR. MARINA DE DEUS MOURA DE LIMA)

Our research group (Buccæ Geminæ) has studied the prevalence of DED in 5-year-old preschoolers from Teresina, Brazil and found that 33.7% have DED (Andrade et al., 2019).

Different varieties of enamel defects can occur, including enamel hypoplasia, caused by the reduction of enamel thickness that takes place during the secretory phase of amelogenesis. An imperfection in enamel translucence can occur if ameloblasts are damaged in the mineralization stage, a defect known as enamel hypomineralization (Andrade et al., 2019; Oliveira et al., 2006; Wada et al., 2017; Wagner, 2016).

In recent years, researchers have detected a particular pattern of enamel hypomineralization that affects primary or permanent molars and incisors. This dental defect is termed hypomineralized second primary molar (HSPM) in primary dentition (Elfrink et al., 2009) or molar incisor hypomineralization (MIH) in permanent dentition (Weerheijm et al., 2001). Clinically, it displays asymmetric severity with demarcated opacities that vary in color from white to brownish yellow, with sharp demarcation between the affected and healthy enamel (Weerheijm et al., 2001).

Hypomineralized enamel has a lower mineral content, which creates a predisposition to bacterial penetration, resulting in pulp inflammation, hypersensitivity and subsequent dental caries (Elfrink et al., 2012). As a consequence of the lower mineral content, these teeth are exceptionally susceptible to loss of enamel due to masticatory forces. Furthermore, the affected teeth can lead to compromised esthetics caused by staining and morphological alterations. Thus, children impacted by DED may experience anxiety and social discomfort due to their dental appearance. In addition, they exhibit occlusal changes. All of these factors can interfere in their quality of life (Andrade et al., 2019).

Despite the associated problems, the etiology of enamel hypomineralization remains unclear (Teixeira et al., 2017). Given that the entire enamel formation process is controlled by genetic factors, it

is hypothesized that genetic variations could be associated with variations in amelogenesis.

Studies have indicated multifactorial complexity involving genetic, epigenetic and environmental factors, although the influence of each component is unclear (Teixeira et al., 2017). Thus far, a modest number of studies have been published regarding the genetic influence on hypomineralized enamel defects.

Fabiano et al. (2013) investigated whether genetic variation in enamel formation genes is associated with molar-incisor hypomineralization (MIH). DNA samples from 234 cases with MIH and 171 unaffected controls from Turkey and Brazil were studied. The comparison between cases and controls demonstrated an association between MIH and ameloblastin, tuftelin, enamelin, amelogenin, and tuftelin-interacting protein 11.

Kuhnish et al. (2014) analyzed the correlation between MIH and possible genetic loci. He concluded that the SCUBE 1 gene was a genetic locus for MIH, thereby playing a plausible biological role throughout tooth development.

Bussaneli et al. (2019) assessed the association between the polymorphisms of these immune response genes and MIH. The investigation found a connection between alterations in this gene and the condition.

An interesting family-based genetic association study evaluated the presence of a genetic correlation in 63 single nucleotide polymorphisms of 21 candidate genes related to amelogenesis. DNA was obtained from 391 individuals who were birth family members of 101 Brazilian nuclear families. Sixty-three single nucleotide polymorphisms (SNPs) were investigated in 21 genes related to amelogenesis. Substantial results were obtained for these genes. It was concluded that the variability in genes involved in amelogenesis was interrelated with the predisposition to develop MIH. This result is in line with the multifactorial perception of MIH etiology, but the authors suggested that additional research is needed to investigate the factors that could influence MIH. (Jeremias et al., 2016).

Specialists have stressed that twin studies provide insights on the contribution of genetic and environmental factors in the manifestation of enamel defects (Lygidakis et al., 2010). Comparison of concordance in monozygotic and dizygotic pairs may help determine the influence of shared, nonshared, and genetic factors on the risk of developing DED (Teixeira et al., 2017). Additionally, these investigations allow the evaluation of genetic and congenital effects on phenotypic variation through the presence of genotypes and similar environments, as well as the possible interaction between them (Wright et al., 2015).

Accordingly, twin studies are the most reliable way of determining the genetic contribution to a given condition (Lygidakis et al., 2010, Teixeira et al., 2017). However, journal articles on this subject are scarce.

Taji et al. (2011) explored the prevalence and site concordance of enamel hypoplasia and opacity in the primary dentition of 2- to 4-year-old twins and singleton controls. They found 80% concordance between monozygotic co-twins and 44% between their dizygotic counterparts, a statistically relevant discrepancy.

Silva et al. (2019) investigated the relative contribution of genes and environment to the etiology of hypomineralized second primary molars (HSPM) and identified the potential environmental risk factors in a longitudinal twin cohort study. A total of 344 6-year-olds underwent dental assessment and HSPM was observed in 19.8% of the cases. The monozygotic twin concordance (0.63) was higher than in dizygotic twins (0.41). However, there was no evidence of additional genetic influence after adjusting for known risk components. The research demonstrated that shared environmental elements are more relevant than genetics in hypomineralized etiology, although the lower number of monozygotic twins likely affected the capacity of the study to identify enhanced concordance in comparison with dizygotic twins.

Our research group (Buccæ Geminæ) carried out a cross-sectional study in order to measure the coincidence of molar incisor hypomineralization between monozygotic and dizygotic twin pairs and the association with environmental factors. The sample consisted of 167 pairs of twins aged 8 to 15 years old (94 monozygotic and 73 dizygotic). A greater concordance in the diagnosis of molar incisor hypomineralization was observed in monozygotic twins, indicating a genetic influence. Although there was greater concordance between pairs of monozygotic twins, in 15 pairs only one individual was diagnosed with MIH. This result demonstrates the multifactorial etiology of the condition (Teixeira et al., 2017).

Later, Dr Alexandre Vieira (2019) calculated the heritability of molar incisor hypomineralization using our data. He discovered a 20% MIH heritability rate and that the difference in concordance between monozygotic and dizygotic twins strongly supports a genetic component in MIH, concluding that further studies are needed to investigate genotypes and environmental risk factors simultaneously.

Some interesting questions that persist are the fact that in some cases only one molar or a part of a tooth was affected, and the differing severity of lesions that can be observed in a patient or even on the same tooth without a "timeline" consistency (Vieira & Manton, 2019).

Thus, the role of genetic factors in the etiology of hypomineralized enamel defects has been little explored. Many questions remain and twin studies may help answer them.

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Chapter 14

Assessment of Oral Health Outcomes among Twins: The Cardec Twins Initiative

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Twin studies are an excellent alternative to investigate the contribution of genetic and environmental factors in the development of individual physical characteristics, as well as in the etiology of diseases (Mucci et al., 2005). Investigating the influence of genetic and environmental components on oral health outcomes can help predict and prevent oral conditions and contribute to planning therapeutic approaches (Otta et al., 2016; Kurushima et al., 2015). To date, however, few studies have investigated the influence of zygosity and fetal parameters on oral health-related outcomes.

Genetic factors appear to have varying degrees of influence on the development of oral diseases (Mucci et al., 2005). A greater correlation was found between identical twins and the presence of dental caries, compared to fraternal twins (Anu et al., 2018). However, an individual's caries experience and the development of dental malocclusion seems to have a strong genetic influence (Rintakoski et al., 2010); the same is true for tooth loss (Kurushima et al., 2015). Other less frequent dental conditions, such as Molar Incisor Hypomineralization (MIH) and dental trauma have also been investigated (Teixeira et al., 2018; Wasmer et al., 2008). MIH has exhibited greater correspondence between monozygotic twins, suggesting a genetic influence (Teixeira et al., 2018). The occurrence of dental trauma in twins has not been genetically associated, given a high concordance of the presence of dental trauma between the twins, despite the type of zygosity (Wasmer et al., 2008).

Although some studies have reported the influence of zygosity on certain oral health outcomes, most have evaluated characteristics of the studied populations only through the individuals' self-reports (Kurushima et al., 2019; Mucci et al., 2005; Rintakoski et al., 2010). In other words, data collection has not been conducted through clinical

examination by a trained professional, which is crucial to providing more accurate information on the patient's clinical situation (Dahake et al., 2015; Dewhurst et al., 1997).

The potential influence of zygosity on children's behavior in response to dental treatment has not been thoroughly investigated. The study of factors influencing children's behavior during dental treatment is important, since dental procedures can be potentially stressful. Fear of dentists is a prevalent complaint worldwide (Thom et. al., 2000) and, together with anxiety, can be defined as a set of negative feelings towards dental care (Sandrini et al., 1998). In this sense, children seem to be the most affected group since they face a different situation from everyday experiences (Possobon et al., 2004). Studies show that at least one in ten children have some degree of anxiety preventing them from tolerating dental procedures. In Brazil, 21.6% of children have reported / shown a certain level of dental fear and anxiety and / or challenging behavior during dental care (Cianetti et al., 2017; Costa et al., 2017).

How and when a dentist intervenes can have either positive or negative physical and emotional repercussions in children due to their stage of growth and development (Possobon et al., 2004). Equipment, instruments, dental routines, and the skill level of the professionals can have a negative impact on physical discomfort. The presence of pain and psychological discomfort are elements of anxiety and fear (Stokes & Kennedy, 1980; Berge et al., 1999; Possobon et al., 2004).

Negative feelings during dental care have multifactorial origins (Campbell et al., 2017). Personal characteristics have the most influence on fear and behavior, but they are also strongly affected by social and family aspects and the environment in which the child lives. All these characteristics are related within the living environment and externalize children's feelings from what they absorb. As such, individuals are seen as active influencers of their environment and are also subject to being influenced by it (Rutter, 1987). In addition, disposition is primarily determined by mechanisms of biological origin and is subject to changes determined by maturation and interaction between the individual, the specific genotype and the environment (Strelau, 1998). However, the association between family environment/style and children's behavior during a dental procedure has been little investigated and results are controversial (Miranda-Remijo et al., 2016).

Faced with this scenario, the origin of difficulties in children's behavior in dental treatment, as well as the determining factors for the child to feel fear and anxiety are a matter of concern. Are these aspects predominantly genetic or environmental? What environmental factors

have the greatest influence on this process? Adequate answers could be obtained by assessing a sample of twins undergoing dental treatment.

Consequently, and considering the studies assessing oral health, patient-centered, and dental treatment-related outcomes in twin children, this study aimed at evaluating the association between twin pair-related factors and oral health outcomes, as well as establishing associations between behavior and successful dental treatment based on a historical cohort of twins born in Sao Paulo, Brazil between 2007 and 2014 at Hospital das Clinicas. This study was designed and planned at the Faculdade de Odontologia, Universidade de São Paulo (FOUSP), Brazil.

The study will include the following aims: i) assess the association between twinning-related variables, pre- and perinatal care, as well as other variables collected and the oral health outcomes; ii) assess whether the impact of oral health conditions on children's quality of life and improvement in quality of life indicators after treatment are comparable between monozygotic and dizygotic twins; iii) assess whether twinning-related factors and other conditions influence children's behavior during dental treatment; and iv) investigate whether these factors affect dental treatment success after 2 years of follow-up.

This project was approved by the Research Ethics Committee of the Faculdade de Odontologia, Universidade de São Paulo, Brazil (CAAE: 31043620.9.0000.0075). The research was conducted in collaboration with the Instituto de Psicologia of the Universidade de São Paulo (IPUSP), more specifically the Painel USP de Gêmeos [University of São Paulo Twin Panel] project, led by Emma Otta, IPUSP professor and researcher in the field of Experimental Psychology (Otta et al., 2019).

Eligible children, aged 6 to 13 years old, enrolled in the Painel USP de Gêmeos, will be invited to take part in this research at FOUSP. After acceptance and both written informed consent and assent is provided, children will be assessed through a clinical examination. This examination will determine several oral health outcomes, such as dental caries, presence of trauma, developmental enamel defects (including fluorosis and MIH), malocclusion, and bruxism. Caregivers will be requested to provide pre- and perinatal-related data and zygosity. Demographic, socioeconomic, and development milestone data will also be collected through a structured questionnaire.

Children's oral health-related quality of life will be evaluated using the Brazilian version of the Child Perceptions Questionnaire (CPQ8-10 and CPQ11-14), a self-report instrument that assesses children's perception of the impact of oral diseases on physical and psychosocial

functioning. (Foster et al., 2013, Barbosa et al., 2011; Jokovic et al., 2004, Goursand et al., 2008). The Parental-CPQ (P-CPQ) questionnaire (Jokovic et al., 2003; Barbosa et al., 2015) will also be implemented to assess caregiver's perception of the children's quality of life. The aim is to analyze the individuality of children based on the parents' responses regarding quality of life, in addition to measuring the impact on oral health-related quality of life according to parents' perception.

After the examination, a treatment plan will be developed considering all child treatment needs. To that end, and if necessary, complementary diagnostic procedures, such as x-rays, may be required. Children will then receive dental care according to the best available evidence-based protocols. All dental procedures will be completed, excluding more complex orthodontic treatments. In such cases, children will be referred to a more specialized service provider. In subsequent sessions, treatments will be provided and the child's behavior recorded as one of the outcomes, as stated in objective iii.

The twins' behavior will be evaluated in phases, based on responses to a questionnaire developed and validated for such an analysis. The first phase will include the patient's first appointment, in which prophylaxis and clinical examination are performed to assess the oral health-related outcomes. This first dentist-patient experience is extremely important for building rapport with the child and understanding his/her characteristics. At this point, the dental practitioner will classify the child's behavior according to the Frankl Scale (Frankl, 1962), which ranges from definitely negative behavior to positive behavior. Caregivers will then complete the Brazilian version of a questionnaire on capacities and difficulties (SDQ-Por) (Goodman (1997; Fleitlich et al. 2000), which aims to assess children's mental health (Saur & Loureiro, 2012).

After the initial examination, and according to the attending pediatric dentist's perception, the child's degree of collaboration will be defined by means of a visual analog scale (VAS) (Nazif, 1971) and again by the Frankl Scale. Both the pediatric dentist and caregiver will register their perception of care through the VAS scale: how satisfied they are with the procedure (interventional or not) performed on the day of care and how stressed they felt when caring for the child. These records will be used for future association with child behavior. At the end of the first appointment, twins will be invited to respond to the FIS (Facial Image Scale) (Buchanan; Niven, 2002) describing how they are feeling at that moment and the parents will complete the VAS.

The second phase will consist of an appointment in which a clinical procedure will be performed. This will be a restorative procedure,

preferably using the atraumatic restorative technique (ART). The child's behavior will be defined during dental treatment sessions determined by the attending pediatric dentist, through the VAS and Frankl scales. In addition, the professional will include: (i) his/her perception of the child's behavior, (ii) satisfaction with the procedure and (iii) perceived stress during the procedure.

Separately, children's caregivers will record their satisfaction and stress with each twin's treatment session. The child, in turn, will answer how he/she is feeling at the moment before and after all procedures until the initial treatment plan is completed. Recall intervals of 6, 12, 18 and 24 months after the treatment will be established. Oral health condition reassessment, guidelines for maintaining oral health, and collection of dental treatment success-related data will be conducted after 2 years through the number of new reinterventions (objective iv).

All recall visits will include professional prophylaxis, clinical examination, and after the end of the session, evaluation of the child's behavior by the pediatric dentist, in addition to their satisfaction with or stress experienced during the procedure. If a need for treatment is detected on return visits, the procedure will be performed by one of the trained operators. Instructions regarding oral hygiene, dietary sugar control, and fluoride use will be repeated at each return visit for all children. Caregivers, in turn, will again record their satisfaction and stress related to the care of each of the twins. Oral health-related quality of life will also be re-evaluated at the end of treatment.

Multilevel regression analysis (first level related to the individual, and second to the pair of siblings) will be carried out to investigate the association between all outcomes and zygosity, pre- and perinatal, as well as other assessed variables.

With this methodology, we expect to meet the aims of the study, welcoming and treating the twins and their respective core family within the scope of the children's dental needs and consequently contributing with new scientific evidence, based on the results of Brazilian research.

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Chapter 15

Overview of the Clinical Branch of the University of São Paulo Twin Panel

Maria Lívia Tourinho Moretto and Gustavo Di Giorgi Ramos

In this chapter, we share the work we have done at the Braço Clínico do Painel USP de Gêmeos [Clinical Branch of the University of São Paulo Twin Panel] and part of the results obtained so far.

At the invitation of Professor Emma Otta, who leads the Painel USP de Gêmeos at the Instituto de Psicologia, Universidade de São Paulo (IPUSP), the first author is very pleased to coordinate the clinical branch of the twin study, which was founded in 2017, and is composed of a group of professionals and students who are equally interested in researching the subject

The clinical branch offers free clinical psychological care for twins enrolled in the Painel USP de Gêmeos and their family at the IPUSP school clinic. From June 2017 to December 2020, we assisted about 30 people and approximately 800 sessions were held.

Earlier last year we started a scientific cooperation partnership with the "Gemelarity and assisted reproduction" research group, which is part of the Clinical and Research Sector of the Departamento Psicanálise com Crianças do Instituto Sedes Sapientiae [Department of Psychoanalysis with Children of the Sedes Sapientiae Institute]. Until 2019, this was a closed group formed by professors Adela Gueller and Ada Morgenstern, who had been researching the subject since 2010 and are the authors of the book "Psychoanalytical care for twins" (2018). After the advent of the partnership with the clinical branch, the group admitted students from the fourth and fifth year of the Psychology undergraduate program at USP, master's and doctoral students, as well as those from the third and fourth year of the Psychoanalysis with Children Training Course and members of the Department, changing its name to "Gemelar" ("twinned").

Last year, the group held weekly meetings. The work began in person at Sedes, but with the arrival of the pandemic, an online meeting format was adopted, with weekly research meetings, consisting of a

reading and reflection group, interspersed biweekly with clinical supervision meetings.

The group is currently composed of three students from the fourth year of the training course in Psychoanalysis with Children, Taísa N. Martinelli, Juliana P. de C. Pedroso and Josseline Capua Rodrigues Sanches; Paulina Mei, third year student; Vanessa Freitas, member of the Psychoanalysis with Children Department; Gustavo Ramos, master's student at LabPsi-Usp; and Maycon A. Fraga, member of LabPsi-Usp, in addition to the professors and coordinators Ada Morgenstern, Adela Gueller and Maria Lívia Moretto.

Clinical care, which usually takes place in person at the USP School Clinic, continued through online meetings. Throughout the year, we held discussions on the texts and clinical cases, and are in the process of producing an article relating twin experiences with those portrayed in the film "The Double Lover" (2018) by François Ozon. Despite the adversities we are facing during this pandemic, the work continues.

After introducing the working group we formed, and all the researchers and clinicians who make it possible, we will present a little of what we have been discussing during our sessions and conversations with the twins under study.

In psychology, there is a consensus regarding the function of the other or of otherness in what we call the constitution of human subjectivity, that is, the Ego is constituted by the Other. The development of the human psyche takes place through a series of processes that we call identification and differentiation. In other words, for me to become myself, I need to have taken another as a strong reference, in order to later build the conditions needed to differentiate myself from it. These identification and differentiation processes are the basis for constructing more complex psychic processes, such as attachment and how the separation occurred, that is, the processes of identification and differentiation are not disconnected from the psychic processes that involve the possibilities of someone bonding and separating.

Some questions guide and motivate our clinical research, especially those that question identification and the psychic possibility conditions of differentiation in twins, and their conditions for binding and separating. The psychological care offered to twins and their parents has allowed us to ascertain the specificities of the demands presented to us in such a way that clinical care is the basis for research that aims to investigate the specificities of the subjectivity of twins and those that must be considered in the clinical care devices for patients in this condition. If the function of the parents is essential to the constitution of subjectivity, our hypothesis is that the co-twin plays a central role in

the construction of identity, in situations in which the parents are truly in the background.

Listening to the twins and their stories has revealed their focus on certain issues, which we will share with you: the difficulties of differentiating themselves from each other; the way they bond with each other seems to be related to the way they bond with other people, in both establishing bonds of friendship and love relationships. The feeling of protection with the existence of the double is undeniable, as is the anguish of separation and the difficulties of mourning in cases of significant loss. Rivalry and resentment between twins is also a relevant issue. The tendency to humor is present in stories that involve some form of cheating and how it is possible to laugh at oneself and one's own image when one has a double as a source of inspiration.

However, most research in clinical psychology emphasizes difficulties that twin siblings have in differentiating themselves from each other, since their intense bond can often be an obstacle to forming relationships with others. This is because clinical psychology studies traditionally focus on cases involving some type of suffering or pathology. Our intention, however, is to fill the gap in studies that target mood and well-being, by examining the playful and pleasant side of having a twin sibling, which undeniably exists, but has only appeared in the psychotherapeutic process of the clinical care we have provided to date.

In the master's research currently conducted by Gustavo Ramos, one of the members of the clinical branch, we started our work with a survey of recent clinical studies on twins produced by psychoanalysts. At the beginning of this work, we wondered whether or not it was possible to think about 'specifics' in the clinical psychoanalysis of twins. Since this is not a recurring theme in psychoanalysis, we asked ourselves if there are specificities in the condition of twins that should be considered and/or justify the singular attention and care provided to these patients in psychoanalytic clinical devices.

Here we have an explanation: since clinical psychoanalysis par excellence is the clinic of singularity, we are far from confusing specificity with specialty, that is, thinking about the clinical and specialized clinical psychoanalysis of twins is not the same thing. Once this important difference is considered, the question arises: are there specificities that should be considered in clinically managing the psychotherapy treatment of twins? If so, what are they?

We started by conducting a survey in the databases of articles published in Scielo and Pepweb (psychoanalytical database) over the last decade. More than a thousand results were found for the descriptors ‘twin’ and ‘psychoanalysis’. However, after refining the

search and analyzing these results, only 27 were considered relevant for the subject investigated because they effectively deal with the intersection between twinning and psychoanalysis, and not with diverse subjects containing only the words researched. In 10 of the 27 publications found, twinning was part of the primary objectives of the article, and the other 17 were mostly clinical articles of cases involving patients who are twins.

In general terms, the authors discussed the following topics: Jeanne Magagna (2007) [psychotherapist and psychoanalyst, lecturer and supervisor at the Tavistock Clinic, London, England] discusses the individuation process of twins in the passage from twin to individual. Althea Hayton (2009) [from London, England, founder of the "wombtwin survivors" or "surviving twins" project, which seeks to study and research the subject] discusses the loss of a twin in utero and the suffering it can engender for the survivor. Elizabeth Wright (2010) [from London, England], in an article entitled "A twin in psychotherapy", recounts her own therapeutic process, and defends the fact that there was no discussion of her specific twin condition. Charlotte Kahn (2012) [PhD in psychology from Columbia University, psychoanalyst and family therapist in New York City] discusses Pierre Lacombe's hypothesis regarding the possible existence of an essential twin neurosis. Based on his personal experience and interviews, Oliver Shirley (2016) [author from London, England] discusses difficulties that opposite sex twins may face in relationships with other people of the opposite sex. Ruth Simon (2016) [US psychotherapist and psychoanalyst working in Oakland and San Francisco] discusses infant twin development based on Winnicott's theory.

Catiesca Pereira Dorneles and Vladia Zenker Schmidt (2015) [psychologists and researchers at FADERGS, in Rio Grande do Sul state, Brazil] conducted a bibliographic survey on twins, psychoanalysis, bonding and maternal care. Mariléia Orn Scalco and Tagma Marina Schneider Donelli (2014) [psychologists and researchers from the UNISINOS graduate program in clinical psychology, in Rio Grande do Sul, Brazil] discuss cases of twins under 1 year of age and psycho-functional symptoms. Marta Knijnik Lucion and Norma Escosteguy (2011) [researchers from the Psychiatry Department of the PUCRS Medical School, in Rio Grande do Sul, Brazil] discuss the condition of mothers and caregivers of one-year-old twins.

In this literature survey, we found that there was no broad discussion about the intersection of twinning with psychoanalysis, and that this discussion emerged through diverse, timely and more specific issues. In these several issues, two main themes caught our attention:

the first was transference within clinical care and the second the status of differentiation in twins.

In regarding to transference, there is a debate on whether the therapist, in the transferential relationship with twins, occupies or not, at certain moments of the treatment, the role of the patient's co-twin, as well as possible maternal and paternal positions, among others. The importance of paying attention to this possible transference in these treatments was also discussed.

Jeanne Magagna (2007) and Klaus Fink (2007) [a German psychologist and psychoanalyst, respectively, living in Hamburg] reported that at a certain moment of their care, they occupied the transferential position of a co-twin of their patients in the sessions. Elizabeth Wright (2010) argued that psychoanalysts who work with twins have to be prepared to occupy the co-twin's position; otherwise, their work will be incomplete. Amanda Dowd (2012) [from Sydney, Australia, a member of the Jungian Society of Analysts, an analyst instructor and private practice psychoanalyst] and Sue Grand (2013) [a clinical psychologist trained at the New School for Social Research in New York City] reported that it took a long time to recognize and pay attention to the uniqueness of the twin condition in the transfer of their cases, and that when they did so it was very important for the progress of those treatments. In opposition to the authors cited above, Joaquin Cañizares (2010) [member of the Scottish Institute of Human Relations] reported that one of his patients "invited him to occupy the empty space left by his missing twin, instead of using him as a therapist" and that this made him feel like a "lonely twin" and any attempt he made to interpret it was unsuccessful, making it difficult to treat this case.

On the subject of differences, Jeanine Vivona (2007) [professor of psychology at the University of Massachusetts] wrote that there is no consensus in the literature as to whether or not twinning leads to the highest degree of differentiation among siblings in relation to siblings who are not twins. The author quotes a survey by Davison (1992) in which analysts separate the effects of twinning into two opposing positions: those who describe that it leads to strong similarity and even symbiosis between siblings and others who notice opposing characteristics and contend that it leads to greater differentiation between siblings.

In this initial part of the research, considering what had been found in recent studies, we were able to answer one of the questions that we initially asked ourselves, concluding that there were indications of relevant specificities in twinning experiences that should be taken into

account in the clinic, and that these indications justified broader and more in-depth research on the subject.

We realized that even in the articles that specifically addressed twinning and psychoanalysis issues, there was no general debate on the status of twinning experiences. This is partly due to the very format in which the articles are published and how knowledge advances in psychoanalysis, with the sovereignty of the clinic and its discoveries in the production of knowledge. As such, most of the publications we found dealt with specific cases. In other words, they were articles written by psychoanalysts who treated cases of twin patients or family members with questions about the twin experience and its effects. In these sessions, the psychoanalysts asked themselves about the twin experiences and how to listen to them amidst the difficulties and possibilities that arose, and decided to write about the subject.

At this point, we would like to return briefly to the issue of specificity in psychoanalysis. The clinic with children, for example, involves specificities. Psychoanalysts who are aware of them can likely do a better job with their patients. Throughout the course of the master's research, we presented our work to the public and debated the issues encountered. At times we have been able to talk to psychoanalysts treating twins and discovered that they had never heard of such issues in their training, and that by learning about them through our research or other sources, they were able to pay more attention to these questions. We would like to underscore that this does not mean that everyone necessarily has questions about their twin experiences (several patients have talked to us about issues other than twinship), but rather that such issues may emerge in the clinic and psychoanalysts who are made aware of them may be better equipped to deal with them.

Since the literature review, our research has advanced and one of the issues we are currently discussing, and would like to briefly share with you, is related to the uniqueness of pair experiences. After all, one of the main characteristics of twinning, both for twins and their families, concerns almost simultaneous births. Compared to other siblings, twins live together at the same ages and for most of their development. Thus, several experiences are shared by the twin pair. Some authors even consider love relationships, or very strong friendships, where even those who are not twins undergo a significant experience or pair relationship.

Historically, the first authors within the psychoanalytic field who contemplated the effects and questions brought about by the twin experience, emphasized the importance of each sibling having his or her own individual experience, beyond what occurs in a dyad. To deny the singular dimension of each of the siblings, and only treat them as

an undifferentiated pair can cause suffering and difficulties. According to psychoanalysis, whether we are twins or not, we experience subjectivity in the field of language and desire, in an inexorably singular way.

However, theorizations about the importance of respecting one's uniqueness have sometimes been interpreted by psychoanalysts and caregivers as prescriptions and followed as if they were absolute norms. We have heard reports of cases where too much concern was expressed by those caring for the individual dimension of each twin, and the entire dyadic dimension was disregarded. The same can occur in clinical care: psychoanalysts excessively concerned about "individuality" and the differences of those they are treating may not be sufficiently attentive to important experiences related to the pair.

We have heard during the sessions, in case reports and clinical discussions, that denying the dimension of the pair can cause suffering, because the pair is fundamental to the twin experience and provides comforting, beneficial and enriching experiences.

If the relationships between parents and children are excessive, there are two poles that cause suffering: on one hand, there would be an attempt to treat the twins in exactly the same way, which would treat difficulties during separation or sibling differentiation. An example of these attitudes are the cases that Gueller and Morgenstern describe of parents who even spend an equal amount of time with each child in order to guarantee no inequality in their relationship with them. At the other extreme is the possibility of parents' treating each child in an excessively "different" and "individualized" way, creating "small differences" where they do not exist, which could be construed as attempts to deny the dimension of their shared experiences.

Adela and Ada denominated this and other challenges that twinning poses "impossibility of twinning", which arise not only from the relationships and experiences of each family, but also reveal our culture and ways of loving, the difficulties and tensions that we encounter between individual experiences, and other transindividual experiences, where such boundaries are unclear.

We would like to conclude by emphasizing that this was a brief summary of our work and the results found in our research thus far, and that there are a number of important topics and discussions taking place in this field. However, these discussions seldom reach a wider audience for clinical and psychoanalytical debate. We believe that one of the main contributions of our psychoanalytic work at the University, in partnership with the researchers and institutions that support it, is to bring these issues to public debate and to a greater number of psychoanalysts, in order to address their challenges and potentialities.

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Tomaz Maranhão created the cover of this book. He is the twin sibling of Gabriel Ferreira, both members of USP's Twin Panel: the first pair of twins enrolled in our registry who grew up apart and reunited in adulthood. They met during the COVID-19 pandemic in 2020, nearing their 23rd birthday, the first one they celebrated together. Tomaz lives in Fortaleza-CE and Gabriel in Uberaba-MG. Both are passionate photographers.The book's cover shows photos of them in different moments of their lives, before and after they met. The invitation to make the cover is an homage, our way to thank them for sharing their life stories with us.