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University of California, Riverside

# Undergraduate Research Journal

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# University of California, Riverside

# Undergraduate Research Journal

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## From the Administration



As a research university, one of UC Riverside's most important duties is the creation of knowledge. Undergraduate research is a hallmark of UCR's scholarly and educational missions and a factor in our university's impressive trajectory.

With faculty-mentored research projects across a breadth of disciplines, UCR provides a wealth of opportunities for students to investigate complex questions and discover the joys of scholarly research.

As you will see in this 13th volume of the *UC Riverside Undergraduate Research Journal*, our students are making the most of these opportunities and accomplishing truly inspiring work. The scholarship that appears each year in this publication represents research excellence and creative endeavors of the highest order. UCR is at the forefront of discovery, with a world-class faculty, including two Nobel laureates, and state-of-the-art instructional and testing laboratories. UCR is creating a home for students, faculty, and community members to collaborate, test, and learn.

At UCR we are stewards of transformation; it is in our DNA to ensure that we create an environment and structure that fosters innovation to solve our community and world's greatest challenges.

I congratulate all the students who contributed to this edition of the Journal, and I express my sincere gratitude to the faculty mentors and staff members that supported these students in their scholarly endeavors.

Sincerely,



Kim W. Wilcox  
Chancellor



The Thirteenth Annual *UC Riverside Undergraduate Research Journal* introduces some of the very best faculty-mentored undergraduate research and scholarship from America's fastest rising university. The Undergraduate Research Journal adds to the university's impressive 65-year legacy of high-impact research, which fully integrates with the undergraduate student engagement experience.

In many ways, UC Riverside represents the university of tomorrow. Our inclusive culture, world-renowned faculty, and rigorous and future-focused environment produces young researchers who are prepared to tackle the dynamic challenges that face not only California's Inland Empire, but the world at large. The students whose work is presented in the journal are enriching the environmental, economic, social, and cultural future of California, the nation, and the world.

Congratulations to each of the scholars featured in this journal as this is the culmination of a lengthy process of discovery filled with excitement, frustration, and anticipation. Thank you to our students, faculty, and staff who made the publication of the Undergraduate Research Journal possible. In particular, I would like to acknowledge the Student Editorial Board for leading the peer-review process, the Faculty Advisory Board for its consultation and advice, and Gladis Herrera-Berkowitz for her role in bringing this journal to fruition.

I hope you enjoy and are enriched by the discoveries revealed in this year's Undergraduate Research Journal.

Wishing you all the best,



Jennifer Brown, Ph.D.  
Vice Provost and Dean of Undergraduate Education



# UCR Undergraduate Research Journal

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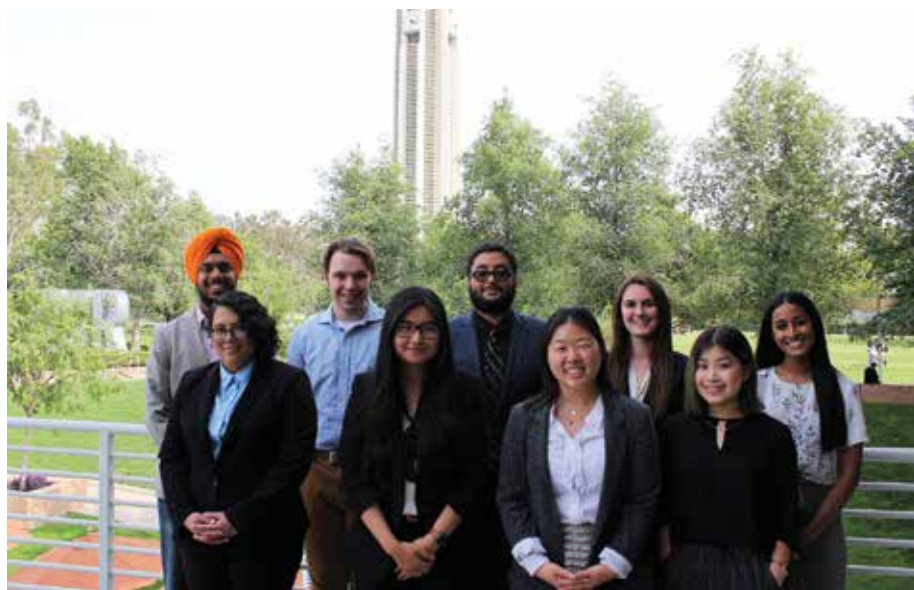
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*It is with great pleasure to present UC Riverside's 13th edition of the Undergraduate Research Journal. I have had the honor of collaborating with brilliant minds across campus in undertaking the endeavor of bringing this publication to fruition this year. It is the collective efforts of everyone involved that ensure the integrity and standards of the Journal reflect the excellence that UCR represents. Congratulations to the authors for your merit – your commitment to undergraduate research and the achievements found in this 13th edition will forever be a part of the Journal's legacy and UCR's academic and creative culture built upon the pursuit of knowledge. Congratulations also to the Student Editorial Board and Faculty Advisory Board – your diligence and dedication to the publication process have ensured the quality and success of the Journal. I am remarkably grateful to have been part of the outstanding team that made this publication possible.*

**SARAH MILLER** — Editor-In-Chief



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Since UCR's Undergraduate Research Journal started, it has published almost 150 scholarly articles across many fields. These papers represent the commitment of many of our undergraduate students to performing independent research as part of their undergraduate experience at UCR. The Undergraduate Research Journal thus fills a critical need. More often than not, undergraduate research forms part of a larger work with many contributors, which can mean a dilution of the student's contributions as well as a longer time between completing the work and publication. With the Undergraduate Research Journal, our students can write about their work and get first-author credit. They can publish before the end of the academic year, and gain the experience of seeing their manuscript go through a peer-review and publication process just like a standard research journal. When the paper becomes a part of a student's professional experience, it contributes to their record of scholarly achievement. The Journal submission and review process is run by undergraduates who form the Student Editorial Board, working with members of the Faculty Advisory Board. We owe a debt of gratitude to the students for their professionalism and dedication for the review and preparation of the articles in this issue. We are also grateful for the participation of the members of the Faculty Advisory Board in guiding the reviewers. If you are interested in publishing your undergraduate research at UCR, consider submission to our next issue!

Chair of the Undergraduate Research Journal Faculty Advisory Board  
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### California Golden Poppies

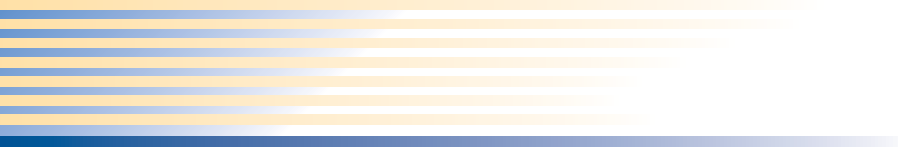
California Golden Poppies found in Walker Canyon near Lake Elsinore during the 2019 Super Bloom. These wildflowers ornamented the fields across Southern California, offering a beautiful sight of vibrant colors and floral diversity. This photo was taken about two miles into the Walker Canyon Ecological Reserve. These poppies, also known as California Sunlight or Cup of Gold, are native to California and Mexico and are the official California state flower.



### Mark Anthony Sebarrotin

*Department of Political Science*

Mark Anthony Sebarrotin is a graduating senior in the Department of Political Science. He is from the Monterey Bay in Central California and hopes to return to serve lower-income communities. He is an avid hiker and spends much of his time outdoors. Mark will serve as a 2019-2020 Coro Fellow in Public Affairs, Northern California region.





## The Time of Day of Conversations and Well-Being

**Daisy Andrade Channell, Lilian J. Shin, & Megan L. Robbins**

*Department of Psychology*

### ABSTRACT

This study explored the potential effects of time of day of conversations on overall well-being. Despite the substantial research on time of day, conversations, and well-being separately, there exists a lack of research on the link between time of day of conversations and well-being. Time of day was defined by examining the average of participants' morningness-eveningness preferences for engaging in conversations, and how that relates to conversation quality and well-being. Morningness-eveningness preference is defined as the degree to which people organize their activities toward the morning or evening (Hasler, Allen, Sbarra, Bootzin, & Bernet, 2010). The present study aimed to explore the link between time of day of conversations and well-being as well as to examine potential conversation quality differences in this association. The duration of the Conversation Study for each participant was 1 month. Participants participated in 2 in-laboratory sessions and completed various surveys outside of the laboratory where they were asked to complete the Subjective Happiness Scale and the Satisfaction with Life Scale to assess overall well-being. Following the first lab session, 12 daily questionnaires were emailed to participants to report the time of day of conversations and conversation length over the course of 2 weeks. Completion of at least 4 questionnaires was required. The results of the present study found a significant association between conversation length and greater subjective happiness. Further, a small trend toward significance was revealed in the association between time of day of conversations and greater subjective happiness as well as between lengthier substantive conversations and greater satisfaction with life. Our study is the first to discuss the importance of time of day of conversations and length of conversations on well-being in conversation research. Further, we hope this research can provide insight on the optimal time of day and duration of time to engage in certain conversation qualities, as time of day of conversations and conversation length may influence one's emotional well-being.

**KEYWORDS:** Conversations, time of day, well-being, emotional well-being, substantive conversations, small talk, morningness-eveningness



### **Daisy Andrade Channell**

*Department of Psychology*

Daisy Andrade Channell is a 4th year Psychology major with a passion for mental health awareness. She is a research assistant in Dr. Megan Robbins's OBSERVE Lab assisting on a research project that studies the relationship between conversation quality and well-being. After graduation, Daisy plans to pursue a PsyD in Clinical Psychology where she hopes to train as a behavioral therapist to provide counseling to young adults.



### FACULTY MENTOR

#### **Dr. Megan L. Robbins**

*Assistant Professor in the Department of Psychology*

Megan L. Robbins is an Assistant Professor in the Department of Psychology at the University of California, Riverside. She is a social-personality psychologist with interests in health psychology who uses naturalistic observation methods to study social interactions, coping, and well-being.

## INTRODUCTION

Happier days are commonly accompanied by moments we spend socializing with others (Sandstrom, Tseng, Costa, Okeke, Choudhury, & Dunn, 2016). Thus, it is unsurprising that the extent to which we spend time socializing plays an influential role in a person's well-being. The relation between socializing and well-being may be due to happier people tending to spend less time alone and more time discussing substantive conversation topics (Mehl, Vazire, Holleran, & Clark, 2010). Conversing with others is an activity most people engage in on a daily basis at different points during their day. The quality and quantity of conversations a person experiences may be influenced by the time of day they engage in these conversations. However, no research has investigated the effects of time of day of conversations on well-being. Discovering one's optimal time of day to engage in certain conversation qualities is important, as it may be beneficial to one's overall emotional well-being. The current study aims to examine the association between time of day of conversations and conversation quality and quantity with overall emotional well-being.

Conversation quality is defined binarily as engaging in either small talk or substantive conversations (Milek et al., 2018). Substantive conversations are defined as conversations where people exchange meaningful information about topics such as emotions, relationships, political views, social ideals, personal matters, and religious preferences (Mehl et al., 2010). In contrast, small talk conversations are defined as engaging in trivial communications about unrelated topics (Shaughnessy, Mislin, & Hentschel, 2015). There is substantial evidence that conversation quality affects well-being in important ways (Milek et al., 2018). For example, prior research has found that conversation quality plays a crucial role in individuals' everyday well-being and overall satisfaction with life (Sandstrom et al., 2016). Specifically, substantive conversations of greater depth may involve debates over profound subject matters that can act as a catalyst for a greater influx of ideas and knowledge. These conversations may lead to a greater sense of well-being, in contrast with discussions surrounding shallow topics. Furthermore, research has demonstrated that people who engage in more substantive conversations experience higher life satisfaction (Milek et al., 2018), which may

be influenced by self-disclosure. Research suggests that disclosing our deepest thoughts and feelings strengthens our interpersonal relationships (Sloan, 2010). Additionally, during substantive conversations, individuals commonly communicate their good fortune and positive experiences, which often leads to prolonged positive emotions (Peters, Reis, & Gable, 2018).

It is not surprising that several studies have been done on conversation quality including substantive conversations; however, there currently exists no previous research on time of day of conversations. There are various ways to define time of day; this study utilized the morningness-eveningness construct. The morningness-eveningness construct is based on an individual's diurnal preference with differences demonstrated in sleep-wake patterns, circadian rhythms, behavioral rhythms, and diurnal variation of mood -- all of which have distinct influences on the expression of psychological disturbances (Randler, 2008). Morningness refers to individuals who prefer daytime activity and show peak performance and alertness in the early morning hours. In contrast, eveningness refers to individuals who prefer nighttime activities and experience peak performance and alertness in the evening (Hasler, Allen, Sbarra, Bootzin, & Bernert, 2010). Eveningness has been found to be associated with experiencing more psychological and psychosomatic disturbances, such as depression and seasonal affective disorder (SAD), as compared to individuals who prefer the morning. People who prefer the morning also report experiencing higher overall satisfaction with life than those who prefer the evening (Randler, 2008). Therefore, the morningness-eveningness construct assists us in evaluating time of day in the context of conversations.

The present study aimed to (1) assess whether time of day of conversations (e.g., morningness-eveningness preference) is associated with overall emotional well-being, (2) identify potential conversation quality (e.g., small talk vs. substantive) differences in the association with time of day and conversation length, and (3) examine the association between conversation length and well-being in undergraduate students. In light of the evidence that substantive conversations are related to well-being, the authors predicted that conversation length would have a dosage effect such that engaging in lengthier conversations

may be related to higher well-being due to spending more time conversing with others and less time alone (Mehl et al., 2010). Further, the authors also predicted that engaging in lengthier substantive conversations would lead to better emotional well-being over time. Additionally, the authors predicted that time of day of conversations would influence participants’ emotional well-being.

**METHODS**

**Participants**

Participants were recruited for the Conversation Study from an introductory psychology course and research methods course. As compensation for participating in the study, participants were awarded 2 research participation credits or extra credit points for their class. In order to be eligible to participate in the Conversation Study, participants had to be at least 18 years old and able to complete questionnaires in English. Participants’ data were excluded from the study if they did not complete 4 or more daily questionnaires and failed to complete both the first (Time 1) and last lab sessions (Time 3). Our final participant sample consisted of 346 undergraduate students ( $M = 20$  years,  $SD = 3$ ) from the University of California Riverside. This sample included 257 females, 88 males, and 1 non-binary participant. Ethnicity was reported by participants as Hispanic/Latino/a (31.8%), Asian (41.3%), African American (4.6%), Caucasian (9.5%), Middle Eastern (5.5%), other (1.7%), or more than 1 race (5.5%).

**Procedure**

Undergraduate participants entered the OBSERVE lab at the University of California Riverside for a pre-intervention session (Time 1) and post-intervention session (Time 3) for a total of 2 in-lab sessions. Participants also completed various surveys in the location of their choosing over the duration of 1 month. Prior to attending the lab sessions, participants were randomly assigned to 1 of 4 conditions (Table 1).

Participants in the substantive conditions were given these instructions:

*“The goal is to have conversations that have the purpose to exchange thoughts, information, or ideas about any topic you like, or conversations in which you reveal your emotions and thoughts about a personal event or feeling that has happened in the past or is currently happening. This might include talking about your feelings (positive or negative) within a current relationship, concerns about your grades, goals for the future, social issues-any topic about which you exchange **personal** or **substantive** information.”*

The difference between Close Other/Substantive and Acquaintance/ Substantive was that participants in Close Other/Substantive were asked to converse with a close friend and participants in Acquaintance/ Substantive were

**Table 1.** Participants’ conversation quality and relationship condition.

<b>Conversation Quality</b>		
	Substantive Conversation	Small Talk
<b>Relationships:</b>		
Close Other	Close Other/Substantive Conversation	Close Other/Small Talk
Acquaintance	Acquaintance/ Substantive Conversation	Acquaintance/ Small Talk

*Note:* Relationship represents to whom the participant is speaking in each condition.

asked to converse with an acquaintance. Participants in conditions Close Other/Small Talk and Acquaintance/Small Talk were given the same instructions as participants in the Substantive condition, with the exception of conversation type:

*“We would like for you to engage in small talk with your chosen acquaintance over the next 2 weeks. The goal is to have a conversation of a light or impersonal nature. This might include talking about something you bought recently, the weather, or sports—any topic that is **light and insubstantial**.”*

For the purpose of the present study, we did not analyze differences between the Close Other and Acquaintance conditions.

After entering the lab, participants sat in a room in groups ranging from 1 to 9 participants. During the first lab session (Time 1), participants were seated at a computer or laptop and were instructed to complete a survey by a trained research assistant. Informed consent, demographics, and emotional well-being were obtained while completing the survey. Following Time 1, participants were emailed a link to complete 12 brief daily conversation questionnaires over the following 2 weeks from a location of their choosing. Daily questionnaires were utilized for participants to report the time and length of their substantive or small talk conversations dependent on the conditions to which they were assigned. Participants were instructed to complete 6 daily questionnaires despite being sent a total of 12 questionnaires. However, we did not disclose that completion of 4 questionnaires was required to move on to the following lab session. After 2 weeks, participants returned to the lab for their second lab session and completed a questionnaire regarding their emotional well-being and conversation length. However, data was not used from the participants' second lab session as it is not relevant to the research questions addressed in this current study. Instead Time 3 was utilized as the only post- intervention session. Time 3 took place 2 weeks after the second lab session where participants completed a survey online that assessed their emotional well-being and conversation length in a location of their choosing outside of the laboratory.

## Measures

**Emotional well-being.** Participants completed the Subjective Happiness Scale (SHS; Lyubomirsky & Lepper, 1999) to assess their happiness levels. This scale consists of 5 items (e.g. “Compared to most of my peers, I consider myself...”) and participants responded on a 7-point Likert scale with the degree to which they agreed or disagreed with the statements (e.g. 1 = *not a very happy person*, 7 = *a very happy person*). Participants also completed the Satisfaction with Life Scale (SWLS; Diener, Emmons, Larsen, & Griffin, 1985), a 5-item measure evaluating life satisfaction that asked participants to rate the degree to which they agreed (1 = *strongly disagree*, 7 = *strongly agree*) with a given statement (e.g. “The conditions of my life are excellent.”).

**Daily questionnaire.** The daily questionnaires included 4 questionnaires. However, only 2 questionnaires relevant to this study were used in the analysis.

**Time of Day.** Time of day was assessed via self-reports. Participants were asked to provide the time (e.g. 11:30 PM) and length of conversation in minutes. Time of day was coded based on the time participants reported engaging in their conversations (e.g. Morning was 6:01am – 12:00pm, coded 0; Afternoon was 12:01pm- 6:00pm, coded 1; Evening was 6:01pm – 12:00am, coded 2; Night was 12:01am- 6:00am, coded 3). Once participants' conversations were coded, the coded time of day data were aggregated. We chose to code time of day on a scale from morning to night to assess the morningness-eveningness construct on a continuum from earlier to later times. For this reason, a correlation analysis was used to assess the relationship between time of day and emotional well-being, conversation length and emotional well-being, conversation quality and time of day, and conversation length and conversation quality.

## RESULTS

### Time of day

#### Is time of day associated with emotional well-being?

Contrary to the authors' expectations, there were weak nonsignificant associations between time of day of participant conversation and subjective happiness as well as satisfaction with life at Time 1. As seen in Table 2, no



significant association between time of day of conversation and subjective happiness or satisfaction with life was found at Time 3. However, despite results not meeting traditional significance, a moderate trend was found in the association between time of day of conversations and subjective happiness at Time 3. Further, a significant association was not found between time of day of conversations and satisfaction with life at Time 1 nor Time 3.

**Conversation quality and time of day.** A correlation between time of day and subjective happiness scores were run before potential conversation quality (small talk and substantive) differences in this association were examined by splitting the data by condition in SPSS. Results revealed that there was not a significant association found between time of day and subjective happiness among participants assigned to small talk ( $r(172) = 0.02, p = 0.81$ ) nor substantive conversations ( $r(174) = 0.05, p = 0.52$ ) at Time 1. Results of running a correlation between time of day and subjective happiness for Time 3 revealed a marginally significant trend in the predicted direction among participants assigned to substantive conversation ( $r(174) = 0.13, p = 0.08$ ). In contrast, among participants assigned to small talk ( $r(172) = 0.04, p = 0.64$ ), time of day and subjective happiness were not found to be significantly associated. Further, assessment of the relationship between life satisfaction and time of day revealed a marginally significant association among participants assigned to substantive conversations at Time 1 ( $r = 0.14, p = 0.06$ ) and at Time 3 ( $r = 0.15, p = 0.06$ ). Among participants assigned to small talk, time of day and life satisfaction were also not associated at Time 1 ( $r = 0.05, p = 0.55$ ) nor at Time 3 ( $r = 0.02, p = 0.78$ ).

**Conversation Length**

**Is conversation length associated with emotional well-being?** As seen in Table 2, a significant association was found between conversation length and subjective happiness at Time 3. Results revealed that life satisfaction was non-significantly related to conversation length at both Time 1 and Time 3. However, a marginal non-significant trend was found in the predicted direction at Time 3 in the association between life satisfaction and conversation length.

**Conversation length and conversation quality.** Correlations were run between well-being measures and conversation length followed by the assessment of potential conversation quality differences in the association between well-being and conversation length. In assessing the relationship between subjective happiness and conversation length at Time 1, results revealed that among participants assigned to small talk conversations, conversation length and subjective happiness were not significantly associated ( $r(172) = 0.03, p = 0.67$ ). Among participants assigned to substantive conversations ( $r(174) = 0.06, p = 0.39$ ), a significant association also was not found between conversation length and subjective happiness at Time 1. However, a weak trend was found towards significance at Time 3 between conversation length and subjective happiness among participants assigned to small talk conversations ( $r(172) = 0.13, p = 0.07$ ) and participants assigned to substantive conversations ( $r(174) = 0.12, p = 0.10$ ). The association between life satisfaction and conversation length at Time 1 was not significant among participants assigned to either substantive conversation ( $r(174) = 0.03, p = 0.65$ ) or small talk ( $r(172) = 0.01, p = 0.82$ ). Further, the same

**Table 2.** Correlations between participants’ overall well-being, time of day and conversation length.

	Subjective Happiness Scale				Satisfaction with Life			
	Time 1		Time 3		Time 1		Time 3	
	<i>r</i>	<i>p</i>	<i>r</i>	<i>p</i>	<i>r</i>	<i>p</i>	<i>R</i>	<i>p</i>
<b>Time of Day</b>	.035	.522	.085	.115	.098	.068	.088	.103
<b>Conversation Length</b>	.049	.363	.131	.015*	.034	.525	.077	.155

Note. \* $p \leq 0.05$ ;  $N = 346$  participants

associations at Time 3 (life satisfaction and conversation length) among participants assigned to small talk ( $r(172) = 0.06, p = 0.42$ ) and substantive conversations ( $r(174) = 0.07, p = 0.33$ ) were not associated.

## DISCUSSION

This study explored the time of day of conversations to (1) analyze its association to well-being, (2) identify potential conversation quality differences with time of day and conversation length, and (3) examine the association of conversation length and well-being. Results revealed that time of day of conversations played a minimal role in overall well-being. However, results revealed that conversation length was associated with greater well-being.

Descriptive findings in the present study revealed that engaging in lengthier conversations over the course of a month resulted in a significant association between those lengthier conversations and better subjective happiness. This finding further supports previous conversation research that happier people spend more time socializing with others as compared to their less happy counterparts (Mehl, 2010). Additionally, among people engaging in substantive conversations over the duration of the study, a weak trend toward significance was found in the association between subjective happiness and the time of day of conversations. This is important to note as participants engaging in substantive conversations at their optimal time of day may facilitate better subjective happiness. The morningness-eveningness construct discusses the idea that individuals experience peak performance and alertness at their optimal time of day (Hasler et al., 2010). If participants engage in substantive conversation at their peak performance and alertness, it is possible that they have a greater ability to engage in profound subject matters as opposed to during other times of day. The results also reveal that engaging in lengthier substantive conversations indeed played a role in better life satisfaction over time regardless of time of day. This is consistent with previous research that found that people who engage in more substantive conversations experienced greater life satisfaction (Milek et al., 2018). It is possible this effect is due to the implications associated with engaging in substantive conversations. For example, participants engaging in conversations of greater depth and concerning subject matters that create an influx of ideas and

knowledge may have resulted in higher life satisfaction. In contrast to the authors' hypothesis, participants engaging in lengthier small talk conversations, rather than substantive conversations, experienced greater subjective happiness over time. Therefore, engaging in small talk conversations regardless of time of day led to greater subjective happiness.

Although most results did not meet traditional statistical significance standards, time of day trended towards significance in association with subjective happiness at Time 3. Therefore, it is possible that asking participants to engage in conversations over a longer period of time in future studies may yield significant results. Future studies should continue to evaluate the link between time of day of conversations and well-being by assessing time of day using alternative measures besides self-reporting to potentially gather more precise data. Self-report limitations, such as reliance on memory, could have affected the time of day data in this study. Further, conversations had in addition to and irrelevant to the 6 conversations participants were instructed to have for the purpose of this study may influence the data as potential confounding variables. Conversations participants had beyond their assigned condition could have affected their emotional well-being and subsequent self-reported data. However, the use of random assignment decreases the likelihood of this occurring. Future studies should ask participants to report all conversations they engaged in that fit the description of their assigned condition, thereby increasing the chances of gathering greater and more precise data.

This current study takes the first step in introducing the variable of time of day to conversation research and its relation to well-being. This is an important first step in conversation and psychological research because time of day plays an influential role in everyday life – potentially determining when individuals sleep, eat, and converse.

## ACKNOWLEDGEMENTS

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# Plastic Consumption and the Individual: Looking at the Personality Traits and Personal Factors Associated with Plastic Consuming Behaviors

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## ABSTRACT

Plastic pollution is a serious issue that has severe implications for our environment. Past studies have examined the associations between individual differences and pro-environmental behavior. However, there is a paucity of research that examines the personal characteristics of individuals who have relatively lower plastic consumption. The present study collected data from 165 undergraduates to investigate the associations between personality traits and plastic consumption. In addition, the study explored the mediation pathways from personality traits (BFI-2) and environmental attitudes (Nature Relatedness Scale) to one's plastic consumption. To measure the criterion, the Plastic Behavior Questionnaire was developed to measure how often an individual performed various plastic consuming behaviors over the course of a year. Results indicate that Conscientiousness and Agreeableness are associated with environmentally friendly plastic consumption. Furthermore, a mediation analysis indicated that environmental attitudes mediate the relationship between Agreeableness and Conscientiousness with environmentally friendly plastic consumption. The results suggest that individuals high in Conscientiousness and Agreeableness tend to engage in lower plastic consumption, and this is mediated by having positive environmental attitudes. Further insights and limitations are discussed.

**KEYWORDS:** Pro-environmental behavior, environmental attitudes, plastic consumption, personality traits



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## Yema A. Conteh

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Yema Conteh is a 3rd year Psychology major minoring in Gender and Sexuality Studies. She is a two-time recipient of the Dean's Academic Distinction Award. Her research interests involve examining the underlying personal and situational motives that drive pro-environmental behaviors. She works as a research assistant in the Personality Assessment Lab under the direction of Dr. Daniel J. Ozer. In the future, she plans to obtain a PhD in Social/Personality Psychology to continue to develop research that promotes sustainable behaviors.

## INTRODUCTION

One of the most environmentally destructive behaviors that humans partake in daily is the consumption of plastics. According to the UN, 300 million tons of plastic were disposed and littered globally in 2015, while only 9% was recycled (United Nations Environment Programme, 2018). Due to human behaviors, marine systems are declining and human health issues are increasing as microplastics accumulate and move up the food chain. Understanding how differences in individual personalities are associated with environmentally friendly plastic consumption may be valuable for developing future interventions that can target those who are prone to the overuse of plastic. While prior research has broadly identified the personality traits associated with pro-environmental behavior, the current project focuses on individuals' tendency to engage in plastic-reducing behaviors.

Within the pro-environmental literature, there is a growing amount of research that examines the relationship between personality traits and environmentally conscious behaviors. A trait can be broadly defined as a unit used to measure an individual's tendencies in their thoughts, feelings, and behaviors (Hong & Paunonen, 2015). They can be broken down to five domain factors: Extraversion, Agreeableness, Conscientiousness, Neuroticism, and Openness to Experience (Soto & John, 2017). The factors are proficient in encapsulating an extensive amount of behavior information within a given domain (Soto & John, 2017).

Past research has investigated the relationship between Openness to Experience and pro-environmental behavior. Openness to Experience can be defined as having an interest in aesthetic, creative, and intellectual pursuits (Soto & John, 2017). There have been consistent findings between Openness to Experience and pro-environmental behaviors (Brick & Lewis, 2016; Markowitz et al., 2012). In both studies, environmental attitudes mediated the relationship between Openness to Experience and pro-environmental behavior (Brick & Lewis, 2016; Markowitz et al., 2012). Those who were higher in Openness to Experience engaged in emission reducing behaviors, such as the utilization of alternative modes of transportation (Brick & Lewis, 2016). Based on past research, the findings suggest that those who

are relatively higher in Openness to Experience tend to engage in pro-environmental behaviors.

Agreeableness and Honesty-Humility have also been found to predict environmental concern and ecological behavior (Hilbig, Zettler, Moshagen, & Heydasch, 2012; Hirsh, 2010). Both Agreeableness and Honesty-Humility involve being selfless as well as respectful and fair towards others (Ashton & Lee, 2007; Soto & John, 2017). Honesty-Humility is associated with having a pro-social value orientation, positive environmental attitudes, and performing more ecologically responsible behavior, such as taking showers instead of baths (Hilbig et. al, 2012). These findings suggest that Agreeableness and related traits are also associated with pro-environmental behaviors.

Likewise, Conscientiousness has also been found to predict pro-environmental behaviors, such as reducing emissions (Brick and Lewis, 2016). Those who are high in Conscientious tend to be organized, responsible, and productive individuals (Soto & John, 2017). Along with Openness and Agreeableness, Conscientiousness also predicts general environmental engagement (Milfont & Sibley, 2012). In addition, Conscientiousness is mediated by environmental attitudes (Brick and Lewis, 2016). Overall, the literature provides support between the link of personality traits, environmental attitudes, and pro-environmental behavior.

The current study investigates the relationship between personality traits and environmentally friendly plastic consumption. This exploratory research aims to specifically address two questions:

1. *Which personality traits are associated with environmentally friendly plastic consumption?*
2. *Do environmental attitudes mediate the relationship between personality traits and environmentally friendly plastic consumption?*

By answering these questions, the findings can be used to expand the pro-environmental literature and to gain additional understanding of individuals who behave in environmentally conscious ways.

## METHOD

### Participants

The sample was comprised of 165 undergraduate students age 18-29 ( $M = 19.56$ ,  $SD = 1.77$ ; 69% female; 44% Asian, 33% Hispanic, 7% White/Caucasian, 5% African American, 6% multiple ethnic backgrounds, and 3% other) enrolled in an introductory psychology course. All participants were recruited from the Psychology Department's subject pool and received research participation course credit upon completion of this survey.

### Procedure

Participants were asked to complete the survey online using Qualtrics survey software. First, the participants were asked to provide demographic information including their age, gender, and ethnicity. Once they provided their demographic information, they moved on to complete the Big Five Inventory 2, Nature Relatedness Scale, and the Plastic Behavior Questionnaire.

### Materials

**Personality.** The Big Five Inventory 2 (BFI-2; Soto & John, 2017) was used to measure the participants' 5 domain-level traits: Extraversion (sociable, outgoing, energetic), Agreeableness (compassionate, selfless, charismatic), Conscientiousness (organized, responsible, productive), Negative Emotionality (emotionally volatile, moody) and Openness to Experience (creative, open-minded, intellectually curious). The items in the 60-item scale were measured using a 5-point Likert Scale, from *strongly disagree* to *strongly agree* ( $\alpha$ 's ranged from 0.79-0.88). Participants were asked to what degree they agreed with the statements provided. A few items from each domain were reverse scored. An example item for Extraversion is "I am someone who is outgoing, sociable."

**Environmental Attitudes.** The Nature Relatedness Scale (Nisbet, Zelenski, & Murphy, 2009) was used to measure respondents' environmental attitudes, such as their connection to nature, their perspective on the environment, and their experiences with nature. The 20-item scale was measured using a 5-point Likert scale, from *strongly disagree* to *strongly agree*. Participants were presented with several statements and were asked to rate them on the

degree to which they agreed with each item. An example item from the scale is "I always think about how my actions affect the environment" ( $\alpha = 0.86$ ).

**Environmentally Friendly Plastic Consumption.** To measure an individual's plastic consumption, the Plastic Behavior Questionnaire (PBQ) was created using information from several environmental organizations' websites and discussion between the authors. The scale initially contained 9 items, but after data collection, the following 3 items were removed to maintain unidimensionality: "I have used reusable containers to pack food," "I have brought reusable bags to the store," and "I have bought food in bulk." Unidimensionality is important for interpreting the denotation of a scale because a measure should only have a single meaning. Using a 5-point Likert-type scale, from *never* to *often*, respondents were asked to rate how often they performed various plastic consuming behaviors over the course of a year. Items examined were "I have used a reusable bottle," "I have used plastic straws" (reversed scored), "I have used plastic wrap" (reversed scored), "I have bought plastic water bottles" (reversed scored), "I have recycled plastic bottles," and "I have brought my own reusable straw out." Unfortunately, the scale has subpar reliability ( $\alpha = 0.58$ ). Despite its substandard Cronbach's alpha, it is still useful in capturing the varying behaviors one may perform to be more sustainable.

## RESULTS

### Big Five and Nature Relatedness Correlations with PBQ

All analyses were performed using R programming language. Zero order correlations between the Big Five traits and PBQ revealed that Conscientiousness had the strongest positive correlation with PBQ ( $r(163) = 0.23$ ,  $p = 0.003$ ). Those who had high scores in Conscientiousness tended to use less plastic. Likewise, Agreeableness was also positively correlated with PBQ ( $r(163) = 0.21$ ,  $p = 0.007$ ). Individuals who were more Agreeable were more likely to use less plastic. Openness to Experience, Extraversion, and Neuroticism were not significantly related to PBQ ( $r_s(163) < 0.12$ ). Lastly, our environmental attitudes measure, Nature Relatedness, had a strong positive relationship with PBQ ( $r(163) = 0.43$ ,  $p < 0.001$ ). Participants with high scores in nature relatedness tended to use less plastic.

### Big Five and Nature Relatedness Correlations

Next, zero order correlations were examined between Nature Relatedness (NRS) and the Big Five traits. Openness to experience had the strongest positive correlation with NRS ( $r(163) = 0.44, p < 0.001$ ). Those who were high in Openness tended to hold strong, positive environmental attitudes. Agreeableness and Conscientiousness were also positively related with NRS ( $r(163) = 0.29, p < 0.001; r(163) = 0.21, p = 0.007$ ). Extraversion and Neuroticism had small but insignificant correlations with NRS ( $r(163) = 0.14$ ).

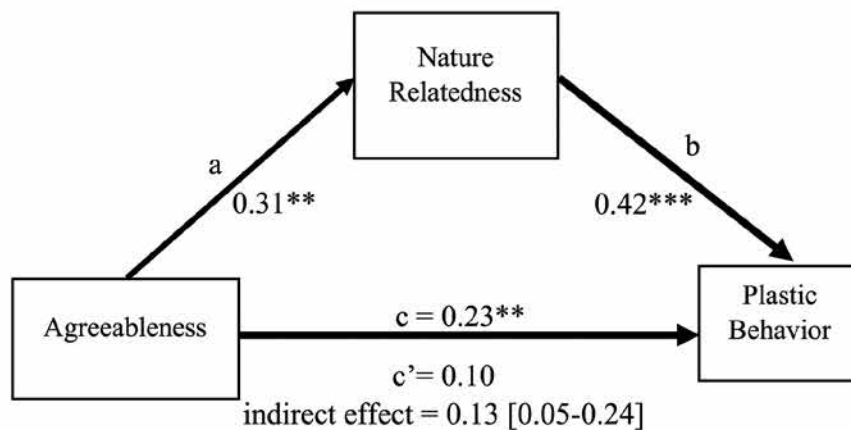
### MEDIATION

Based on the results from the zero order correlations, mediation was tested using the Preacher and Haynes (2008) nonparametric bootstrapping method, resampled 10,000 times, to explore the potential causal pathways from traits to behaviors. Nature Relatedness was added as the potential mediator in the model to explore the relationship between Agreeableness and PBQ. As shown in **Figure 1**, the total effect (**c**) of Agreeableness on PBQ was significant ( $\beta = 0.23, t(162) = 2.71, p = 0.0074$ ). The indirect effect (**ab**) of Agreeableness and Nature Relatedness ( $\beta = 0.13, 95\% \text{ CI } [0.05, 0.24]$ ) was also significant. Examining paths (a) and (b) respectively, the effects of Agreeableness on Nature Relatedness (**a**) ( $\beta = 0.31, t(163) = 3.82, p < 0.001$ ) and Nature Relatedness on PBQ (**b**) ( $\beta = 0.42, t(162) = 5.51, p < 0.001$ ) were both significant. In addition, the direct effect (**c'**) of Agreeableness on PBQ when controlling for Nature Relatedness was not significant ( $\beta = 0.1, t(162) = 1.24, p = 0.22$ ). Results indicate Nature Relatedness fully mediates the relationship between Agreeableness and PBQ.

In addition, a mediation analysis was also performed between conscientiousness and PBQ to explore the potential causal pathway through Nature Relatedness. The indirect effect (**ab**) of Conscientious and Nature Relatedness ( $\beta = 0.09, 95\% \text{ CI } [0.02, 0.16]$ ) was significant. The total effect (**c**) of Conscientious on PBQ was also significant ( $\beta = 0.25, t(162) = 3.06, p = 0.003$ ). Examining paths (a) and (b), the effects of Conscientiousness on Nature Relatedness (**a**) ( $\beta = 0.22, t(163) = 2.75, p = 0.007$ ) and Nature Relatedness on PBQ (**b**) ( $\beta = 0.42, t(162) = 5.6, p < 0.001$ ) were both significant. In addition, the direct effect (**c'**) of Conscientiousness on PBQ when controlling for Nature Relatedness was also significant ( $\beta = 0.16, t(162) = 2.08, p = 0.039$ ), signifying Nature Relatedness as partially mediating Conscientious and PBQ.

### DISCUSSION

The findings from this project suggest that individuals in our sample who were relatively higher in Agreeableness and Conscientiousness tended to engage in environmentally friendly plastic consumption. Nature Relatedness fully mediated and partially mediated the effects of Agreeableness and Conscientiousness, respectively, on plastic consuming behaviors. Thus, those higher in Agreeableness and Conscientiousness were more likely to hold more positive environmental attitudes, which in turn led to more environmentally friendly plastic consumption. Our results provide support to past research that examined the relationship between personality traits and pro-environmental behaviors.



**Figure 1:** Mediation diagram of Agreeableness on PBQ through Nature Relatedness. Confidence intervals of the indirect effect are indicated in brackets. \* $p < 0.05$



Our results support other research that has found associations between Agreeableness and environmental concern and environmental engagement (e.g. Hirsh, 2010; Milfont & Shelby, 2012). The study participants' characteristics of selflessness and concern for others may extend to the environment, which can result in engaging in environmentally conscious behaviors. As suggested in Milfont and Shelby (2012), those who take action and have concern regarding environmental issues tend to also work well with others and have the will to compromise their own interests. Those authors' explanation may also extend to environmentally friendly plastic consumption. Those who possess concern for others and the environment may make active efforts to reduce their consumption.

Our findings also provide support to the literature that found associations between Conscientiousness and outcomes like reducing emissions and general environmental engagement (Brick & Lewis, 2016; Milfont & Shelby, 2012). Individuals high in Conscientiousness may feel they have an environmental responsibility to consume less. Milfont and Shelby (2012) also suggest that those high in Conscientiousness have concern for the future and are therefore more likely to perform more environmentally friendly behaviors. Compared to Brick and Lewis (2016), our findings did not result in a full mediation. This may be due to using different environmental attitudes measures. However, other studies have found correlations between Conscientiousness and other variables like Political Cynicism and Machiavellianism (Swami, Chamorro-premuzic, Snelgar, & Furnham, 2011). Those with higher Conscientiousness, lower Political Cynicism, and lower Machiavellianism tended to engage in eco-friendly household waste management (Swami et al., 2011). Based on the results from the current study, future studies should further explore the relationship between Conscientiousness and other individual difference variables to fully account for this relationship.

Openness to Experience was not related to plastic consumption despite past studies finding a relationship with other pro-environmental behaviors (Brick & Lewis, 2016; Hilbig et al. 2012; Markowitz et al., 2012; Milfont

& Shelby, 2012). The conflicting findings might result from a unique association between plastic consumption that cannot be accounted for by Openness to Experience. Our results found a strong positive relationship between Openness to Experience and Nature Relatedness but not with the PBQ. While open people tend to be intellectually curious, this trait may not extend to more specific behaviors like reducing one's plastic consumption.

While the results of our study support existing literature, some of the limits should be addressed. First, the scale used to measure plastic consumption has relatively low internal consistency. Though we tried to maintain unidimensionality, participants tend to not see their plastic consumption as a single dimension. For example, as participants, it may be easier to associate plastic consumption with reducing one's use of plastic water bottles but not using reusable straws. As this is the first study that specifically focuses on plastic consumption, the scale is a good first step. Future studies should develop ways to improve the existing scale in a way that better accounts for one's plastic consumption. Another limitation to the study is that our sample population was undergraduate students enrolled in a psychology course, so the results may not accurately represent other populations. Despite this limitation, the findings are interesting because they allowed us to explore the possible relations between personality traits and plastic consumption within a population that has not been previously tied to being environmentally conscious.

## CONCLUSION

The current study adds to the pro-environmental literature by examining the relationship between personality traits and an individual's plastic consumption. Conscientiousness and Agreeableness were associated with environmentally friendly plastic consumption, and these relationships were mediated by environmental attitudes. As this is the first study to examine the relationship between individual personality differences and plastic consumption, we provided further insight to the types of people who engage with the environment in a sustainable way. However, future studies should investigate other variables that may influence this relationship.

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## Head Width is Correlated with Learning Ability in *Formica* Ants

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### ABSTRACT

Although insects possess small nervous systems, they exhibit behavioral plasticity, defying the idea that organisms with small brains are incapable of performing sophisticated behaviors. Eusocial insects are an ideal system for studying learning and memory because their ability to retain information aids in several key functions, including navigation and nestmate recognition. We trained 77 *Formica integroides* individuals from 5 different colonies to associate either a citrus or conifer odor with a sucrose reward. We exposed each worker ant to 1 odor along with the reward and the other odor along with water for 3 hours, alternating between each odor every 30 minutes. Twenty-four hours later, we carried out a learning assay in a Y-tube with 1 odor presented in each arm in the absence of the sucrose reward and observed which odor each ant worker visited first. Here, we focus on the relationship between associative learning and brain size using the measured head width of each worker as a proxy for brain size. While the idea that insects can learn, retain, and integrate various sensory cues has previously been established, our study provides the first behavioral evidence that learning ability is associated with brain size in ants. Our data shows that ants are capable of learning to associate olfactory cues with a reward in controlled laboratory conditions using an associative conditioning paradigm. Moreover, our data indicates that ants with larger heads demonstrate stronger associative learning abilities. In particular, larger ants demonstrate a higher successful learning rate than smaller ants.

**KEYWORDS:** *Formica* ants, head width, brain size, associative learning, learning and memory, eusocial insects, sensory cues



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Dr. Purcell investigates the proximate and ultimate drivers of transitions in social organization by integrating population genomics, field ecology, and manipulative experiments both in the lab and field as well as individual-based modeling approaches. Her primary study organisms have been social spiders from the genus *Anelosimus* and socially polymorphic ants from the genus *Formica*.

More recently, she has been working on yellowjacket wasps (*Vespula pensylvanica*) in collaboration with Erin Rankin (UCR Entomology). Students in her lab are answering related questions about social insects, including how ant workers partition tasks, why some ant species are more likely to become invasive than others, and how ants manipulate the soil in and around their nests.



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Department of Entomology

Jeneane is a graduating 4th year studying Neuroscience. Jeneane has been working in a cognitive neuroscience lab since her 2nd year using electroencephalogram equipment to record the electrical activity of the brain to better understand learning processes. After conducting research in Switzerland, Jeneane merged neuroscience with entomology to study learning and behavior in insects. She is a residential advisor working to mentor students and build communities. She teaches neuroscience in the Inland Empire to inspire underprivileged students to pursue higher education in STEM. Jeneane is interested in addressing health care disparities in underserved communities and intends to pursue a career in medicine.

## INTRODUCTION

One long-standing question in neurobiology is whether learning ability is associated with brain size. While research on the relationship between cognition and brain size has a strong taxonomic bias toward vertebrates, this pattern in insects still remains unknown (Chittka and Nevin, 2009). In mammals, relative brain size is measured by the encephalization quotient defined as the brain-to-body weight ratio (Cairo, 2011). This can be seen in humans who have the largest encephalization quotient, but not the largest brain. Across the phylogeny of mammals, for example, species with larger brains relative to their body size tend to possess greater cognitive ability (Willemet, 2013). Although understanding the link between size and cognition in insects requires further development, there is already substantial evidence that insects can learn and remember information in a broad range of contexts (Dukas, 2008; Chittka and Levin, 2009). For example, insects are capable of individual recognition (Sheehan, 2012), information transfer (Leonhardt, 2016), complex navigation (MacDonald, 2016), and associating sensory cues with positive and negative stimuli (reviewed by Dukas, 2008).

Several insect species have served as dominant models for empirical tests of learning ability and the evolution of cognition. Through studies of *Drosophila melanogaster*, we now know that learning ability is an evolving trait that can be the target of selection (Mery and Kawecki, 2002) or indirectly influenced by extrinsic interactions such as mating system (Hollis and Kawecki, 2014) or intraspecific aggression (Yurkovic et al., 2006). Honey bees and bumblebees have also been used extensively to study learning in a field setting and under controlled laboratory conditions. The honey bee waggle dance, for instance, remains one of the most striking examples of social learning and information transfer among any organism (von Frisch, 1967; Riley et al., 2005). In fruit flies, bees, and beyond, researchers have consistently found that insects can utilize visual cues for navigation (Collett, 2010), olfactory signals like pheromones for communication (Horst et al., 2002), gustatory reception to avoid toxic chemicals and detect nutrient rich food (Montell, 2009), and tactile cues for information about the environment (Uzsak et al., 2014). Insects also frequently integrate information from multiple

cues. For example, foraging insects use visual and tactile cues to navigate through their environment toward targets and away from threats (Heinze and Pfeiffer, 2018).

Research on learning in social insects has been biased toward bees, perhaps because they excel at learning to interpret complex visual cues that are relatively easy for researchers to manipulate (Nityananda et al., 2014). Broadening this focus to more social insect systems will be beneficial for a variety of reasons (Leonhardt et al., 2016). First, social insects are ubiquitous in terrestrial ecosystems, and gaining a more comprehensive understanding of their learning abilities and limitations will have implications for maximizing ecosystem services while limiting detrimental effects on managed systems (Wilson, 1990). Second, social insects provide the unique opportunity to measure learning in individuals that come from a common social environment and similar genetic background and to replicate across multiple scales (nestmates, different colonies, and different species). Finally, many social insects exhibit dramatic intraspecific and intracolony variation in body size (and, as a result, brain size), which facilitates research on the relationship between brain size and learning ability (Gouws et al., 2011).

Here, we investigate whether learning and memory in an ant species exhibiting continuous size variation is associated with head size variation. Specifically, we ask whether ants can learn to associate an odor with a reward and whether an ant's ability to learn and remember this association is correlated with its head width. We chose to use an olfactory cue because ants rely heavily on their well-developed olfactory systems to navigate, locate food, identify mates, and avoid predators (Knaden et al., 2015). A recent study demonstrated that *Camponotus* ants can learn to associate olfactory cues with sugar rewards (Udino et al., 2017), but previous research has not explored which traits are associated with learning ability. We expect to observe an association between learning ability and head width for several reasons. First, olfactory sensitivity is associated with body size in other social insects. Spaethe et al. (2007) demonstrated a size-based odor sensitivity difference in bumblebees. In this case, larger workers have larger antennae, and these contain proportionally more olfactory sensilla than smaller antennae. Larger



workers also have a stronger response to standard odor concentrations in electroantennogram assays. If the same pattern applies to ants, larger workers should be more sensitive to odor cues. In *Formica*, head width and scape length are strongly correlated (Tawdros et al., unpublished results). Second, given what we know about how insects process information, we propose that there may be a trade-off between investment in larger brains and stimulus processing speed. Nityananda et al. (2014) carried out an experiment testing how rapidly bumblebees can process simple and complex visual cues and found that they need to observe complex cues longer and more continuously than primates presented with similar cues in order to respond correctly. Another study determined that large dogs have better short-term memory and self-control, indicating that the increase in absolute brain size is associated with variation in executive function (Horschler et al., 2019). If the ability to process information is associated with the absolute neural capacity, this could lead to intraspecific size-based differences in cognitive ability.

In this study, we train individual *Formica integroides* workers to associate 1 of 2 novel olfactory cues with a sucrose reward. We then test workers in an odor choice assay to determine whether they will visit the reward-associated odor in the absence of the reward. We expect many ants to learn this association during the study. We further ask whether the ants that visited the reward-associated odor (“learners”) were larger than those that did not (“non-learners”). The genus *Formica* is highly amenable to this study because colonies harbor workers of continuously variable body size in the absence of morphological castes (West and Purcell, in review; Tawdros et al., unpublished results). To our knowledge, no previous study has examined whether head width is associated with learning in ants.

## MATERIALS AND METHODS

### Sampling

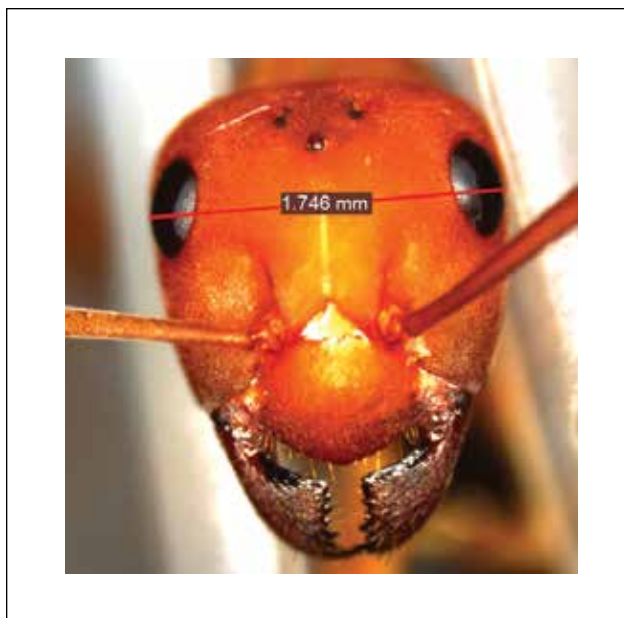
We collected *Formica integroides* workers from 5 colonies in the San Bernardino Mountains of California (34.1300°N, 116.8800°W). This species is a mound building wood ant found in western North America. We provided groups of nestmates with *ad libitum* water and maintained them in boxes for at least 24 hours at room temperature in the laboratory, after which we initiated the training phase.

### Training Phase

We trained individual ants to associate either citrus or conifer odor with a sucrose reward. We achieved this by placing fragrant orange peels or crushed *Pseudotsuga macrocarpa* needles in a small box, along with a 1.7mL tube containing 70% sucrose solution and plugged with cotton. In order to eliminate a potential bias resulting from innate odor preference, half of the workers from each training colony were given sucrose in combination with citrus, while the other half were given sucrose in combination with conifer. Workers were initially placed alone in a box with their training cue and sucrose solution for 30 minutes and were then transferred to a control box with the alternative odor with water for 30 minutes. They were exchanged between the treatment and control box every 30 minutes for 3 hours. As a result, each worker was exposed to the odor associated with a sucrose reward for three 30-minute periods and with the alternative odor along with water for three 30-minute periods. We provided both the sucrose solution and the water control inside a 1.7mL tube with a cotton wick. Trained ants were placed together with nestmates trained on the same odors with *ad libitum* access to water for another 24-hour period. Most ants were observed feeding on the sucrose solution during the training period.

### Testing Phase

Twenty-four hours after the training phase, we presented each worker with a choice of the 2 odors in a Y-tube in the absence of any reward. We released the ant workers into the empty chamber and observed whether they first visited the arm with the citrus or conifer odor. We also measured the time that each worker took to choose which arm to enter. We considered ‘learners’ workers that went to the odor that they were trained to associate with sucrose, while ‘non-learners’ were workers that entered the arm with their control odor or did not enter either arm within 30 minutes. After each learning assay, we collected workers into ethanol for subsequent analysis. We cleaned the Y-tube between each worker introduction by rinsing it with warm water and soap in order to eliminate any potential non-target cues from previous worker ants. We randomized the orientation of the conifer and citrus odor for each trial by placing conifer odor in the right arm of the tube and citrus odor in the left arm of one Y-tube and in the opposite



**Figure 1. The head of a *Formica integroides* worker ant**  
A photo of the head of a *Formica integroides* worker indicating the area used for head measurement. Photo was taken using a Leica S8AP0 microscope with an attached Leica DMC2900 camera.

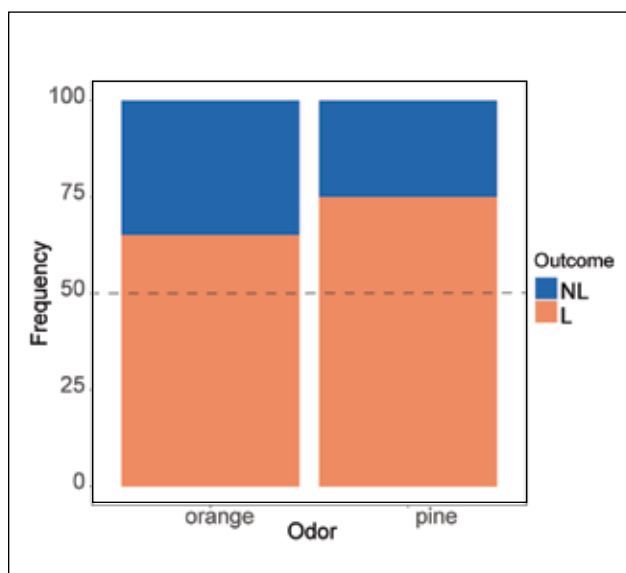
orientation in a second Y-tube. Each worker was randomly selected to perform the test in the first or second Y-tube in order to eliminate orientation bias.

### Head Measurement

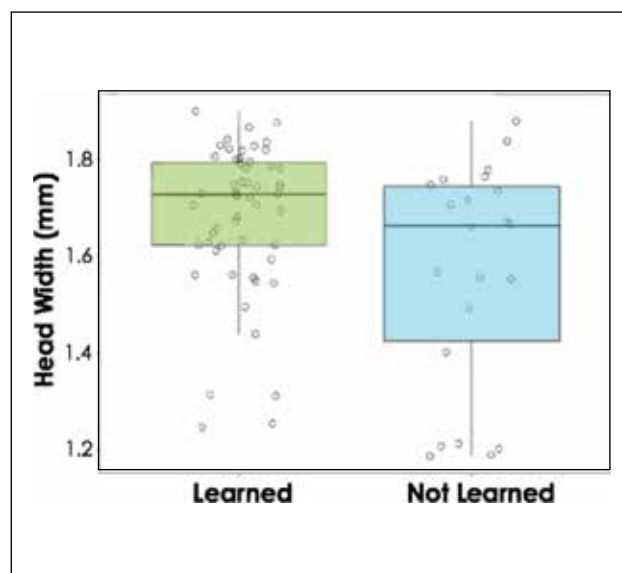
We photographed 77 workers from 5 colonies at 25x magnification with a Leica S8AP0 microscope and an attached Leica DMC2900 camera. We used the Leica Application Suite (V 4.6.2, Leica Microsystems) to measure the head width of each worker across the widest point of its eyes to the nearest thousandth of a millimeter (Figure 1).

### RESULTS

The chi-squared analysis was performed to show that there is no preference bias between the citrus and conifer odor ( $\chi^2 = 1.2031, p = 0.27$ ; Figure 2). We examined whether learners had a larger head width than non-learners. We determined that head width was not normally distributed, so we used a non-parametric Wilcoxon test to show that learners have larger heads than non-learners ( $W = 782.5,$



**Figure 2. Learning outcome on trained odor**  
*Formica integroides* workers learned to associate 2 different odors with sucrose rewards and responded positively to those odors in the absence of the reward. N=77 worker ants; 55 worker ants learned, and 22 worker ants did not learn. The probability of exactly, or more than,  $K_{30}$  out of  $N_{45}$  is  $p = 0.0178489$ . The probability of exactly, or more than,  $K_{25}$  out of  $N_{32}$  is  $p = 0.0010512$ . The number of worker ants that learned is shown in black. The number of worker ants that did not learn is shown in blue.



**Figure 3. Head width by learning outcome**  
Ants with a larger head width were more likely to learn to associate an olfactory cue with a sucrose reward. There was a significant difference in head width of workers that learned and workers that did not learn.

DF = 76,  $p = 0.046$ ; **Figure 3**). We used a linear regression model to show that the relationship between latency and the head width of ant learners is insignificant. (Multiple R-squared = 0.009248, Adjusted R-squared = -0.009446, F-statistic = 0.4947 on 1 and 53 DF, p-value = 0.4849). We compared the proportion of learners to the null expectation that 50% of ants would choose the left vs. the right arm of the Y-tube using a binomial test (**Figure 2**). The probability of exactly, or more than,  $K_{30}$  out of  $N_{45}$  is  $p = 0.0178489$ .  $N_{45}$  is the total number of ants trained on orange odor,  $K_{30}$  are the “learner” ants (**Figure 2**). The probability of exactly, or more than,  $K_{25}$  out of  $N_{32}$  is  $p = 0.0010512$ .  $N_{32}$  is the total number of ants trained on conifer odor, and  $K_{25}$  are the “learner” ants (**Figure 2**).

Our results demonstrate that ants are capable of perceiving and learning olfactory cues in controlled laboratory conditions using an associative conditioning paradigm. Through this study, *Formica integroides* workers learned to associate olfactory cues with a reward, showing that ant workers could retain information for at least 24 hours. Moreover, our results suggest that large ants were more likely to learn to associate an odor cue with a reward than small ants.

Although we did not measure brain size, we are using head width as a proxy. Cole (1985) showed that head width is strongly correlated with brain size across a diverse group of ants. This relationship appears to be conserved in social Hymenoptera, as Riveros and Gronenberg (2010) found a significant allometric correlation between head width and body mass with total brain volume in the bumblebee *Bombus occidentalis*. Despite this link, few studies have investigated the functional differences in behavior associated with brain size. Future research could focus on investigating the relationship between behavioral plasticity and brain volume using different cognitive tasks.

While most of the research on insect learning and memory has focused on associative learning, few studies have investigated the relationship between brain size and learning. Although our data suggests that head width is correlated with associative learning ability, it is important to consider intraspecific size-based task performance differences as a potential factor. West and Purcell (in

review) recently found that *Formica* workers utilize a size-based task allocation strategy in which differently-sized workers perform different tasks. Therefore, it is possible that large ants are better learners than small ants because the tasks on which they specialize in the field require a skillset similar to the challenge we created for them in the lab (Chittka and Muller, 2009). Thus, our results may be the product of successful skill transfer among large workers rather than a difference in cognitive ability between workers with differently-sized brains. However, because both large and small *Formica* workers specialize on foraging tasks (protein foraging and honeydew collection) that likely require similar navigation skills, we suggest that this explanation is unlikely (West and Purcell, in review). Further, it is important to note that we are unaware of what tasks the workers we collected for this study performed in the field. Future studies may investigate the role of task performance on differences in learning ability by subjecting differently-sized workers that are known to perform the same task in the field to similar cognitive tests.

Some limitations during the training phase include imprecision of timing when transferring the ants between the control and experimental conditions every 30 minutes. During the testing phase, we placed the ant in the middle of the Y-tube; however, this warrants a small amount of inconsistency due to human error. Additionally, ants learned to associate olfactory cues without showing any bias to the conifer or orange odor, despite the likelihood of prior exposure of the ants to conifer in their natural habitat and the common use of citrus as an insect repellent (Oshaghi, 2003). The lack of a bias towards either odor can be related to a previous study demonstrating that the presentation of a reward overrides a usually aversive response to an odor (Carcaud et al., 2009).

Furthermore, we assessed the duration of ants that navigated to the right or left arm of the Y-tube. Given our previous hypothesis that stated that larger ants are better learners, we also hypothesized that larger ants would locate the reward-associated odor with a smaller latency than smaller ants. We found no association between head width and the amount of time it took each worker to navigate to the reward-associated odor. This finding suggests that brain size is not associated with the speed of navigating

this laboratory assay. Future studies might explore latency and behavioral performance in association with brain size to investigate whether ants with a bigger brain size exhibit a faster and stronger adaptation to learning.

Our experimental aim was to develop an associative learning paradigm to analyze the relationship between brain size and learning through behavioral assays in *Formica integroides* worker ants. Here, we have shown that large ants yield a higher learning outcome than small ants. We exhibited that *Formica integroides* workers are capable of learning and retaining the olfactory conditioned stimulus and the unconditioned stimulus for at least 24 hours. *Formica* workers were shown to exhibit behavioral plasticity by successfully choosing the correct reward-based odor. Our study provides a method for analyzing the relationship between learning and brain size through associative learning tasks using olfactory cues at a behavioral level. This opens up new experimental possibilities to investigate the association between brain allometry and intraspecific size differences, the neural mechanisms underlying cognitive ability, and the association between head size and learning ability using different sensory cues. Future studies may also explore the relationship between the encephalization quotient and learning ability by measuring the encephalization quotient in learner and non-learner worker ants. An insect's ability to detect and interpret stimuli in a complex sensory environment plays a role in survival, and the factors underlying this mechanism may depend on brain size (Vet, 2006).

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## Chaucer's Latin: Misuse, Misinterpretation, and Critique in *The Canterbury Tales*

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### ABSTRACT

Reserved for the educated in medieval society, the Latin language was, for the better part of the period, the primary language of literature. However, by the end of the fourteenth century, Latin's preeminent status was waning with the growing regard for the potentials of the vernacular languages as sources of literature. In this period, the author Geoffrey Chaucer served as a legitimizing force for the English vernacular. However, in spite of this status, Chaucer's *The Canterbury Tales* continues to cite Latin excerpts and quotations throughout the text. While these minor citations are seemingly insignificant, this paper explores the motivations behind the inclusion of these excerpts and concludes in one interpretation that Latin is cited in the *Tales* as a language that is misused by the various members of medieval English society. This misuse involves ideas of misinterpretation and deception, and is, in summation, a criticism levied by Chaucer against the medieval clergy, the denomination that Chaucer associates most with the misuse of Latin.

**KEYWORDS:** Latin, quotation, Geoffrey Chaucer, social critique, clerical criticism, *The Canterbury Tales*, vernacular



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## INTRODUCTION

In all that has been written about *The Canterbury Tales*, there has been little discussion on the significance of the Latin quotations peppered throughout the text. The study of Chaucer's use of Latin in *The Canterbury Tales* is important, as the *Tales* are renowned as a great work of the English vernacular. Though the use of Latin is infrequent and confined to a few notable instances, the insertion and juxtaposition of Latin to the vernacular English is jarring and the text calls attention to it. Furthermore, the scholarship concerned with *The Canterbury Tales'* Latin has focused on the examination of Latin as it relates to an idea of misinterpretation, and primarily confined itself to a discussion of "The Nun's Priest's Tale." A few works such as Piero Boitani's "My Tale is of a Cock' or, The Problems of Literal Interpretation" and Melissa Furrow's "Latin and Affect," deal with a selection of the implications of the Latin quotations. However, Piero's article deals with the multitude of interpretations of Latin, but only within "The Nun's Priest's Tale," while Furrow's explores the use of Latin quotations as evidence for the Middle-English reader's facility with Latin. Neither of these examples deals with Chaucer's use of Latin in either the "General Prologue" or in relation to the characters of *The Canterbury Tales'* frame narrative.

With these quotations in mind, the question of the *Tales'* Latin becomes: what is the literary function of these quotations and can these quotations be linked to any of the prevalent themes that run throughout the *Tales'*? I have found that Chaucer uses it in order to criticize it. In this paper, I identify two predominant uses of Chaucer's Latin within *The Canterbury Tales'*: the characters of the *Tales'* use the language either in ignorance of its meaning or to deceive. Together, these two qualities relate to a criticism of the denomination of medieval society associated with Latin -- the Clergy. I look to augment the analysis of these

two uses with a discussion relating them to additional themes of Chaucer's scholarship, namely clerical criticism, abuse of authority, and speech as deception. Since I will be dealing with clerical criticism, I will focus on five clerical characters: the Prioress, Friar, Summoner, Pardoner, and Parson. Specifically, the focus will be on their respective descriptions in the "General Prologue," though I will also consider the remaining *Tales'* in my discussion.

## Clerical Criticism

On a close reading of the Prioress' characteristics, her aura of refinement reveals itself as a facade of sophistication, and she is presented as a social climber rather than a devout cleric. This concern with social status and secular concerns over religious issues serves as a recurring trend among Chaucer's clerical characters, seen in the juxtaposition of their descriptions with the ideal qualities of a member of the clergy. Instead of living a life dedicated to God or the poor, she concerns herself with secular matters, such as her table manners,<sup>1</sup> pet dogs,<sup>2</sup> and the accent of her French.<sup>3</sup> However, what is most damaging to her spiritual pretensions is her adornment with a golden brooch that reads "*amor vincit omnia*,"<sup>4</sup> or "love conquers all." For a cleric, this is a problematic presentation, as this line of Latin belongs not to any original creation of Chaucer's but to the classical writer Virgil and his *Eclogues*.<sup>5</sup> With the scandalous connotations of this Latin and given its source, there are two possible readings: either she misinterprets the line as implying a notion of divine love<sup>6</sup> or it further evidences her concern with secular issues. Taken in conjunction with the entirety of the Prioress' character, the Latin quotation of her portrait augments an interpretation of the character's depiction with a notion of clerical criticism -- namely that the Prioress is concerned with secular matters, such as her social standing, to the point that she is unable or unwilling to consider the implications of her adornments.

<sup>1</sup> "At mete wel y-taught was she with alle;/ Ne wette hir fingres in hir sauce depe;/ Wel coude she carrie a morsel, and wel kepe/ That no drope ne fille upon hire brest./ In curteisye was set ful muchel hir lest./ Hir over-lippe wyped she so cleane,/ That in hir coppe was no ferthing sene/ Of grece, whan she dronken hadde hir draughte" ("General Prologue," lines 127-135).

<sup>2</sup> "Of smale houndes hadde she, that she fedde/ With rosted flesh, or milk and wastel-breed./ But sore wepte she if oon of hem were deed,/ Or if men smoot it with a yerde smerte..." (ibid., 146 - 149).

<sup>3</sup> "And Frensh she spak ful faire and fetisly/ After the scole of Stratford atte Bowe, /For Frensh of Paris was to hire unknown" (ibid., 124 - 126). note. n.6. "I.e. she speaks in the English fashion, as it was spoken at Stratford at the Bow - a suburb some two miles east of London and home of the Benedictine nunnery of St. Leonard's."

<sup>4</sup> "And theron heng a broche of gold ful shene,/ On which ther was first write a crowned A./ And after, *Amor Vincit Omnia*" (ibid., 160-163).

<sup>5</sup> Chaucer, Geoffrey. *The Canterbury Tales*. Introduction and Notes by Robert W. Hanning and Translated by Peter Tuttle, (p. 823), originally the phrase referred to an erotic passion, although in its new context it could reference divine love.

<sup>6</sup> McGowan, Joseph P's "Chaucer's Prioress: Et Nos Cedamus Amori" deals with the variety of contradictory interpretations of this Latin quotation, but McGowan considers the Virgilian context and whether there is a double meaning in the original Latin. However, his findings are relatively inconclusive in regard to the character of the Prioress.

In a manner similar to the Prioress, the Friar is on a surface level lavished with descriptors<sup>7</sup> that with a shallow reading create a positive impression. However, underneath this description of his qualities, the presentation of the Friar is no kinder than that of the Prioress as he is rendered simultaneously as a beggar and a charlatan who uses spoken language, both English and Latin, to swindle. Chaucer describes the Friar's language as both "fair" and "plesaunt" in two instances relating to the roles of his vocation: once in reference to absolution, "And plesant was his absoluciou" ("General Prologue," line 222), and again in reference to his reading of scripture, "So plesant was his *In principio*" (line 254). In these instances, the Friar is less a preacher and more an actor, one with a conscious regard for speech who knows by no accident that he can modify how he speaks to have a greater effect on his audience. This awareness is seen in the line "Somwhat he lisped... To make his English sweete upon his tonge..." (lines 264-265). Hence the Friar understands that the more pleasing his speech, the more the nobility he preaches to will reward him in "pitaunce" or donations.<sup>8</sup> These actions strain the medieval expectations placed upon the clergy and imply the Friar's misuse of clerical authority to enrich himself with gifts while simultaneously ignoring the poorer members of his society.<sup>9</sup> This rendering of the Friar continues in the treatment of his vocation within "The Summoner's Tale" as one notorious for corruption. That is not to say that the Summoner's account is unbiased; indeed he comes across as vindictive against the Friar's own insults towards him. Nonetheless, the acknowledgement of the Friar's corruption, on the part of the characters of the *Canterbury Tales*, labors the point that the Friar is a disreputable character who uses speech and authority to live beyond his vows of poverty. This misuse and abuse continues in the manner of the Friar's Latin and use of scripture.

The singular reference to the Friar's Latin in the description of his *in principio* further compounds the qualities of

his speech. The line, "So plesant was his *in principio*" establishes this connection between these three notions of speech, Latin, and misuse of scripture. In regard to speech, the description of the Friar's *in principio* as "plesaunt" refers back to the idea of the Friar's conscious regard for the qualities of his language and the implications of corruption that accompany that sentiment. The reference and the misuse of the *in principio* (the opening to the book of Genesis and the Gospel of John) is particularly damning to the Friar's character, as it exemplifies a misuse not only of Latin but also of scripture. When taken with the other aspects of the Friar's character, he is effectively using Latin, scripture, his speech, and his office to enrich himself rather than living the expected poor life of a clergyman. This is a criticism emblematic of the clerical abuses of Chaucer's England, as the clerical bodies and mendicant orders of Chaucer's time similarly reneged on their vows of poverty. As such, the Friar's misuse of Latin supports this criticism insofar as he compounds his speech and antique learning in service to his own devices and standing.

The Prioress and the Friar both display pretensions to living beyond their social status, either emulating or supplicating to the aristocracy, respectively, but the Summoner does not. Noted as a drunkard and described with special attention to his unsavory physical appearance,<sup>10</sup> the Summoner does not portray any pretensions to being of a higher social standing; he instead conjoins this physical persona to his mannerisms, particularly in regard to his abuse of his vocation. Described as illiterate in Latin,<sup>11</sup> the Summoner, as detailed in the "General Prologue," compounds and exemplifies the aforementioned qualities of the Prioress' ignorance through his own illiteracy and the duplicity of the Friar, which again feeds Chaucer's clerical criticism by painting a church official -- particularly a despised church official -- as both ignorant and corrupt. This criticism extends to the Summoner's Latin, as, though he is unaware of its meaning, he uses it to enhance the effect of his own speech. Notably, he frequently quotes

<sup>7</sup> The friar is described as "wantowne" (distinguished) ("General Prologue," line 210), and as knowledgeable in clerical matters "In alle the ordres foure is noon that can" (211). His generosity is also mentioned in the line "He hadde maad ful many a mariage/ of yonge wommen, at his own cost." (212-213), however some scholars have interpreted this line with the double meaning that the Friar seduced the women. (p.7, n6).

<sup>8</sup> "He was an esy man to yeve penaunce/ Ther as he wiste to have a good pitaunce" (ibid., 223-224)

<sup>9</sup> "Accorded nat, as by his facultee./ To have with seke lazars aqueyntaunce" (ibid., 245).

<sup>10</sup> "That hadde a fyr-reed cherubinnes face, /For sawcefleem he was, with eyen narve./ As hoot he was, and lecherous, as a sparwe;/ With scalled browes blake, and piled berd;/ Of his visage children were aferd" (ibid., 624 - 628).

<sup>11</sup> "Than wolde he speke no word but Latyn./ A fewe termes hadde he, two or three,/ That he had lerned out of som decree..." (ibid., 638-640).

<sup>12</sup> "Thanne wolde he speke, and crye as he were wood" (ibid., 636); here "wood" can be taken as meaning mad.

"*Questio quid iuris*" (line 646) or "the question is, what point of law applies," and he does so as if mad<sup>12</sup> in order to enhance the terror of his position and potentially the terror of his appearance. A use of Latin that Chaucer parallels to a bird in the lines "And eek ye knowen wel how that a jay/ Can clepen 'Watte' as well can the Pope" (Chaucer, "General Prologue", lines 642-643).<sup>13</sup> Essentially, Chaucer is remarking that the Summoner's use of Latin is limited to mimicry without understanding, which is a use similar to the misuse and ignorance of the Prioress' own Latin quotation. Therefore, because the Summoner compounds both aspects of Chaucer's criticism, it is not surprising that Chaucer reserves the greatest criticism for the most controversial clerical figure. He paints a portrait of an illiterate and corrupt church official who is ignorant of Latin but still uses it without regard to correctness in order to enhance the effectiveness of his own character.

Paired with the Summoner and contributing to the Summoner's characterization as an unsavory church official, the Pardoner complements this grouping of the clerical characters through his display of hypocrisy in the telling of his tale. As a character the Pardoner is comparable to the Summoner; therefore, it is surprising that the Pardoner gives a moral tale on the nature of greed rather than a raunchy fabliau. Within the first lines of his tale's prologue, the Pardoner establishes the theme as relating to the biblical proverb "*Radix malorum est Cupiditas*"<sup>14</sup> or "the root of evil is greed." Such a thematic statement already indirectly criticizes the self-interests of characters such as the Friar. However, it more directly indicts the Pardoner, as within a few lines of giving his theme he describes himself as a avaricious conman.<sup>15</sup> The ending to his exemplum tale furthers his blatant hypocrisy, as upon finishing his lecture on greed he offers to sell pardons and relics to the other pilgrims.<sup>16</sup> This act is jarring considering the content and message of his earlier theme and even more so given that the Pardoner admits that his relics are a sham.<sup>17</sup> As such, the Latinate and biblical morals of the Pardoner's

theme are constructed through the actualities of his self-indulgence. Nonetheless, the Pardoner while amoral offers something of an anti-exemplum, as his presentation is deplorable but also straightforward and honest. Through the blatancy of his corruption and admission of vice, the Pardoner provides a more straightforward depiction of the worst clerical excesses but without the pretensions of the Prioress or Friar, which would have obscured a truthful portrayal.

### Clerical Exempla

Chaucer has supplied these four characters as a collective unit to criticize the worst excesses of the Clergy in Chaucer's society, and this critique is compounded when the corruption of these characters is juxtaposed with a proper exemplum: the Parson. Compared to the Prioress' obsession with social advancement, the Parson is a humble character, brother of the Plowman,<sup>18</sup> and makes no pretensions to living above his station. Contrary to the neglectful Friar, the Parson is devout to the common people of his parish,<sup>19</sup> and the text cites him as lacking the mercenary qualities of both the Friar and Pardoner: "He was a shepherde and noght a mecenarie" ("General Prologue, line 514). In summation, the portrait of the Parson provided within "The General Prologue" depicts a rather unassuming character, which is a quality that continues in "The Parson's Tale" through the conscious rejection of poetics and the tale's truthful moral quality. In actuality, "The Parson's Tale" does not assume the form of a typical tale, as he instead gives a treatise on penitence,<sup>20</sup> a moral lesson that comes across as genuine given the Parson's character in contrast to the Pardoner's hypocritical didactic. This idea of contrast and continuing rejection of obscurity is seen in the Parson's Latin given this tale's overall theme is given in English rather than the Pardoner's "*Radix malorum est Cupiditas*". This is an abstinence that characterizes the Parson's relationship with the language in the instances in which Latin does appear. A notable appearance of such a relationship is in the line "I ne can seye it noon otherweyes in English, but in Latin

<sup>13</sup> "And you also know well how a jay can say "Walter" as well as the Pope" (My own transliteration).

<sup>14</sup> Noted as being cited from 1 Timothy 6:10 Chaucer, Geoffrey. *The Canterbury Tales: Seventeen Tales and the General Prologue*. Ed. V.A. Kolve and Glending Olson. Third Norton Critical Edition. W.W. Norton & Company, Inc. New York, pg. 257 (n.5.).

<sup>15</sup> "Thus can I preche agayn that same vyce/ Which that I use, and that is avaryce." ("The Pardoner's Prologue", lines 426-427).

<sup>16</sup> "But sirs, o word forgat I in my tale,/ I have relikes and pardon in my male,/ As faire as any man in Engelond,/ Whiche were me yeven by the popes hond." (ibid., 919-922).

<sup>17</sup> "Reliks been they, as wenen they eechoon./ Than have I in latoun a sholder-boon/ Which that was of an holy Iewes shepe./" "Good men," seye I, "tak of my wordes kepe;/ If that this boon be wasshe in any welle,/ If cow, or calf, or sheep, or oxwelle" (ibid., 349-354). These lines imply that the relics he is selling are animal bones, rather than the saints' bones he claims.



it highte *centesimus fructus*,” which he translates literally as “the hundred fruit” in the preceding line (“The Parson’s Tale” § 76). Where the Summoner and Friar would obscure and enhance their speech with Latin, the Parson does not. He instead qualifies that because the idea he is speaking of is beyond his ability to translate with respect to idiosyncrasies he will give it in the original language though he gives the English prior to the Latin, only giving the Latin because of the inadequacies of translation.

### Use of Latin Outside of the Clerical Group

Beyond “The General Prologue,” “The Pardoner’s Tale,” and “The Parson’s Tale,” the Latin quotations of *The Canterbury Tales* fall predominantly into two categories: as either continuing similar notions of misinterpretation or misuse, or as one of the seemingly inconsequential mentions of “*benedicite*,” which are scarce, but their use in the text may warrant greater investigation. However, in the former thematic category there are three larger instances in which Latin appears in regard to an issue of misuse: “The Reeve’s Tale,” “The Miller’s Tale,” and “The Nun’s Priest’s Tale.” Furthermore, within the fabliaux narratives of the Reeve’s and Miller’s Tales, this misuse takes a sacrilegious quality by utilizing religious language and Latin quotation in a sexualized context. Within “The Reeve’s Tale,” the Latin comes in an unsanctimonious reference to prayer, as one of the characters (the wife of the miller character within “The Reeve’s Tale”) cries “‘Help, holy croys of Bromeholm, . . . *In manus tuas!* lord, to thee I calle!” after realizing that she slept with the wrong man the previous night (“The Reeve’s Tale,” lines 4286-4287). In this scene, there exists a juxtaposition of sacred language (in this instance, a prayer) with a sexualized scenario that can be read as rape and demonstrates a misuse of a sacred language.

The reference to Latin in “The Miller’s Tale” is less direct in establishing a similar juxtaposition, but it evokes in its subtlety the qualities of the Friar’s deceptive speech. “The

Miller’s Tale” focuses on the character Nicholas, a live-in scholar who attempts to use his learning and his rhetoric to seduce the wife of his host. However, it is the description of his speech qualities that I would like to emphasize, as the language used to describe Nicholas’ speech bears marked similarity to the Friar’s. He is detailed as singing “*angelus ad virginem*”<sup>21</sup> and singing it “so swetely.” This idea of sweet language conjoining with “sacred speech” renders the hymn sacrilegious while also continuing the discussion of the seducing qualities of speech. Though within the context of the frame narrative neither the Reeve nor the Miller are clerical figures, the characters of their tales utilize Latin in a manner that parallels and satirizes the clerical use by repeating the same tropes seen in the “General Prologue” descriptions.

“The Nun’s Priest’s Tale” and its character of Chauntecleer offer the most comprehensive commentary on Latin in *The Canterbury Tales* outside of the “General Prologue.” The Nun’s Priest is a near non-entity in “The General Prologue” mentioned only in passing.<sup>22</sup> Within his tale, however, Chauntecleer comes across in much the same manner as Nicholas from “The Miller’s Tale,” with the distinguishing difference being that Chauntecleer is a talking rooster. The comparison is apt because both characters represent a parody of the medieval intellectual society through their attention to rhetoric, and both characters use Latin and rhetoric to seduce and flatter. The discussion of Latin in “The Nun’s Priest’s Tale” is thematically similar to the previously discussed instances in the sense that the idea of misinterpretation also returns in Chauntecleer’s use of Latin. Chauntecleer notably quotes the Latin phrase to his mistress, hen, Pertelote “*In principio, mulier est hominis confusio*,” or “In the beginning, woman is the ruin of man.” However, Chauntecleer renders the translation as “woman is mannes joye and al his bliss” in order to flatter Pertelote (line 4356). And given Chauntecleer’s knowledge and insight into the different levels of medieval rhetoric,<sup>23</sup> it is more likely that the mistranslation is an attempt at flattery

<sup>18</sup> “With him (the Parson) ther was a Plowman, was his brother . . .” (“General Prologue,” line 529).

<sup>19</sup> “That Cristes gospel trowly wolde preche;/ His parissshens devoutly wolde he teche” (ibid., 481-482).

<sup>20</sup> “Of whiche weyes, ther is a ful noble wey and a ful covenable, which may nat faile to man ne to womman, that thurgh sinne hath misgoon fro the righte wey of Ierusalem celestial; and this wey is cleped Penitence” (“The Parson’s Tale,” § 1).

<sup>21</sup> A known annunciation hymn (pg. 72) Chaucer, Geoffrey. *The Canterbury Tales: Seventeen Tales and the General Prologue*. Ed. V.A. Kolve and Glending Olson. Third Norton Critical Edition. W.W. Norton & Company, Inc. New York.

<sup>22</sup> “Another Nonne with hire (the Prioress) hadde she;/ That was her chapeleynne, and Preestes three” (“General Prologue,” lines 163-164).

<sup>23</sup> This idea is the essential thesis of Susan Gallick’s article “Styles of Usage in ‘The Nun’s Priest’s Tale.’”

through the misconstruing of translation rather than an ignorant mistake. As a result, Chauntecleer serves as an additional parallel to the Friar and Nicholas from "The Miller's Tale," as all three characters use Latin for flattery, which supports the idea of Latin being used as a tool for deception.

In contrast to these themes of deception, ignorance, and abuse of authority, another concept that persists throughout the *Tales* is the idea of the normative and the text's subsequent preference for the status quo. Representing this conservatism is the Knight, the only aristocrat of Chaucer's characters. Unlike the clerics who merely emulate high-society, the Knight is established as a "lover of chivalrie, trouthe, honor, fredom, and courteise,"<sup>24</sup> proclaiming the values of medieval courtly culture. The idea of *trouthe* is most relevant to this discussion because of the juxtaposition it offers to the corrupt and less than truthful qualities of the clergy. The Parson alone represents an ideal cleric, and in the end, the Knight complements the qualities of the Parson with a representation of a secular ideal that contrasts the secular qualities of Chaucer's clerical characters.

## CONCLUSION

The Prioress, Friar, Summoner, and Pardoner all engage in forms of deception and use Latin for this end. This *trouthe* relates to Chaucer's possible preference for English over Latin, as, to paraphrase a quote from another of Chaucer's works, "English is just as good for the instruction of an Englishman as Greek or Latin."<sup>25</sup> In this discussion of English, the text presents the use of English as a type of *trouthe*, and Latin is included to be criticized rather than for any notion of its prestige.

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<sup>24</sup> ("General Prologue," line 46).

<sup>25</sup> Paraphrased from Paxton Hart's article "Chaucer's Regard for English" pg. 7.

## Examining the Relationship Between Personality, Anxiety, and Self-Handicapping

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### ABSTRACT

University student engagement in self-handicapping strategies, such as procrastination, remains a prevalent problem. Previous studies have found these behaviors to be detrimental to students' academic performance, which raises the question about what kind of factors influence people to engage in self-sabotaging behaviors. Literature suggests that anxiety and personality are variables involved in self-handicapping. The current study examines the role of test anxiety as a moderator of self-handicapping and, additionally, considers the relationship between measures of personality and academic performance. This study utilized 844 undergraduate students who completed a survey containing several personality measures. Results suggest that test anxiety acts as a moderator in the relationship between personality and self-handicapping. This indicates that personality may predispose one to self-handicapping behaviors, but test anxiety is the driving force behind them. This research is very beneficial to college campuses, which should consider encouraging students to engage in behaviors aimed to reduce their test anxiety rather than attributing these behaviors to student laziness or personality.

**KEYWORDS:** Self-handicapping, test anxiety, personality, procrastination, self-esteem, self-efficacy



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## INTRODUCTION

For most students their future employment relies heavily on their college academic performance. Unfortunately, a sizeable number of students experience anxiety, which can result in academic self-handicapping (Rappo, Alesi, & Pepi, 2017) and be detrimental to their academic performance. Self-handicapping behaviors are unconscious forms or manifestations of self-sabotage that people engage in to externalize their failures (Jones & Berglas, 1978). For example, when a student chooses to go out with friends the night before an exam, the student blames the resulting failure on going out, rather than attributing it to his or her (lack of) ability. One of the most common forms of academic self-handicapping is procrastination, which is also known to negatively impact academic performance (Beswick, Rothblum, & Mann, 1988). Despite the consequences of engaging in these behaviors such as self-handicapping and more specifically procrastination, they remain as prevalent problems for students. This raises the question as to what kind of influences can cause people to engage in self-sabotaging behaviors despite their known harmful consequences. Common practice in today's society is to attribute procrastination to laziness and lack of motivation. However, this does not provide a clear root cause analysis and may be a source of discouragement to students as it appears to blame the problem on a lack of character (Ellis & Knaus, 1977). However, in psychology, researchers often emphasize that self-handicapping and procrastination are rooted in an individual's personality and their level of particular emotions, such as anxiety (Rothblum, Solomon & Murakami, 1986).

### Test Anxiety

Among college students, one of the most crippling forms of anxiety is test anxiety, which is the fear of failure while taking an exam. Previous studies have found that test anxiety is not only prevalent but also negatively impacts academic performance (Hill & Wigfield, 1984). Previous literature has examined the influence of test anxiety on procrastination, which is a common form of self-handicapping. Research has found those who self-report high levels of procrastination also tend to report higher levels of test anxiety. Additionally, high self-reported procrastinators tend to report more state anxiety and anxiety-related symptoms as compared to low self-reported

procrastinators (Rothblum, Solomon, & Murakami 1986). This indicates that both test anxiety and state anxiety are factors related to study habits and self-handicapping. As such, if researchers hope to find effective methods to combat self-handicapping, it is worth taking the time to understand the relationship between anxiety and self-handicapping.

### Personality

This analysis will operationalize personality using multiple self-concept variables that reflect on the beliefs one holds about oneself. The self-concept variables examined are self-esteem, self-efficacy, self-clarity, and impostor syndrome. These variables reflect on an individual's feelings and beliefs about themselves, so therefore it is reasonable to group them together as aspects of personality. For the purposes of this analysis, they will be referred to as "personality characteristics."

Self-esteem is best described as one's belief in one's self-worth as well as a reflection of an individual's confidence or cynical opinions of oneself. Previous literature has found self-esteem to be negatively correlated with self-handicapping. For example, Shen (2007) found a correlation between the two variables of ( $r = -0.57$ ). In other words, individuals who have low self-esteem also tend to report higher levels of self-handicapping, which suggests that belief in one's self-worth is related to one's academic success.

A concept strongly related to self-esteem is self-efficacy or an individual's belief in their ability to achieve goals by virtue of their own efforts. A specific form of self-efficacy is college/academic self-efficacy. Academic self-efficacy is an individual's belief in their ability to succeed at a range of challenges and tasks associated with college and college life. Literature has found using correlational data that academic self-efficacy is strongly related to better academic performance ( $r = 0.29$ ; Chemers, Hu, & Garcia 2001).

Another self-concept variable of interest is self-clarity. Self-clarity is the extent of the individual's self-confidence, consistent viewpoints, and consistent beliefs. Literature has found that those who have high self-clarity tend to self-handicap less. Additionally, correlational data studies have

also found that those who have high self-clarity tend to have less test anxiety ( $r = -0.30$ ; Thomas & Gadbois 2007).

Finally, impostor syndrome is the least studied self-concept variable in existing literature. Impostor syndrome involves individuals who are persistently doubting their accomplishments, suffer from the recurring thought that they “don’t belong” within their social or academic groups, and have a secret fear of being exposed as a fraud. Many who suffer from impostor syndrome tend to attribute their successes to external factors, such as luck. Research has indicated that those who have higher levels of impostor syndrome tend to report higher levels of self-handicapping ( $r = 0.53$ ; Want & Kleitman 2006).

### Predictions

This analysis will examine the relationship between personality characteristics and academic outcome variables. Test anxiety, self-handicapping, and GPA will form the academic outcome variables. Self-esteem, self-efficacy, self-clarity, and impostor syndrome will form the personality characteristics. While anxiety and personality may be predictive of self-handicapping, it is reasonable to assume that one may act as a moderator in the relationship. Additionally, meta-analyses have shown a strong negative relationship between GPA and variables such as self-handicapping and test anxiety (Schwinger, Wirthwein, Lemmer & Steinmayr, 2014), which allows the assumption that predictors that are positively related to GPA should be negatively related to self-handicapping and anxiety. Therefore, we predict:

- *Hypothesis 1:* There will be an interaction effect between test anxiety and self-esteem when predicting self-handicapping.
- *Hypothesis 2:* Self-esteem will be negatively related to the academic outcome variables self-handicapping and test anxiety, and positively related to GPA;
- *Hypothesis 3:* Self-efficacy will be negatively related to the academic outcome variables self-handicapping and test anxiety, and positively related to GPA;
- *Hypothesis 4:* Self-clarity will be negatively related to the academic outcome variables self-handicapping and test anxiety, and positively related to GPA;
- *Hypothesis 5:* Impostor syndrome will be positively related to the academic outcome variables self-

handicapping and test anxiety, and negatively related to GPA.

## METHODS

### Participants

Participants consisted of 844 Southern California college undergraduate students recruited via the institutional SONA system in exchange for research credit. Participation in research was mandatory to receive a course grade, but students were allowed to opt out if desired by completing an alternative assignment (attending a campus sponsored research lecture) in accordance with university policy. Prior to being posted on the institutional system, the current study was reviewed and approved by the university IRB to ensure adherence to research standards. Overall, 536 females and 308 males participated in the study. The mean age was 19.26, ( $SD=1.49$ ). The sample was ethnically diverse, consisting of 40.89% Asian Americans, 36.45% Latino, 8.87% Caucasian, 7.07% who identified as other, 5.28% African Americans, and 4.92% Mixed-ethnicity. The sample consisted of 51.3% freshmen, 26.1% sophomores, 13.6% juniors, and 7.7% seniors. Additionally, the sample included participants from many different academic backgrounds. Broken down, the participants ranged from 49 different majors, 49.2% social science majors, 27.5% hard science majors, 8.6% business majors, 7.7% engineering majors, and 7% undeclared.

### Measures

**Self-Handicapping.** Self-Handicapping was measured using the Self-Handicapping Survey (SHS; Urdan & Midgley, 1995). The SHS consists of 6 items measured on a Likert scale, ranging from 1 (Strongly Disagree) to 6 (Strongly Agree). The scale consists of questions that assess levels of self-handicapping. For example, one of the items asked “Some students fool around the night before a test so that if they don’t do well, they can say that is the reason. How true is this of you?” To assess the reliability of the questionnaire, we utilized the Cronbach’s alpha statistic to assess the close relationship of scale items and therefore interpret how much they are assumed to capture the same construct. Cronbach’s alpha values of 0.7 or higher indicate acceptable reliability. The Cronbach’s alpha for the SHS was 0.87, indicating the scale had a high level of reliability.



**Test Anxiety.** Test-anxiety was measured using the Sarason Test-Anxiety Scale (STAS; Sarason, 1980). The STAS consists of 37 items, each measured on a Likert scale ranging from 1 (Strongly Disagree) to 5 (Strongly Agree), and 7 of the 37 items were reverse coded. For example, some of the items on the scale included “During a test, I find myself thinking of the consequences of failing” and “I really don’t see why some people get so upset about tests” (reverse coded). Cronbach’s alpha was 0.91, indicating the scale had a high level of reliability.

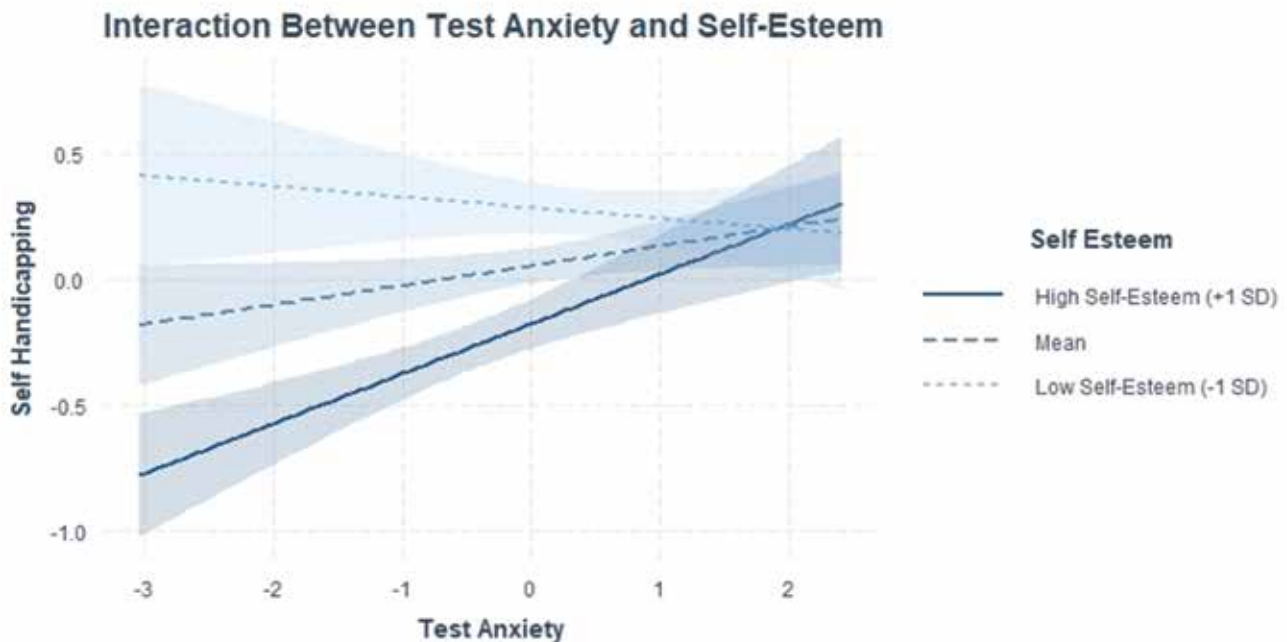
**Self-esteem.** Self-esteem was measured using the Rosenberg Self-Esteem Scale (RSES; Rosenberg, 1965). The RSES consists of 10 items, each measured with a Likert scale ranging from 1 (Strongly Disagree) to 4 (Strongly Agree), and 6 of the 10 items were reverse coded. Items on the scale included “I feel I have a number of good qualities” and “I wish I can have more respect for myself” (reverse coded). Cronbach’s alpha was 0.89, indicating a high level of reliability.

**Self-clarity.** Self-clarity was measured by using the Self-Concept Clarity Scale (SCC; Campbell et al., 1996). The SCC consists of 12 items and is based on a Likert scale

ranging from 1 (Strongly Disagree) to 5 (Strongly Agree), and 2 of the 12 items were reverse coded. On this scale, high self-clarity scores indicate that participants have less consistent beliefs. Some of the items on the scale include “My beliefs about myself often conflict with one another” and “I spend a lot of time wondering what kind of person I really am.” Cronbach’s alpha was 0.90, indicating the scale had a high level of reliability.

**College Self-efficacy.** College self-efficacy was measured using the College Self-Efficacy Inventory (CSE; Solberg et al., 1993). The CSE evaluates the degree to which participants feel confident accomplishing college-related tasks and challenges. The scale consists of 22 items, each measured on a Likert scale from 1 (Very Unconfident) to 7 (Very Confident). Some of the items on the scale include “Socializing with others you live with” and “Participate in class discussions.” Cronbach’s alpha was 0.90, indicating the scale had a high level of reliability.

**Impostor Phenomenon.** To measure Impostor Phenomenon, participants filled out the Clance Impostor Phenomenon Scale (CIPS; Clance, 1985). The CIPS consists of 20 items, each measured on a Likert scale that ranges



**Figure 1.** Graph of test anxiety’s moderating role between personality and self-handicapping. Shaded bars represent 95% confidence intervals. Low self-esteem (-1 SD) slope= -0.04. High self-esteem (+1 SD) slope =0.19. Mean slope =0.08.

from 1 (Not at all true) to 5 (Very true). One of the items involved states “It’s hard for me to accept compliments or praise about my intelligence and accomplishments.” Cronbach’s alpha was 0.80, indicating the scale had a high level of reliability.

## Procedure

After signing up for the study through the SONA system, participants reported to a reserved computer lab to complete multiple measures of personality and academic achievement. Researchers then greeted participants, obtained their consent to participate, and informed participants that the focus of the study would focus on the collegiate participants’ beliefs about success in college and college life. Afterward, the researchers read the directions for the study, and the participants were given sixty minutes to complete the survey. All survey measures were administered online.

## Results

Hypothesis 1 predicted that there would be an interaction effect between self-esteem and test anxiety, predicting self-handicapping. To examine this hypothesis, a standardized regression analysis was performed on the predictor variable self-esteem, the moderating variable test anxiety, and the outcome variable self-handicapping. A moderator variable is a variable that influences the strength and/or direction of the relationship between two other variables. The results found a significant main effect of self-esteem ( $\beta(837) = -0.230, p < 0.001$ ), a significant main effect of test anxiety ( $\beta(837) = 0.0070, p < 0.005$ ), and a significant, though weak, interaction effect between self-esteem and test anxiety ( $\beta(837) = 0.12, p < 0.001$ ; **Figure 1**). This suggests that those low in self-esteem and those high in self-esteem both handicap at similar levels when test anxiety reaches its peak.

Hypothesis 2 stated that self-esteem would have a negative relationship with self-handicapping and test anxiety, as well as a positive relationship with GPA. Pearson bivariate correlations were conducted to assess the relationship between the variables. As hypothesized, self-esteem had a moderate negative relationship with self-handicapping and test anxiety and a positive relationship with GPA (**Table 1**). In other words, those low in self-esteem are likely to self-handicap more and have more test anxiety.

Hypothesis 3 predicted that self-efficacy would have a negative relationship with self-handicapping and test anxiety and a positive relationship with GPA. Pearson bivariate correlations were conducted on self-efficacy and the academic outcome variables. As hypothesized, self-efficacy results indicate a moderate negative relationship with self-handicapping and test anxiety and a positive relationship with GPA (**Table 1**). To illustrate this relationship, those low in self-efficacy tend to be high in self-handicapping and test anxiety.

Hypothesis 4 predicted self-clarity would have a negative relationship with self-handicapping and test anxiety and a positive relationship with GPA. Pearson bivariate correlations were conducted to assess the relationship between self-clarity and the academic outcome variables. As hypothesized, the results indicate that self-clarity had a moderate negative relationship with self-handicapping and test anxiety and a nonsignificant positive relationship with GPA (**Table 1**). More specifically, those who report higher self-clarity tend to report that they self-handicap less and experience less test anxiety.

Hypothesis 5 predicted impostor syndrome would have positive relationship with self-handicapping and test anxiety and a negative relationship with GPA. Results of Pearson bivariate correlations indicate impostor syndrome correlates to a weak positive relationship with self-handicapping, a strong positive relationship with test anxiety, and a weak negative relationship with GPA (**Table 1**). Additionally, those low in self-efficacy tend to self-handicap considerably more and have slightly increased test anxiety.

## DISCUSSION

This study sought to explain some of the reasons people engage in self-handicapping behaviors. To do so, test anxiety was examined as a moderator in the relationship between personality and self-handicapping. Consistent with previous research, results found those low in self-esteem are more likely to self-handicap. Results also indicate that those who have high self-esteem are less likely to self-handicap at low levels of test anxiety. One surprising result from the study is that among those who are high in self-esteem, the incidence of self-handicapping increases rapidly along with test anxiety, while among

those who are low in self-esteem there appears to be no relationship between test anxiety and self-handicapping at all. Lastly, as test anxiety reaches its highest levels, self-handicapping differences between those high and low in self-esteem nearly disappear. Results indicate personality can make one more susceptible to self-handicapping; however, test anxiety is the driving force behind the initiation of self-handicapping behaviors. This suggests that the root of the problem, concerning self-handicapping, may not necessarily lie within the student’s personality. This research is useful to college campuses, which should consider encouraging students to engage in behaviors aimed to reduce their test anxiety rather than attributing these behaviors to student laziness. Additionally, this analyzed how personality characteristics and test anxiety relate to self-handicapping and the results of Pearson bivariate correlations between the academic outcome variables and personality characteristics are consistent with previous research.

An alternative explanation for the findings in this study indicate that test anxiety may act as a proxy for uncertainty. In many situations associated with high test anxiety, there is also a high level of uncertainty surrounding the outcome. For example, when preparing for an exam, students may

experience anxiety because they are unsure of their abilities, the test content, or their performance. Therefore, literature frequently examines the role of uncertainty in relation to other variables associated with self-handicapping.

**Limitations**

One limitation of this study is the reliance on self-reported data. As previous studies have indicated (Hirt, Deppe, & Gordon 1991), there is a distinction between self-reported and behavioral self-handicapping. More research is needed before we can assume that this data, gathered by means of self-report, generalizes to students’ behavior. Another important limitation of this study is the use of university freshman undergraduates in the participant sample. It is common practice at universities for the majority of the participant pool to be comprised of freshmen undergraduate students. However, this limits the extent to which the results generalize the personality and academic success of higher level college students or even high school students. This study is no exception. More research is needed before we can propose that the results found in this study generalize the mass populations of students at a collegiate level or secondary schooling. Finally, future research should consider the role of anxiety and uncertainty in the relationship between personality and self-handicapping.

For hypotheses 2-5, **Table 1** (below) provides an overview of the analyses that was conducted for each. Pearson bivariate correlations were conducted to test the relationship between personality characteristics and academic outcome variables.

Measure	1	2	3	4	5	6	7
1. Self-Handicap	—						
2. Test Anxiety	0.19**	—					
3. GPA	-0.14**	-0.21**	—				
4. Self-Esteem	-0.26**	-0.44**	0.09**	—			
5. Self-Efficacy	-0.22**	-0.29**	0.13**	0.45**	—		
6. Self-Clarity	-0.21**	-0.48**	0.05	0.52**	0.31**	—	
7. Impostor Syndrome	0.22**	0.60**	-0.10**	-0.54**	-0.34**	-0.66**	—

**Table 1.** Bivariate correlations between the academic outcome measures and personality characteristics.

Note: N = 844, \* =  $p(2\text{-tailed}) < 0.05$ , \*\* =  $p(2\text{-tailed}) < 0.01$

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# The Effects of Followers' Ethnicity on Asian Americans' Leadership Aspirations, Leadership Qualification, and Leadership Competence

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## ABSTRACT

There is currently not enough research on Asian American leadership. Specifically, there is little on the reasoning for Asian Americans' high educational attainment, yet low acquirement of managerial positions in companies. We investigated the influence that ethnicity of followers had on Asian Americans' leadership aspirations, perception of their leadership qualification, and the perception they believe their followers will have on their competence as a leader. We surveyed 299 Asian American undergraduate students from the University of California, Riverside using a  $3 \times 2$  factorial design. As hypothesized, Asian Americans did have higher leadership aspiration when their followers were European American, as opposed to Asian American. We also found that when Asian Americans were asked to lead Asian Americans, as opposed to European Americans, they believed their followers were better qualified to be a better manager. Of note, we failed to find evidence that Asian Americans would believe their European American followers to have a negative perception of their competence as a leader. These findings suggest that the ethnicity of followers may play a role in Asian American leadership aspiration and perception of their leadership qualification. By identifying factors—such as ethnicity—that influence Asian Americans' leadership aspiration and perception of others' leadership skills, companies can become more inclusive and aware of diverse perspective on leadership.

**KEYWORDS:** Ethnicity, followers, Asian American, European American, leadership aspiration, leadership competence, leadership qualification



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## INTRODUCTION

The United States has become a diverse country in terms of race and ethnicity. As of 2017, the Asian population has the highest population growth of any ethnic group (U.S. Census Bureau). Despite their increasing population, there is a lack of Asian Americans (AAs) in leadership positions, even though they are highly educated and competent (Eagly & Chin, 2010). They are the least likely among all other ethnicities to be promoted to managerial positions; this is not to say that AAs do not desire to be managers, rather they face organizational barriers which may prevent them from growing within a company (Xin, 2004). The imbalance between high education status and low managerial positions may be related to a miscommunication that occurs when AAs use culturally influenced tactics to impress their superiors (Xin, 2004).

Individuals can react differently to leadership strategies and interpret their effectiveness as negatively or positively based on one's culture (Hanges, Lord, & Dickson, 2000). According to the implicit leadership theory, individuals have mental representations and preconceived notions of what embodies a leader. These mental representations can be influenced by culture and may be used to predict who individuals will consider a 'good' leader (Burriss, Ayman, Che & Min, 2013). For example, Western (e.g., United States) and Eastern countries (e.g., China) have varying ideologies of what a 'good' leader entails and this can be rooted back to cultural thinking. In individualistic cultures, such as the U.S., people are self-oriented and focused on their individual goals (Wagner & Moch, 1986). Individuals in individualistic cultures would be more likely to believe that leaders should focus on personal goals and emphasize competition (Offermann & Hellmann, 1997). On the other hand, collectivist cultures, such as China, are group-oriented and focus on societal unity through cooperation (Wagner et al., 1986). As such, individuals in collectivist cultures would be more likely to have notions that 'good' leaders should focus on cooperation and care for their followers (Offermann et al., 1997).

AAs may adhere to their ethnic culture, as well as embrace their American identity, making it likely for them to be a part of both individualistic and collectivist cultures (Benet-Martinez, Leu, Lee & Morris, 2002; Jung & Yammarino,

2001). Bicultural individuals, such as AAs, may use cultural frame shifting, "shifting between two culturally based interpretative lenses in response to cultural cues" (Benet-Martinez et al., 2002, p. 492). Cultural frame switching allows bicultural individuals to access either their individualistic or collectivist orientation through primes—variables that influence an individual's cognition—which may influence their leadership aspirations. AAs may access their individualistic self when surrounded by European Americans (EAs), utilizing the ethnicity of their followers as a prime, and aspire to be a manager to promote their personal self-esteem. When AAs are surrounded by other AAs, however, they may access their collectivist self and not aspire to be a manager to maintain group unity.

Through the theoretical framework of individualism, collectivism, and implicit leadership, it is possible to predict whether AAs will be more or less likely to strive for managerial positions in light of the different ethnicities of their followers (Burriss et al., 2013). Studies suggest that AAs have lower leadership aspiration—motivation to obtain leadership positions—than EAs, which is mediated by the impressions of oneself as a leader. It is not to say that race itself makes AAs less likely to want to be a leader, but rather affects their impressions of themselves as a leader (Festekjian, Tram, Murray, Sy & Huynh, 2014). Studies done on AA executives indicated that there are three factors that influence an individuals' leadership aspiration: "biased perception of AA leaders, lower leadership aspiration of AAs, and failure of leadership imagination" (Sy, Tram-Quon & Leung, 2017, p. 150). AAs agree that there is a perception by other ethnicities that AAs do not aspire to be leaders; executives also believe that it is the case that some AAs show low motivation to be a leader (Sy et al., 2017). In Sy et al.'s study (2017) other AAs reported it was not a lack of leadership aspiration, but merely that they were not actively pursuing leadership positions and instead waiting for management to approach them. It is possible that AAs may have higher leadership aspirations around EAs, especially when ethnicity is used as a prime to the individualistic culture.

While it is possible that ethnicity influences an individual's leadership aspiration, it may also affect an individual's perception of others. Mental representations influenced

by culture can shape one's perception of what qualifies as a 'good' leader. Studies have shown that AAs view EA managers as less authentic and less social than successful managers (Burriss, et al., 2013). These results suggest that EAs may not fit AAs' mental representation of what a 'good' leader looks like because they tend to make decisions by themselves instead of including the group (Burriss et al., 2013). As studies have shown, when leadership strategies match the group's mental notions of a 'good' leader, the managers are seen as more effective (Thomas & Ralvin, 1995). It is possible AAs may believe their AA followers, as opposed to their EA followers, to be better qualified for a leadership position.

In the same way that mental representations shape AAs' perception of what a 'good' leader is, stereotypes also influence an individual's belief of how others perceive them. Studies have indicated that AAs are often stereotyped as not being assertive, social, or having the qualities of a leader (Chin, 2013). When compared to EAs, AAs are perceived as less ideal leaders (Sy et al., 2010). Specifically, when AAs were perceived to not fit the expectations for the types of occupations AAs should occupy, such as a salesperson, they were evaluated lower in competence compared to EAs (Sy et al., 2010). Based on additional studies that suggest there is a miscommunication in leadership tactics used to impress superiors (Xin, 2004), we hypothesized that AAs would believe their EA followers, as opposed to their AA followers, would not perceive them as competent managers.

Prior analyses have demonstrated that expectations of what a 'good' leader looks like in the United States frequently does not match the perception EAs have of AAs (Rosette, Leonardelli & Phillips, 2008). The current study sought to fill in gaps in the literature by focusing solely on AA leaders' perspectives. First, there are very few studies on AA leadership and most focus on the perception of AAs from the viewpoint of non-Asian ethnic groups (Chung-Herrera & Lankau, 2005). The current study aims to evaluate whether the followers' ethnicity influence AAs' leadership aspiration. Furthermore, we look at whether the followers' ethnicity influence AAs' perception of their followers' leadership abilities. We also evaluate whether the followers' ethnicity affects the perception AAs think

their followers would have on their competence.

Considering previous investigations suggesting that cultural frame switching can occur in bicultural individuals and that race of one's followers does not directly affect leadership aspiration, we hypothesized that AAs would have higher leadership aspiration when their followers were EA, as opposed to AA (Hypothesis 1). In addition, based on studies of AAs mental representation of what a 'good' leader looks like, we hypothesized that AAs would not view EAs as better qualified to be a good manager than them, as compared to AAs (Hypothesis 2). Finally, because previous analyses demonstrated that individuals' have a negative view on AAs' competence specifically when the occupation does not fit the expectations of others, we hypothesized that AAs would believe their EA followers would not perceive them as a competent manager, as opposed to their AA followers (Hypothesis 3).

## METHODS

### Participants

The study consisted of 299 (158 women) PSYC 1 and 2 undergraduate students from the University of California, Riverside. On average, participants were 19.35 ( $SD = 1.32$ ) years old. Participants completed the study in exchange for research credit. Participation was restricted to AAs, as we were focused on intrapersonal leadership perceptions of AAs.

### Measures

Leadership aspiration was measured by using a forced choice item, "Which of these two roles would you prefer? *Manager or Employee.*" Festekjian's (2014) Intrapersonal Leadership Perception (ILP) measure was used to assess leadership aspiration, AAs' perception of other's leadership skills, and the perception AAs believe their followers have on their competence. This study examined items 1, 7, and 8 (e.g., "I would like to be the manager," "I believe my colleagues would be better qualified to be good manager than me" and "I believe my colleagues would think I am competent if I were the manager"). Participants responded to the ILP items using a 6-point Likert Scale (1 = "Strongly Disagree" and 6 = "Strongly Agree").

### Study Design & Procedure

A 3 (Asian prime vs. American prime vs. Control) × 2 (EA vs. AA followers) factorial design was used. The first manipulation of the study primed participants to a specific cultural background or the control by having them complete a 5-minute reflective writing prompt based on their condition. Participants in the Asian prime wrote about their Asian cultural background. Participants assigned to the American prime wrote about their opinions and emotions on mainstream American culture. Participants in the control condition wrote about animals in nature; the writing was not to include any emotions or opinions. The second factor manipulated was the group composition (i.e., who the participants interacted with). The experiment always required 3 AA participants; if the requirement was not met, EA or AA, confederates were used. For the AA follower condition, if one AA participant signed-up, there were two AA confederates and an AA experimenter. If two Asian American participants signed up, there was one Asian American confederate present, and the experimenter would still be Asian American. In the EA follower condition the same would occur but the confederates and experimenter were EA, and the participants would still be AA.

Undergraduate students arrived at the lab to take part in what they believed were two different studies within their hour timeslot (“Writing Study” and “Career Study”). Prior to beginning the experiment, participants signed-in

and received an assigned letter (e.g., “A”, “B”, or “C”) for the study; confederates also took part in this process. Once signed-in, participants were first asked to complete the “Writing Study,” where each participant was randomly assigned to a writing prompt. The “Writing Study” also included a consent form and demographic information. Once completed, participants were asked to raise their hand to indicate that they were ready to move on to the next study. Participants were asked to turn their chairs around to face the experimenter before starting the next survey (to create an environment where participants noticed the ethnic backgrounds of the other participants or confederates). The experimenter then read a script indicating that the participants would be asked to develop a business plan with their colleagues in the room. They were also told that the following surveys would be used to assign the roles of manager and employees (no actual business plan task took place). Participants then completed the “Career Study,” which was utilized for the dependent measures. Once both surveys were completed, participants were asked for consent and debriefed.

### RESULTS

In order to examine possible relationships between follower type and the dependent measures related to leadership aspiration, self-perception of leadership qualification, and self-perception of competence, we ran multiple independent-samples t-tests. **Table 1** presents the results

	<i>M<sub>AA</sub></i>	<i>SD<sub>AA</sub></i>	<i>M<sub>EA</sub></i>	<i>SD<sub>EA</sub></i>	<i>t</i>	<i>df</i>
Leadership Aspiration	1.71	0.46	1.63	0.49	1.56	296
Leadership Aspiration (ILP)	3.56	1.14	3.84	1.25	-2.06*	297
Qualification (ILP)	3.62	1.01	3.83	1.05	-1.759	290
Competence (ILP)	3.96	0.91	3.67	1.08	2.48**	297

**Table 1:** Comparing Leadership Aspiration, Perception of Qualification and Competence based on Followers’ European American or Asian American Ethnicity.

Note: \**p* < 0.05; \*\**p* < 0.01. *M<sub>AA</sub>* and *SD<sub>AA</sub>* are the means and standard deviations when followers are Asian American. *M<sub>EA</sub>* and *SD<sub>EA</sub>* are the means and standard deviations when followers are European American. ILP are the Intrapersonal Leadership Perceptions items.



of the independent-samples t-tests comparing measures of participants' leadership aspiration, qualification, and competence based on their followers' ethnicity.

### Leadership Aspiration

We compared differences in leadership aspiration in the AA follower and EA follower conditions with the Leadership Aspiration item using an independent samples t-test. The means for AA followers ( $M = 1.71$ ;  $SD = 0.46$ ) and EA followers ( $M = 1.63$ ;  $SD = 0.49$ ) did not differ significantly,  $t(296) = 1.56$ ,  $p = 0.12$ . These results indicated that those who led AA followers versus EA followers did not differ in their leadership aspiration. Another independent-samples t-test was conducted to compare leadership aspiration in the AA follower and EA follower conditions using an ILP item to analyze whether similar results would be found using different items. Leadership aspiration did significantly differ for AA follower ( $M = 3.56$ ;  $SD = 1.41$ ) and EA follower ( $M = 3.84$ ;  $SD = 1.24$ ) using the ILP item,  $t(297) = -2.05$ ,  $p = 0.04$ . These results showed that the ethnicity of followers' does have an effect on leadership aspiration. Specifically, our results indicated that those who were asked to lead AAs, as opposed to EAs, were less likely to want to be a manager.

### Perception of Self Leadership Qualification based on Followers' Ethnicity

We examined differences in AAs' self-perception of their leadership qualification as compared to their followers with an ILP item using an independent-samples t-test. Perception of leadership qualification did significantly differ for AA follower ( $M = 3.96$ ;  $SD = 0.91$ ) and EA follower ( $M = 3.67$ ;  $SD = 1.09$ ) conditions,  $t(290) = 2.48$ ,  $p = 0.014$ . These results showed that the ethnicity of followers does have an effect on perception of leadership skills. Specifically, our results indicated that when AAs were asked to lead AAs, as opposed to EAs, they believed their followers were better qualified to be a better manager than them.

### Perception of Self Competence based on Followers' Ethnicity

We also compared differences in the perception AAs believed their followers would have about their competence as a leader with an ILP item using an independent-samples t-test. Perception of competence was marginally significant

for AA follower ( $M = 3.62$ ;  $SD = 1.01$ ) and EA follower ( $M = 3.83$ ;  $SD = 1.05$ ) conditions,  $t(297) = -1.76$ ,  $p = 0.08$ . These results indicated that there is a trend in a non-predicted direction. Specifically, our results suggested that when AAs are asked to lead AAs, as opposed to EAs, they were more likely to believe their followers would not view them as competent managers.

## DISCUSSION

This study set out to investigate whether the ethnicity of AAs' followers influenced their leadership aspiration, their perception of their followers' leadership qualification, and the perception AAs believe their followers had on their competence as a leader.

We found that there were differences in the results between leadership aspiration using the one forced-choice item and 6-point Likert scale ILP item. The forced choice Leadership Aspiration item yielded no difference between EA and AA followers; however, differences were found in the ILP item. Therefore, hypothesis 1 was supported. The difference may suggest that the forced-choice item put more pressure because they were told that the responses to the survey would determine their role. A definite answer seems like a commitment to being manager; this pressure may have led participants to select 'employee' instead of 'manager.' On the other hand, the Likert scale opened a door for the possibility of being manager without the commitment which may have eased the pressure of being manager. The Likert scale made it more likely for the participants to select in-between options such as 'somewhat disagree' and 'somewhat agree' to being manager. A possible explanation for this result may be that AAs are using the ethnicity of their followers as a signal to either access their individualistic self or collectivistic self and act according to those cultures (Benet-Martinez et al., 2002).

As expected hypothesis 2 was supported, AAs believed they were better qualified to be a good manager than their EA followers, as opposed to their AA followers. The results presented here extend findings from the literature on collectivist and individualistic cultures as well as implicit leadership. For instance, previous research has demonstrated individuals in collectivist cultures prefer collaborating, listening, and empowering other members



in the group (Kawahara, Pal, & Chin, 2013). A potential explanation for this behavior is that collectivist culture emphasizes the unity of the group and by stating that one is better than an individual in the group, unity can be disrupted (Wagner et al., 1986). Another possible explanation for these results is that EAs do not fit the mental representations that AAs have of what a 'good' leader is; therefore, they did not believe EAs would be better qualified to be a good manager than them (Burriss et al., 2013).

We also predicted that AAs would believe that their EA followers would see them as a less competent leader, as opposed to their AA followers. Although hypothesis 3 was not supported, there was a non-predicted trend in the opposite direction, indicating that AAs believed that their EA followers would see them as a more competent leader than their AA followers. A potential reason for this trend could be the influence of gender. In this experiment, all the confederates used were females and as studies have shown society views women as warm and compassionate towards others (Huddy & Terkildsen, 1993). It is likely that the participants, specifically males, did not solely focus on their followers' ethnicity, but also took into consideration the stereotypes surrounding women's behavior and therefore believed they would not view them incompetent. Future studies should examine the role gender has on AAs' belief that their followers will view them as more or less competent. The study should include all male AA followers versus all female AA followers to see if this trend is being influenced by gender or ethnicity.

Nevertheless, the evidence presented in this paper is not without limitations. This study was conducted on university students with a mean age of 19.35 ( $SD = 1.32$ ), which would not generalize to the Asian workforce because it is comprised of 65.8% AAs over the age of 20 (U.S. Census Bureau). Future studies should include a more diverse subject pool in terms of age and education to have a more representative sample of the U.S. workforce. Furthermore, the current study consisted of only two measures and four items. The four items were analyzed individually making it more likely that the observed results we found may be influenced by moderators (i.e., gender) or a third variable. Future studies should decrease the influence of other factors by analyzing more than one item at a time.

## CONCLUSION

The phenomenon of AA leadership has rarely been investigated through an AAs' perspective of their own leadership ability; therefore, in this study, we sought to provide a basis for future research in this area "(Chung-Herrera et al., 2005; Sy et al., 2010). By identifying factors, such as the ethnicity of their followers, that influence their leadership aspiration and perception of others leadership skills, we can combat negative stereotypes that AAs lack managerial desire. It is also important to recognize that the U.S. workforce is becoming more diverse and not everyone has the same mental representation of what a 'good' leader looks like; therefore, it is important to consider different leadership strategies. Companies becoming more inclusive and aware of diverse ethnicities' perspective of leadership is important for workplaces to cultivate and prosper.

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## Dads: When Is Emotional Involvement Too Much?

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### ABSTRACT

High paternal involvement is linked to positive emotional and psychological outcomes among daughters (Barker, Iles & Ramchandani, 2017). Meanwhile, children of absent fathers are at an increased risk of developing psychopathology such as depression (Culpin et al., 2013) and low self-esteem (Luo, Wang, & Gao, 2011). However, over-involvement has also been seen to contribute to negative child developmental outcomes (Schiffrin et al., 2013). Most research focuses on the link between maternal emotional over-involvement (EOI) and psychopathology and has revealed mixed evidence. Evidently, impacts of *paternal* EOI on daughter mental health and self-esteem have been understudied (Gar & Hudson, 2008; Lindhout et al., 2008; Nelson, Hammen, Brennan, & Ullman, 2003). The present study examines whether paternal EOI is associated with daughter depression and self-esteem in a sample of 8- to 12-year-old Mexican girls and their fathers. Mothers of participants were also included as primary caregivers. Daughter self-esteem and depression were measured by self-report and primary caregiver report. We measured paternal EOI based on *Expressed Emotion* captured by father's five-minute speech sample.

The hypothesized links between fathers' EOI and daughter depression and self-esteem were not supported. However, there was a marginally significant relationship between higher self-rated depression for those girls whose fathers received a borderline EOI rating ( $M = 59.6, SD = 6.06$ ) than those who received a low EOI rating ( $M = 49.6, SD = 6.07$ );  $t(8) = -1.99, p = 0.08$ . Our research supports the need to further examine the threshold of optimal paternal emotionally over-involved attitudes on daughter emotional development.

**KEYWORDS:** Paternal over-involvement, daughters, expressed emotion, self-esteem, depression, FMSS, EOI



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Fernanda Sandoval is a 3rd year Psychology major. Intrigued by the aspects of parenting and emotional development that lead individuals to be resilient while undergoing adversity, she hopes to utilize research for the development of educational programs that aid vulnerable children and their families. After participating in research at the KIND Lab for three years under the guidance of Dr. Kalina Michalska, she hopes to receive a PhD in School Psychology and develop future policies beneficial to all families.



### FACULTY MENTOR

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Professor Michalska earned her doctorate in Developmental Psychology from the University of Chicago. She is an Assistant Professor in the Department of Psychology at UCR and the director of the Kids Interaction and Neuro Development (KIND) Lab, where she leads a research program on the brain bases of socioemotional development. Professor Michalska holds additional appointments in the Interdepartmental Graduate Program in Neuroscience and the Department of Psychiatry at the School of Medicine at UCR. In her work, she combines neuroimaging (fMRI), physiological measures, and behavior observations to understand the brain systems underlying typical and atypical emotion development processes. In addition, Professor Michalska has a longstanding interest in policy and education programs targeting socioemotional development and social mobility. She is a board member of Acceso Academy, a nonprofit organization that provides affordable college test preparation assistance for underprivileged students in Southern California.

## INTRODUCTION

Depression of children in the United States has increased dramatically in the last several decades (Twenge, 2000), as has the absence of fathers in the family (Cabrera, LeMonda, Bradley, Hoffeth, & Lamb, 2000). There is growing evidence of the adverse influence of paternal absence on children's mental health, but the role of paternal involvement in child mental health has received less attention. Paternal involvement has been linked to daughter's emotional development (Barker, Iles, & Ramchandani, 2017; Sarkadi, Kristiansson, Oberklaid & Bremberg, 2008), primarily as a protective factor against depression (Chang, Halpern, & Kaufman, 2007; Flouri & Buchanan, 2003). It is also thought to impact daughters' self-esteem, subjective emotional evaluation of her self-worth, in a positive manner (Deutsch, Servis, & Payne, 2001). All in all, father involvement appears to be beneficial to important socioemotional outcomes. However, excessive involvement may also interfere with a child's social competence and autonomy, potentially leading to poorer mental health and lower self-esteem. These findings therefore beg the question: what daughter outcomes are associated with fathers who are *emotionally over-involved* (EOI)?

Prior research on maternal-child relationships suggests that there might be an optimal level of parental involvement. In particular, Shiffrin and colleagues (2013) have described the negative effects of maternal over-involvement. Such behaviors may be problematic as they convey to the daughter that she is incapable of handling challenging situations independently (Kins, Beyers, Soenens, & Vansteenkiste, 2009; Soenens et al., 2007), thereby increasing feelings of insecurity and depression (Schiffrin et al, 2013). Less is known about this relationship in the context of the father-daughter dyad. Our study addresses this question directly. Given that higher levels of overinvolved maternal parenting predict lower levels of well-being for daughters, we test whether a link also exists between paternal EOI and daughter depression and self-esteem (Kouros, Pruitt, Ekas, Kiriaki, & Sunderland, 2017).

Associations between paternal EOI and daughter self-esteem are understudied; however, available studies suggest that EOI may be inversely related to child self-

esteem (Santos, Saraiva, & De Sousa, 2009). Positive self-esteem characterizes an individual who is confident in her abilities, has a sense of self-worth, and is able to cope with life challenges. A person with low self-esteem, on the other hand, frequently exhibits a lack of confidence and feelings of worthlessness and inadequacy. It has been reported that parenting characterized by high levels of overprotection, a component of EOI, negatively predicts self-esteem in adolescents (Herz & Gullone, 1999). To our knowledge, no research to date has examined whether this association also manifests at younger ages. Longitudinal research has demonstrated that high self-esteem can be beneficial to child development in the form of more likeability amongst peers, perceived attractiveness, better interpersonal relationships, increased happiness, enhanced initiative, and pleasant feelings (Baumeister, Campbell, Krueger, & Vohs, 2003). Conversely, low self-esteem has been linked to lower relationship satisfaction, lower positive affect, higher negative affect, higher depression, and lower health trajectories (Orth, Robins, & Widaman, 2011). Despite the importance of self-esteem for healthy socio-emotional development and the known role of mother-child relationships on the development of self-esteem, we know very little about the extent to which fathers contribute to children's self-esteem. The current study therefore aims to contribute to acquiring knowledge in this research area by testing associations between father involvement and daughter self-esteem.

Paternal involvement research is typically framed around three components: engagement, accessibility, and responsibility (Lamb, Pleck, Charnov, & Levine, 1987). One index of family engagement that has received considerable research attention is the Expressed Emotion (EE) measure. The EE measure provides an index of the level of criticism and overinvolvement expressed by parents when describing their children and the relationship between the child and parent (Magana, 1986). EE ratings are based on the Five-Minute Speech Sample (FMSS), (Magana, 1986) an audio recorded five-minute sample of the parent's voice tone, verbal content, and affective displays when describing the child. We focus on the EE, which has been documented to predict outcomes among depressed children (Asnarow, Goldstein, Tompson, & Guthrie, 1993) and is a concurrent marker of risk for psychiatric disorder



(Asnarow, Goldstein, Tompson, & Guthrie, 1993). In the EE measure, paternal EOI is comprised of overconcern, unusual self-sacrificing behavior, emotional displays, excessive praise (5 or more positive remarks), and lack of objectivity towards one's daughter.

The present study addresses the following questions: (i) what is the impact of father emotionally over-involved attitudes on daughters' self-esteem; and (ii) is there a relationship between father emotionally over-involved attitudes and daughter depression? We focus specifically on Latino fathers and their daughters, as about 49% of Riverside County identifies as Hispanic or Latino yet is under-represented in research. Latinos, the largest ethnic group in the United States, are highly diverse in nativity, socioeconomic status (SES), and immigration experience (Garcia & Jensen, 2009). Understanding the role of the father in Latino families is especially important because the majority of Latino children live in two-parent households where fathers share in parenting responsibilities and day-to-day care of their children (Cabrera & Bradley, 2012).

## METHODS

### Participants

Participants were fathers ( $N = 10$ ), mothers ( $N = 7$ ), and their daughters ( $N = 10$ ,  $M = 9.70$  years,  $SD = 1.34$ ) recruited from the sample pool of a larger, longitudinal study at the University of California, Riverside. Initial recruitment for the larger study was done through the UC Riverside Child Studies Database. Families in the database were recruited through community events in the Inland Empire. Families with daughters were screened via phone and were invited to participate if the daughter was between 8 and 13 years of age, Mexican, and pre-menarche, as our study attempts to understand emotional development of girls before they experience the changes of puberty. If not the primary caregiver (PCG), fathers were contacted separately via contact information shared by mothers at the initial visit. Adult participants who consented were enrolled. Minor participants who verbally assented and whose PCGs gave written consent were enrolled. Procedures were approved by the University of California Riverside Institutional Review Board. For each visit, families were compensated for their time in the form of gift cards.

### Procedures

Through the longitudinal study, data was collected from each child subject and PCG biweekly. Child subjects and PCGs completed a series of surveys in person. Fathers, if not the PCG, completed a separate set of surveys online. In addition, participating fathers provided a five-minute, free form speech sample about their daughters and their relationship with them. Data was collected on a rolling basis and participants were compensated for their time.

### Measures

The Five-Minute Speech Sample (FMSS) was administered to capture paternal emotions and attitudes towards daughters. The FMSS has demonstrated satisfactory reliability and validity comparable to those of other expressed emotion measures (Magana et. al, 1986). The FMSS was utilized as it is concise, easier to administer and code as it is a shorter version of the Camberwell Family Interview (CFI) (Calam & Peters, 2006). The father was prompted to speak for five minutes about what kind of person his daughter is and his relationship with her (Magana, et. al, 1986, p. 205). Speech samples were recorded and later transcribed.

*Coding and Expressed Emotion.* The FMSS collected from fathers was coded based on the Expressed Emotion (EE) Coding System (Magana et. al, 1986, p. 205). Each sample was coded twice by trained raters; any discrepancies were discussed and resolved by a third coder. EE ratings were based on the absence or presence of the following components: lack of objectivity, over-protective, self-sacrificing behavior; emotional display, excessive details about the past, positive remarks frequency, statements of attitude frequency, quality of initial statement, quality of relationship, criticisms frequency, and dissatisfaction.

High EOI was determined if any of the following indicators were present in the speech sample: (i) self-sacrificing, overprotective behaviors, lack of objectivity, (ii) an emotional display, or (iii) a combination of 2 or more elements: statements of attitude, excessive detail of the past, or excessive praise (5+ positive remarks). Borderline EOI is determined by the presence of overprotective, self-sacrificing behaviors; statements of attitude; excessive detail about the past; or 5+ positive remarks. Low classifications of EE were assigned to those who did not satisfy any of the

specified criteria for High or Borderline EE. No father in the sample met the requirements for high EOI.

*Self-esteem.* Child global self-esteem was assessed utilizing the Single-Item Self-Esteem measure (SISE, Robins, Hendin, & Trześniewski, 2001). The SISE demonstrated high reliability ( $\alpha = 0.75$ ) and validity (0.72 to 0.76) (SISE, Robins, Hendin, & Trześniewski, 2001). The statement, “*I have high self-esteem,*” was rated on a 5-point Likert scale ranging from 1 to 5 (1 - *not very true of me*; 5 - *very true of me*).

*Depression.* Daughter depression symptoms were assessed using the 12-item Children’s Depression Inventory II Short Form (CDI II, Kovacs, 2011). The CDI II demonstrated acceptable levels of reliability ( $\alpha = 0.67$  to 0.91) and good discriminant and convergent validity (Bae, 2012). It quantifies symptoms such as depressed mood and self-evaluation in the context of emotional and functional problems. Each item consisted of three statements regarding the child’s mood and behavior over the past two weeks in order of increasing severity from 0 to 2 (0 -- *I am sad once in a while*; 1 -- *I am sad many times*; 2 -- *I am sad all the time*). Higher total scores demonstrated a greater depressive state. PCGs also reported on their child’s depressive symptoms using the 17-item, CDI II Parent Form two weeks later. PCGs indicated which response best described their child for a number of statements over the past two weeks on a scale of 0 to 3 (0 -- *Not at all*; 1 -- *Some of the time*; 2 -- *Often*; 3 -- *Much or most of the time*). For our analysis, we utilized daughter-reported, PCG-reported depressive symptoms, as well as a combined score. Due to previous research confirming that parent-child rating discrepancies of mood disorders exist, separate independent t-test analyses are conducted between child-rated, parent-rated and combined scores for the CDI II and paternal EOI (Van Roy, Groholt, Heyerdahl, & Clench-Aas, 2010; Doorn et al., 2018).

## RESULTS

### Paternal EOI and Daughter Depression

It was predicted that paternal EOI would be related to daughter depression. Analyses did not show a significant relationship between EOI and parent-rated depression of the child; nor did it show a significant relationship between EOI and combined depression ratings. However,

an independent t-test demonstrated a marginally significant relationship between higher child-rated depression for subjects whose father received a borderline EOI rating ( $M = 59.6$ ,  $SD = 6.06$ ,  $N = 5$ ) than those who received a low EOI rating ( $M = 49.6$ ,  $SD = 6.07$ ,  $N = 5$ );  $t(8) = -1.99$ ,  $p = 0.08$ ,  $d = 1.65$ ).

### Paternal EOI and Daughter Self-Esteem

It was hypothesized that a link would exist between paternal EOI and daughter self-esteem. An independent t-test showed no significant difference for child-rated self-esteem between subjects whose fathers received low and borderline EOI ratings. These results do not support the hypothesis that paternal EOI has an effect on daughter self-esteem.

## DISCUSSION

The goal of this study was to examine the relationship between paternal emotional over-involvement and daughter emotional development outcomes: (i) self-esteem and (ii) depression. No significant findings emerged from our analyses, and we were unable to establish clear link between paternal EOI, depression, and self-esteem. Worth noting is a marginally significant relationship found between higher child-rated depression for subjects whose father received a borderline EOI rating than those who received a low EOI rating. This result, even though not significant, reveals that even borderline paternal over-involved attitudes may have negative implications on a daughter’s risk for depression. Fathers may be conveying to daughters that they are incapable of handling challenging situations independently (Kins, Beyers, Soenens, & Vansteenkiste, 2009; Soenens et al., 2007), thereby increasing feelings of insecurity and depression (Schiffrin et al., 2013).

The present study aimed to contribute to the understudied research on paternal emotional over-involvement and their young daughters’ emotional development. However, several limitations should be noted. First, our sample size was small, which may have limited our ability to reveal significant relationships between paternal emotional over-involvement and daughter emotional outcomes. Our sample was further restricted due to language barriers, family conflict, and father lack of involvement impacting father participation. Also, it should be noted that there was

lack of EOI variability in the current sample. Because our fathers were recruited due to their daughter's typically developing status, there was not much variability in EOI. Most fathers were rated as low EOI or borderline EOI, but none satisfied the requirements for high EOI. Perhaps more variability would exist with greater access to clinical populations. Lastly, other limitations related to the stability of our findings may be affected by daughter self-esteem and depression not collected during the same visit.

Future research on Hispanic father-daughter dyads would benefit from more subjects and continuously collecting evaluations of self-esteem and depression across visits. Our study supports the need to examine further the nuances and attitudes fathers may have on daughter longitudinal, behavioral outcomes.

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## Reproductive Behaviors of Live-Bearing Poecilids

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### ABSTRACT

The numerous species of the *Poeciliidae* family are known to possess certain shared characteristics regarding anatomy, physiology, and behavior. Over time, scientists researching the live-bearing poecilids have compiled a database detailing the mating behaviors of each species. This database, however, contains gaps of information regarding the mating behaviors of several species, including *Poecilia gillii*, *Poecilia butleri*, *Poecilia salvatoris*, and *Poeciliopsis infans*. We sought to ascertain the missing statistics for these four species and definitively determine the presence of mating behaviors unique to the *Poeciliidae* family. Observational tank environments were constructed specific to each species and periodic recordings taken for each tank over an allotted time period. Mating behaviors were compiled into five common subcategories observed across each poecilid species and the number of occurrences for those five representative subcategories tabulated from each trial. Observations taken throughout the duration of the experiment revealed an absence of the courtship display subcategory for all four species and varying degrees of activity for the remaining four subcategories. Recordings from this study presented previously unobserved demonstrations of reproductive behaviors from the four species, i.e. ambiguous displays of mating behaviors from *P. salvatoris* or melanic exterior shiftings in response to presence of offspring in *P. infans*. This paper provides a comprehensive report on definitive mating behaviors of four previous unobserved species within the guppy family and serves as a basis for further exploration into the evolution of traits and behaviors as a response to reproductive activity for a more cohesive understanding of joint morphology and evolution.

**KEYWORDS:** Mating behavior, biology, evolutionary ecology, Poecilid, behavioral interactions



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Tamara Tang is a 3rd year Bioengineering major. Her research looks into rapid evolution and the morphology of the gonopodium through live-bearing *Poeciliidae* under the mentorship of Dr. David Reznick. Tamara is involved in other research pursuits involving neurocomputational modeling and neuroimaging in the department of Bioengineering under Dr. Megan Peters. She is the Recruitment Chair for the Biomedical Engineering Society and Co-Director for UCR Biohack. Tamara intends to enter industry after graduating before returning to academia to pursue a Master's degree in Bioengineering.



### FACULTY MENTOR

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David Reznick is a Distinguished Professor in the Department of Evolution, Ecology and Organismal Biology. He is an evolutionary biologist that specializes in experimental studies of evolution performed on natural populations. He is also studying the evolution of life histories in the fish family *Poeciliidae*. One product of this work is the discovery that these fish have evolved the functional equivalent of a mammalian placenta nine times and that the evolution of the placenta is associated with the evolution of male traits associated with sexual selection. Tamara Tang's project contributes to this larger research program.



## INTRODUCTION

The *Poeciliidae* are a family of freshwater fish commonly characterized by their ability to give birth to live young. This ability differentiates them from most other aquatic species who reproduce through the external fertilization of eggs. The appx. 250 species of the *Poeciliidae* family possess different traits and characteristics regarding behavior and physiological or anatomical function [8]. For example, male *Poeciliidae* develop a hooked gonopodium, which is the intromittent organ and is a metamorphosed anal fin. Males often display brighter pigmentation upon maturation than other individuals of the species [3,5,6]. Reproductive behaviors, such as the development of sneak copulation tactics within less aggressive males or elaborate courtship displays within more aggressive males, have also emerged as a result of this adaption [2,7].

Evolutionary ecology researchers have long sought to characterize the numerous species in the *Poeciliidae* family as well as quantify the common reproductive behaviors shared amongst the species. One recent characterization is the relationship between the evolution of male traits associated with sexual selection (e.g. bright coloration, enlarged dorsal fins, swords on tails) and elaborate courtship behavior [4]. Goldberg et al. performed phylogenetic analyses in which they evaluated how the evolution of these traits were related in the genus *Poecilia* and how sexual selection shaped this evolution. These analyses integrate a DNA-based family tree with data that characterize every species in the tree, allowing us to make inferences about how traits evolved and how the evolution of one trait may have influenced the evolution of others. However, many species within the *Poecilia* family were not included in his analysis. We sought to build upon Goldberg's results, provide a more complete data set in order to increase the strength of his conclusions, and better understand the joint evolution of these traits, as well as how and why they evolved in the first place.

The goal of the Reznick lab is to provide comprehensive knowledge on the behaviors of the *Poeciliidae* family. We are compiling a database that details how females provision offspring (either fully, before the egg is fertilized, or continuously after fertilization via the equivalent of a mammalian placenta), male-male aggression, the presence

of courtship displays as a form of reproductive behavior, sexual dichromatism, and male ornamentation. Frequently studied species, such as sailfin mollies, *Gambusia* spp., or guppies, are well described for these traits, but no or only fragmentary information is available for most other species in this family [8]. This study will provide the missing data for some of the lesser known species – in particular, *Poecilia gillii*, *Poecilia butleri*, *Poecilia salvatoris*, and *Poeciliopsis infans*. The slight variances in size, pigmentation, and other factors across these four species will provide a better understanding of reproductive behaviors within the livebearer family, as well as the different nuances and variations it can take on across genera. For instance, *P. infans* males have two morphs: males with a melanic outer skin and males with silvery scales. This melanic difference in males is a trait that we observe unique to *P. infans* and is predicted to hold some influence on reproductive behaviors or male-female dynamics.

In this study, we will determine the presence of courtship displays, or alternative behaviors in place of courtship displays, as well as other common behaviors associated with the evolution of viviparity (e.g. the ability to produce live young) 4 species within the *Poeciliidae* family to better understand their distinct evolutionary characteristics. This research holds implications in the morphology of the gonopodium as well as other reproductive traits specific to *Poeciliidae*. By investigating the development of certain behaviors in response or with context to changes in reproductive activity, this study hopes to provide a more comprehensive profile on behavioral interactions of the *Poeciliidae* family and subsequently establish a protocol for evolution work that seeks to understand the development and causal relationships behind the evolution of traits.

## METHODOLOGY

### Animals

A total of four species in the live-bearing *Poeciliidae* family were used: *Poecilia gillii*, *Poecilia butleri*, *Poecilia salvatoris*, and *Poeciliopsis infans*. For the *P. gillii* and *P. infans*, a sample size of n=6 subjects were used (n=4 females, n=2 males), whereas a sample of n=5 subjects were used (n=3 females, n=2) for the *P. salvatoris* and *P. infans*. Restrictions in population size for particular populations dictated the choice in slight variances in

sample size across populations. At time of retrieval and initiation of recordings and observations, subjects were found in relatively healthy condition from existing stock tanks from the Reznick Lab. Subjects were fed a diet of TetraMin Flake, Community Crave, and brine shrimp at regular intervals twice a day. 45% water changes were done once a week with UV-treated deionized water in order to optimize water quality and subject health.

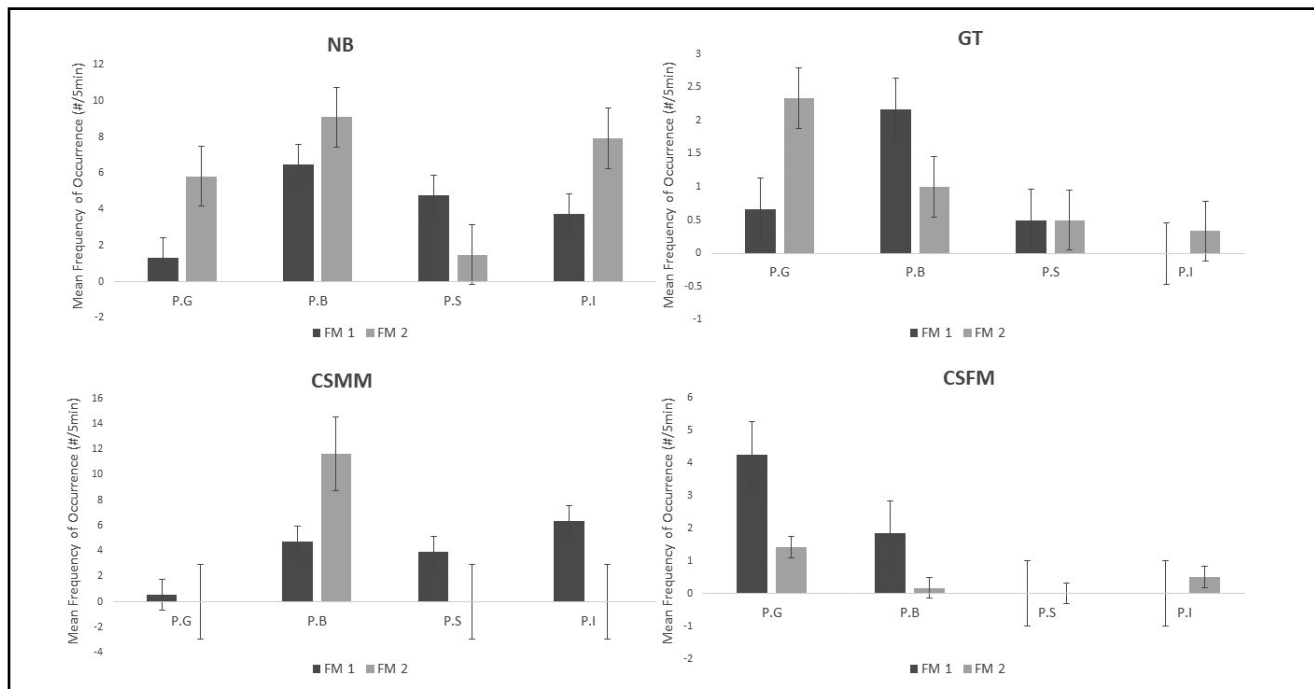
### Environment Assimilation

Four experimental tanks separated by species were prepared three days prior with gravel, an aquatic plant, a sponge filter, and connecting air tube. Blank sheets of paper were added in between tanks for isolation and prevention of external influence on behavior. Subjects were retrieved from their respective stock tanks and placed in the according experimental tanks. Subjects were allowed three to five days to acclimate to the change in environment and differing levels in water quality before the initiation of observations and recordings. To record presence of offspring, baby dividers composed of plastic mesh were introduced into the tank environment halfway through the duration of the experiment. All procedures met the U.S.

National Institute of Health guidelines for care and use of laboratory animals and were approved by Institutional Animal Care and Use Committee of the University of California, Riverside.

### Observations

Video recordings were taken for five-minute intervals per trial via video camera (iPhone XR). A focal male (denoted FM 1/FM 2) was chosen prior to the commencement of the trial and its quantitative and qualitative behaviors observed for a five-minute period. Movement by the researcher was restricted for the entirety of the trial in order to prevent external influences on observational data. Quantitative observations taken were divided into five subcategories, designated as representative traits of reproductive behavior: courtship displays (CD), nibblings (NB), gonopodal thrusts (GT), chasings from a chosen focal male towards the context male (CSMM), and chasings from females towards the focal male (CSFM). Quantitative traits were tabulated after the completion of the five-minute trial and observations on the behavior of the focal male as well as the associated context were noted: interactions between focal and context male, interactions between focal male



**Figure 1.** Average values of frequency of occurrence of selected representative mating behaviors over 5-minute trials. (Mean  $\pm$  SEM) Behaviors not observed or recorded during trials are represented in the absence of values for chasings (CSFM, CSMM), gonopodal thrusts (GT) in the *P. infans* population, chasings from focal male to context male (CSMM) in the *P. gillii*, *P. salvatoris*, and *P. infans* populations, and chasings from females to focal male (CSFM) in the *P. salvatoris* and *P. infans* populations.

and context females, and general observations of note regarding context subjects and environment.

### Statistical Analysis

Data was analyzed using Microsoft Excel and MATLAB 2018b. Results are expressed as the mean  $\pm$  SEM. Graphs were constructed using Microsoft Excel in order to compare results across populations and focal males.

## RESULTS

### Averages Values of Representative Mating Behaviors

The mean number of incidences of each representative mating behavior were calculated for each male across tanks over a period of 46 trials (**Figure 1**). Instances of courtship displays were not observed during trials, no quantitative mean values could be gathered, and the graph for the CD subcategory was omitted entirely. Quantitative CSFM values from the males in the *P. salvatoris* tank and the melanic male from the *P. infans* tank were not observed and are represented in the graphs accordingly. No CSMM values were recorded from the less dominant males in the *P. gillii*, *P. salvatoris*, and *P. infans* tank and are displayed accordingly. There was also an absence of evidence for the GT subcategory by the melanic dominant male in the *P. infans* tank and is represented as such.

### Behavioral Observations

Similar mating behaviors were observed across all populations and trial tanks, with varying levels of frequency of aggression, activity, and mating attempts. FM (Focal Male) 2 for *Poecilia gillii* was observed to be the more dominant male of the two, contrary to initial predictions that FM 1 would be more dominant based on larger relative size and ornamentation. FM 2 was observed to be more aggressive regarding attempts at copulation and would forego courtship rituals and nibbling for active aggressive behaviors via gonopodal thrusts. FM 1 held little to no interest towards the other fish in the tank, regardless of gender or context behavior. Male preference towards the three smaller females was found in favor of the aggressive largest female. Both males avoided the largest female, thus no reproductive interactions were observed. Females in the tank were observed to be relatively aggressive in comparison to other populations, both towards each other and towards the males, with the largest female as most aggressive.

Observations of extremely aggressive interactions between males were found from Tank 2 involving *P. butleri* subjects. FM 2, possessing more vibrant coloration and a comparatively longer gonopodium length, was noted to be the more dominant or aggressive of the two (e.g. incessant chasing and unprovoked aggression). FM 2 was equally proactive in terms of displays of mating behaviors and copulation attempts. Halfway through the duration of the experiment (trial n~35) FM 2 experienced a decrease in aggressive copulation attempts and FM 1 experienced an increase in aggression and NB and GT attempts. Aggression between males remained consistently significant throughout the duration of the experiment. Of the four trial tanks, Tank 2 was the only tank to not yield offspring by the conclusion of the experiment.

Little behavioral data was gathered from Tank 3 housing the *P. salvatoris* population. Most observation trial periods found subjects idle and unmoving during recordings. Approximately two outlying trials presented satisfactory representative mating behaviors; one particular recording (trial n=36) found subjects particularly interactive. Rather than male-male aggression, extreme aggression between FM 1 and a context female were observed, with the context female seemingly extremely unresponsive to FM 1 displays and attempts. The context female displayed signals (e.g. dilated pupils and rigid body structure from) signifying negative receptivity and aggression. Behaviors were ultimately concluded to be too ambiguous to accurately categorize specific mating behaviors, but were clearly defined as reproductive behaviors regardless.

Ample recordings of mating behaviors were obtained for Tank 4 containing the *P. infans* population, which exhibited constant and consistent displays of courtship and reproductive behaviors throughout each trial period for the entire duration of the experiment. The melanic FM 1 was noted to be the dominant male in the tank, shown through constant copulation attempts towards females by the nibbling of their gonopore area. FM 1 was observed to be extremely aggressive towards FM 2, using intimidation tactics on FM 2 upon swimming within a certain radius of any context females. FM 2 exhibited little to no reproductive behaviors and was often found hiding behind or under the plant placed in the tank or in the corner or walls

of the tank for the majority of recorded trials. Periodically FM 2 would attempt sneak copulation out of visual range of the melanic male, but would retreat upon being sighted by FM 1. Females in Tank 4 became apparently pregnant at approximately trial n~20, which was when recordings observed an increase in aggression of mating behaviors.

*P. infans* males seem to possess a different method of mating in comparison to the other *Poecilia* – rather than quick nibbling or gonopodal thrusting attempts, *P. infans* males would latch on to the gonopore area of the female for extended periods of time. After observing a lack of presence of offspring past the time period they were expected to give birth, it was concluded that the *P. infans* subjects were likely giving birth and subsequently eating their offspring, thus baby dividers were added into the tank environment (trial n=34). Presence of offspring was then observed (trial n=35). Succeeding the addition of the baby dividers and offspring birth, shifts in coloration from silver to melanic were observed for FM 2, in addition to a slight increase in reproductive aggression. A full transition to a melanic coloration was not observed, due to the FM 1 forcing FM 2 back to its initial coloration through displays of increased aggression and intimidation.

## DISCUSSION

The observations recorded during this study produced unexpected mating behaviors from focal subjects than initially predicted, observing that less ornamented males displayed comparatively higher frequency of mating behaviors as opposed to their counter male. The smaller *P. gillii* FM 2 clearly demonstrated to be the more aggressive male versus the larger FM 1 with a longer gonopodium length. Results may have been altered due to the general health of FM 1 being slightly malnourished relative to the other males from the stock tank. *P. butleri* FM 2 was also observed to be more aggressive despite lower ornamentation and a longer gonopodium length. Observations from both tanks suggest that males with disadvantageous traits (i.e. less pigmented ornamentation) adapt other mating behaviors to compensate -- i.e. increases in aggression.

General health and longevity of the *P. salvatoris* subjects may have played a factor in the lack of mating behaviors observed throughout the duration of the experiment. The

*P. salvatoris* population were comparatively older than the other subjects, which may have resulted in decreased aggression and behaviors given overall lack of activity observed during trials. However, the few trials recording evidence of movement returned surprisingly confounding results. Extremely aggressive male-male behavior as well as male-female behavior was observed, such as context females displaying a rigid body structure signifying aggression and non-receptivity, ambiguous courtship displays in the form of body tilting from the male, and excessive aggression or tagging between male and female suggesting that the female may be dodging attempts at copulation. Actions could not be clearly denoted as aggression or courtship and were surmised to actually be behaviors derived from either classification. The mating behaviors observed from the *P. salvatoris* imply to be too ambiguous to be clearly segregated into previously established mating behaviors categories but were conclusively classified as mating behaviors regardless.

*P. infans* FM 2 displayed shifts in pigmentation from its original silver to the melanic coat resembling FM 1 following the insertion of the baby divider into the tank environment, possibly in response to the presence of offspring. It could not be confirmed whether presence of offspring was the trigger for the changing of pigmentation or the slight increase in aggression, but could be connected to implications from previous studies [1] in which submissive males would experience melanic shiftings following the removal of the previous dominant male within the environment. We were unable to record definitive time-stamps as to when females gave birth due to the fish in the tank because they subsequently ingested their offspring, which prompted the insertion of mesh dividers halfway through the experiment. Therefore, it could not be confirmed as to the exact cause for changes in behavior for *P. infans* males.

Results from this experiment suggest that male behaviors may change drastically subsequent to offspring birth. Increases in aggression and mating behaviors were observed across all tanks following the insertion of the baby divider and the presence of offspring, excluding *P. butleri* subjects which did not give birth at all. This experiment splits the data too finely to draw conclusive

connections to offspring and behavior, but would be possible to determine with multiple replicates of trials for more accurate regression analysis values. Similar results have been observed in studies with different species, such as *Gambusia* and *Poecilia chica* [1]. More indicative data in the form of an extension of the study utilizing different subjects, multiple populations, and combinations could draw conclusive evidence connecting increases in mating behavior to the female reproductive cycle.

### CONCLUSION/FUTURE WORK

Ultimately, observations and recordings throughout the duration of this experiment revealed evidence of absence of courtship displays for *Poecilia gillii*, *Poecilia butleri*, *Poecilia salvatoris*, and *Poeciliopsis infans*, which are consistent with Goldberg et al.'s findings utilizing other species in the *Poeciliidae* family. Further revelations include correlations between reproductive behaviors, particularly in response to presence of offspring, changes in coloration in *P. infans* regarding the non-dominant, non-melanic context males within the tank environment, and observed increases in mating behavior and aggression in response to female fertility and positive receptivity. Though much definitive data was retrieved affirming presence and absence of representative mating behaviors, previously unrecorded behavioral demonstrations bring to light new queries – specifically, the evolutionary reasoning behind the shiftings in exterior coat in *P. infans* and the nature of the ambiguous reproductive behaviors observed in *P. salvatoris* – which remain unable to be answered within the

current scope of the study. Further studies utilizing multiple replicates of trials would thus be beneficial to confirm conclusive triggers or reasonings behind these observations. Overall, the data from the four *Poecilia* species provides conclusive information of livebearer mating behaviors and lends itself as a supplement to larger-scale studies and papers on the morphology of the gonopodium within the *Poeciliidae* family – more importantly, the causal relationships and reasoning behind the evolution of traits. The data from three of the observed species – namely, *Poecilia gillii*, *Poecilia salvatoris*, and *Poecilia butleri* – prove consistent with Goldberg's observations and serves to strengthen his conclusions on the evolution of male behavior regarding male ornamentation and other co opted traits. From this study, we effectively establish a precedent for how evolution work concerning the emergence of traits and behaviors is approached, and contribute to efforts to visualize and better understand integrated evolution and morphology within the animal kingdom.

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## Gut Microbiome and its Effect on HNF4 $\alpha$ Isoform Expression in Mouse Liver and Colon

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### ABSTRACT

A large and diverse population of microorganisms that resides in and on the bodies of all animals plays a significant role on the overall health of the organism. The nuclear receptor Hepatocyte Nuclear Factor 4 $\alpha$  (HNF4 $\alpha$ ) plays an important role in the normal development and function of both the liver and intestines. The expression of HNF4 $\alpha$  is regulated by two promoters (P1 and P2). Currently, there is little known about the relationship between the gut microbiome and the expression of HNF4 $\alpha$ . Here, we compared wild-type C57BL/6J male mice raised under either conventional (Conv) or germ-free (GF) conditions for changes in histology and HNF4 $\alpha$  protein levels in proximal colon, distal colon, and liver tissues. Our results show that the morphology of both proximal and distal colon, but not the liver is significantly impacted due to the lack of microbes in GF mice. Expression of both P1- and P2-HNF4 $\alpha$  is significantly higher in the distal colon of conventional mice as compared to that in germ-free mice. In the liver, P1-HNF4 $\alpha$  protein levels were significantly higher in Conv mice compared to GF mice. In contrast, the expression of P2-HNF4 $\alpha$  trended higher in GF mice. The proximal colon showed no difference in HNF4 $\alpha$  expression between Conv and GF mice. Since the two HNF4 $\alpha$  isoforms have distinct and opposing physiological roles, our results suggest that gut microbiota may play an important role in modulating intestinal and hepatic physiology due to their effect on HNF4 $\alpha$  expression.

**KEYWORDS:** Gut microbiome, HNF4 $\alpha$ , colon, liver, mice, germ free



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## INTRODUCTION

The microbiome of an organism consists of bacteria, viruses, fungi, and archaea that play an important role in health as well as disease.<sup>1</sup> Germ free (GF) animals are raised under gnotobiotic conditions (i.e. without being exposed to any germs) therefore, there is a lack of all species of microorganisms that naturally populate the body.<sup>1,2</sup> This means that GF mice lack microorganisms that may be needed for proper gut function. Hepatocyte nuclear factor 4alpha (HNF4 $\alpha$ ) is a highly conserved nuclear receptor required for the development and proper functioning of the liver, kidney, stomach, pancreas, and intestine.<sup>3</sup> It has two promoters, P1 and P2, which are responsible for expression of at least 9 isoforms total;<sup>4</sup> P1-driven HNF4 $\alpha$ 2 and P2-driven HNF4 $\alpha$ 8 are the predominant forms in most tissues.<sup>5</sup> On the protein level, the difference between these two isoforms is just 16 more amino acids on the N-terminal of P1-HNF4 $\alpha$ . Dysregulation of HNF4 $\alpha$  has been implicated in many diseases such as gastrointestinal disorders, colon cancer, liver cancer and diabetes.<sup>5-7</sup> P1-HNF4 $\alpha$  is known to be a tumor suppressor, while P2-HNF4 $\alpha$  is not.<sup>7,8</sup> Additionally, P1- and P2-HNF4 $\alpha$  also have different roles in the regulation of circadian rhythms.<sup>8</sup> These isoforms also showcase differential expression in relation to different tissues and exhibit somewhat different functions. In the colon, P1-HNF4 $\alpha$  is primarily expressed in the differentiated part of the crypt, while P2-HNF4 $\alpha$  is primarily expressed in the proliferative part of the crypt.<sup>6</sup> Crypts in the colon are important for regeneration of intestinal epithelium and absorption of nutrients like water and salt.<sup>9</sup> While it is clear that HNF4 $\alpha$  plays a critical role in proper gut function, there is not much known about the relationship between HNF4 $\alpha$  and the gut microbiome, which also plays a key role in gut function. In the liver, HNF4 $\alpha$  plays an important role in regulating gene expression and proper development and function. P1-HNF4 $\alpha$  is mainly expressed in the adult liver, while P2-HNF4 $\alpha$  is mainly expressed in the fetal liver and liver cancer.<sup>7,10</sup> This study will characterize the expression levels of HNF4 $\alpha$  in the liver and colon of GF mice by comparing the results to conventional (Conv) mice in order to determine the effect of microbiota on liver and colon function.

## METHODS:

### Ethics Statement

Care and treatment of animals was in accordance with guidelines from and approved by the University of California, Riverside's Institutional Animal Care and Use Committee (AUP #A20180067 and #A20170020). All mice had *ad libitum* access to food and water. At the end of the study, mice were euthanized by carbon dioxide inhalation followed by cervical dislocation, in accordance with stated NIH guidelines.

### Tissue Embedding, Sectioning and Staining

Male C57BL/6J mice were weaned at 3 weeks and maintained in a normal 12:12 hour light-dark cycle in either a conventional vivarium or a germ-free isolator at the University of California, Riverside. At least six mice were included in each group at the start of the study; this sample size is normal for animal studies, because it is difficult to generate germ-free mice. Liver, proximal colon (PC), and distal colon (DC) tissues were collected and snap-frozen in liquid nitrogen before storage at -80°C or fixed in 10% neutral-buffered formalin. Weight and length measurements were taken for spleen and small intestine. Tissue fixed in 10% formalin was washed with phosphate-buffered saline (PBS) and then stored in a 30% Sucrose+PBS solution for 24 hours at 4°C. This tissue was then subsequently embedded in optimal cooling temperature (OCT) compound, sectioned at 5 $\mu$ m on a Microm Cryostat onto positively charged slides, and stored at 20°C. All slides were rehydrated in 95% ethanol for 7 minutes, tap water for 7 minutes, ddH<sub>2</sub>O for 2 minutes, and stained in hematoxylin (Ricca) for 40 seconds. Slides were then dipped in tap water for 30 seconds, running tap water for 90 seconds, 95% ethanol for 15 seconds, counterstained in eosin (Sigma-Aldrich) for 3 seconds, and finally dipped in 95% and 100% ethanol two and three times, respectively, for 20 seconds each time. Slides were left in Citrisolve (Fisher Scientific) for at least 40 seconds. This staining process is completed all at once in succession. Slides were then fixed and preserved with Permount (Fisher Chemicals). Histology images were captured at 10x for proximal and distal colon and 20x for liver on an Evos Microscope (Life Technologies). Crypt length and submucosal thickness were measured using SPOT Imaging software (Sterling Heights, MI).

### Immunoblot Analysis

Whole cell extracts were prepared from tissues stored in liquid nitrogen and protein concentration was determined by a Bradford Assay.<sup>6,11</sup> The protein extracts (35 $\mu$ g) were separated by 10% sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and transferred to Immobilon Membrane (EMD Millipore, Billerica, MA). The membrane was then blocked with 5% nonfat milk for 30 minutes and incubated in primary antibodies (mouse monoclonal anti-HNF4 $\alpha$  P1 and P2; catalog no. PP-K9218-00 and Cat PP-H6939-00, respectively, R&D Systems) in 1% milk, overnight at 4°C. After several washes in 1x TBST (mixture of tris-buffered saline and polysorbate 20), the blots were incubated in horseradish peroxidase (HRP)-conjugated goat anti-mouse (GaM-HRP) secondary antibody (Jackson ImmunoResearch Laboratories) for 40 minutes at room temperature and then followed by several washes: 5 minutes with 1x TBST (mixture of tris-buffered saline and polysorbate 20), 10 minutes with 1x TBST, and 10 minutes of 1x TBS (tris-buffered saline). Blots were developed using SuperSignal™ West Pico PLUS Chemiluminescent Substrate (ThermoFisher Scientific)

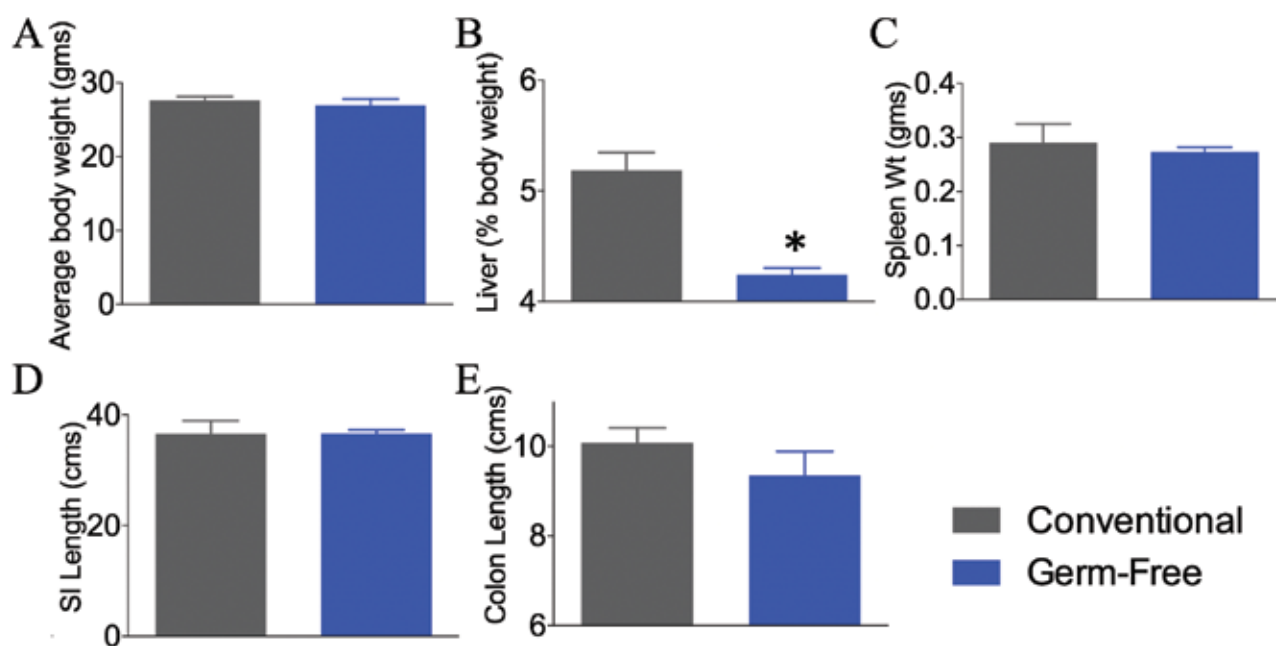
and imaged in a Chemi-Doc imaging system (Bio-Rad). Coomassie staining of blot was done to verify equal protein loading.

### Statistical Analysis

Data is presented as mean  $\pm$  standard error of mean (SEM). Statistical significance, using GraphPad Prism (GraphPad Prism version 6.00 for Mac, GraphPad Software, La Jolla California USA, www.graphpad.com), is defined as  $P \leq 0.05$  using Student's t-test, unless indicated otherwise.

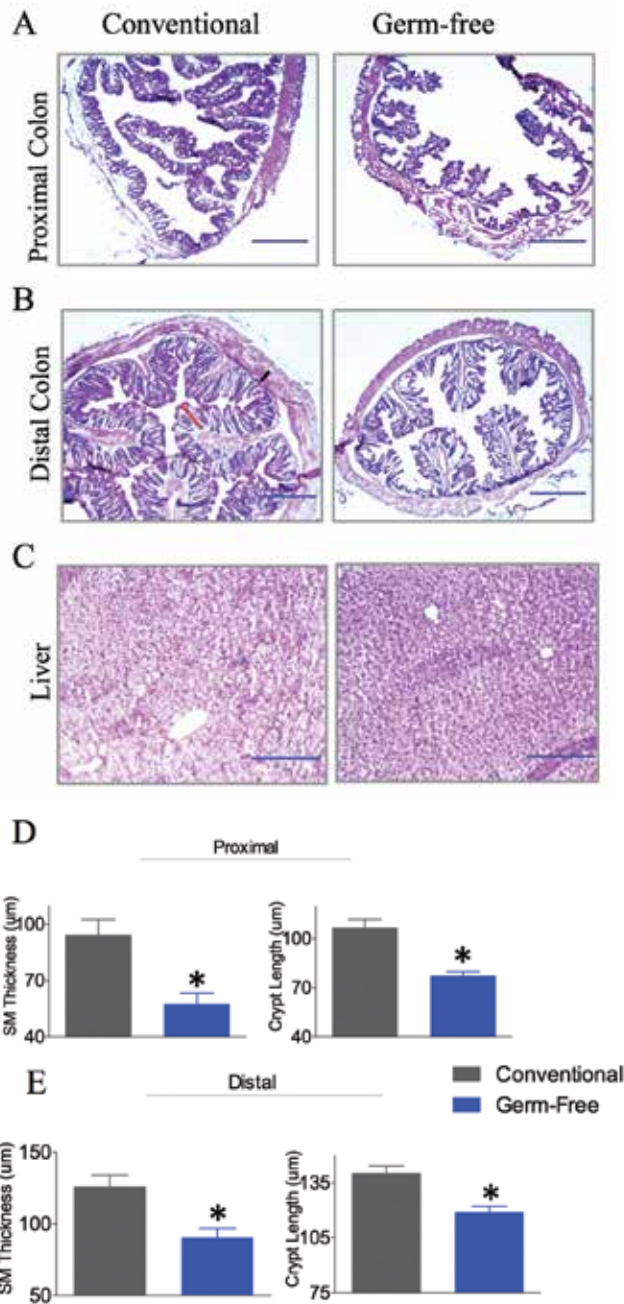
### RESULTS

There was no difference in average body weight, spleen weight, small intestine (SI) or colon length for the mice raised under gnotobiotic or conventional conditions (**Fig. 1A, C, D, E**). In contrast, liver weight, normalized to body weight, was significantly lower in the GF mice (**Fig. 1B**). Histological examination of the proximal and distal colon tissues indicates that the intestinal epithelium in the GF mice appears less healthy than Conv mice, with markers such as shrunken and sparse crypts (**Fig. 2A, B**). These observations are supported by measurements of crypt



**Figure 1. Effect of gut microbiota on organ size.** A) Average body weight, at harvest, of 3-4 months old male C57BL/6J mice on vivarium chow raised under conventional or germ-free conditions. N=6-7 per group. B) Liver weight recorded as percent of body weight. C) Spleen weight. D) Length of small intestine. E) Colon length. Data are presented as  $\pm$  SEM. \*Significantly different,  $P < 0.05$ .





**Figure 2. Histological analysis of liver and gut tissue from Conv and GF mice.**

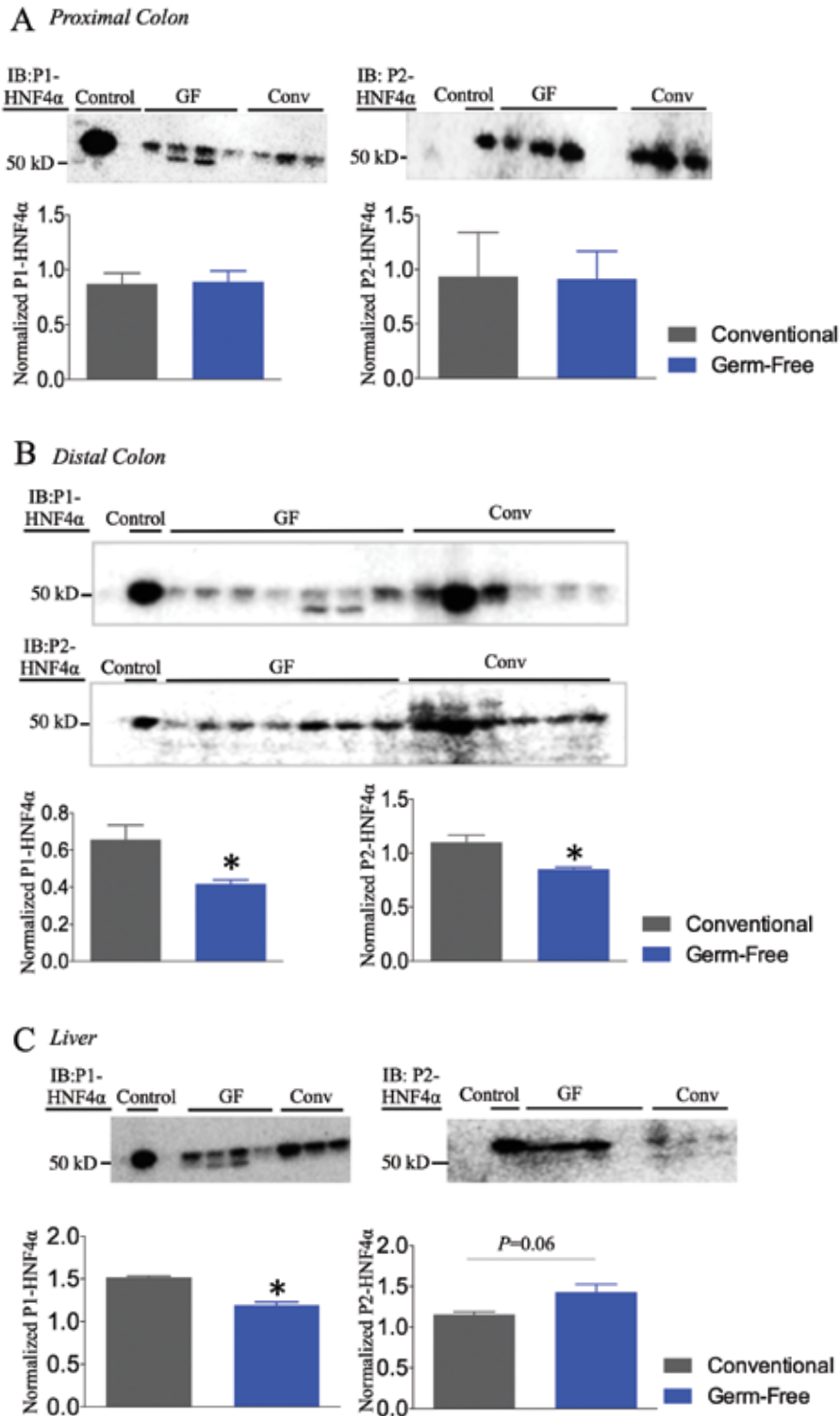
A-C) Representative H& E sections of proximal colon, distal colon and liver tissues from male C57Bl6/J mice raised under conventional or germ-free conditions. Images taken at 10x for proximal and distal colon and 20x for liver. Scale bar (horizontal blue line) is 400 µm in proximal and distal colon and 200 µm in liver images. D-E) Average submucosal (SM) thickness and crypt length measurements in proximal and distal colon tissues. Black line in left panel B indicates what was measured as submucosal thickness and red line indicates typical crypt length measurement. N=2-3 mice per group for proximal colon and N= 5-7 mice per group for distal colon and liver. Difference in sample size is because tissues were not saved at the time of harvest or difficulties in properly processing the tissue.

length and thickness of the submucosa, both of which are significantly lower in the proximal and distal colon of GF mice (Fig. 2 D, E). Histologically, liver tissues do not show any significant differences between Conv and GF mice (Fig. 2C). These results suggest multiple significant findings. The lack of microbiome in GF mice decreases liver size, while liver tissue morphology remains unaffected. Additionally, colon length remains the same between GF and Conv mice; however, histologically there are significant differences between the two.

Next, the comparison of P1- and P2-HNF4α protein expression in the liver, proximal and distal colon of GF mice to that in Conv mice identified no significant observational difference in the expression of either isoform of HNF4α in the proximal colon (Fig. 3A). However, both P1- and P2-HNF4α are expressed at significantly lower levels in the distal colon of GF mice (Fig. 3B). Additionally, in the liver, a significant decrease was observed in P1-HNF4α expression in GF mice accompanied by an increase (trending significant, P=0.06) in P2-HNF4α levels compared to Conv mice (Fig. 3C). These results suggest that the microbiome, or lack of, affects the hepatic and colonic expression of HNF4α.

**DISCUSSION**

Conventionally raised mice have trillions of microorganisms residing in or on their bodies.<sup>2</sup> These microbes not only help in the digestion of food, but also play an vital role in the maintenance of almost all physiological functions such as metabolism, immunity, reproduction, and even behavior.<sup>12</sup> Gnotobiotic animals are important tool that can be utilized to elucidate the role of various microbial species in health or disease.<sup>12</sup> This study explored the effect of gut microbiota on the intestines and liver in male mice by comparing germ-free (GF) to conventional (Conv) mice through the analysis of histology and characterized levels of HNF4α expression in various organs. In terms of the effect of lack of microbiome on organ physiology, results remain consistent with what has been reported previously, i.e. spleen weight and liver weight either decrease or show no change in GF mice compared to Conv mice.<sup>1</sup> An effect of microbiome on these organs could be due to metabolites that are produced by the gut microbiota which travel via the portal blood to the liver and other organs.<sup>13</sup> The effect of gut bacteria and their metabolites, such as short-chain fatty acids (SCFAs), on the morphology of the colon has



**Figure 3. Immunoblot analysis.** A) Top IB of WCE from the proximal colon of germ-free (GF, n=4) or conventional mice (Conv, n=3). Bottom- Quantification of the P1- and P2-HNF4α signals from blots shown above normalized to total protein, as determined by Coomassie staining of the same blot. B) Top-IB of WCE from distal colon of GF (n=7) or Conv (n=6) mice. Bottom- Quantification of the P1- and P2-HNF4α signals as in A. \* P < 0.01. C) Top-IB of WCE from liver of GF (n=4) or Conv (n=3) mice. Bottom- Quantification of the P1- and P2-HNF4α signals as in A. \* P < 0.01. Each lane is from a different mouse. The position of the molecular weight marker (50 kD) is shown. P1- and P2-HNF4α specific positive controls were included on respective blots. Difference in sample size is because some of the tissues were not saved at the time of harvest.

been described extensively. For example, it has been shown that infusion of microbially-derived SCFAs into the colon can increase crypt length.<sup>14,15</sup> This is in agreement with our observation that crypts in both proximal and distal colon of the GF mice are shorter than those in Conv mice. Crypts in the colon are responsible for the regeneration of the intestinal epithelium and for absorption of important nutrients, including water and salt.<sup>9</sup> Based on the histological analysis of the GF mice, the villi seem to be damaged compared to the Conv mice, suggesting that the colons of GF mice may have significantly decreased nutrient absorption, imbalanced water and electrolyte absorption, and a disturbed secretion process. Another interesting finding includes a decrease in the thickness of the submucosal layer in both proximal and distal colon of GF mice. We found no previous reports of this in the literature; although, decreased thickness of muscularis in the cecum and of the lamina propria layer in the small intestine has been reported previously.<sup>1</sup>

A proper balance of HNF4 $\alpha$  isoform expression is required for normal development and functioning of the colon.<sup>6,16</sup> We report here that there is a decrease in both P1- and P2-HNF4 $\alpha$  protein in the distal colon of GF mice. This suggests impaired differentiation (function of P1-HNF4 $\alpha$ ) and decreased proliferation (function of P2-HNF4 $\alpha$ ) of epithelial cells in the distal colon.<sup>6</sup> This dysregulation affects the ability of the mice to respond effectively to any challenges to the colonic epithelium, both in health and disease.

HNF4 $\alpha$  plays a significant role in regulating gene expression as well as proper development and function of the liver.<sup>3,4</sup> The two isoforms of HNF4 $\alpha$  play opposing roles in the modulation of liver health and opposing expression, with P1-HNF4 $\alpha$  expressed in the normal adult liver and P2-HNF4 $\alpha$  mainly expressed in the fetal liver and also during liver cancer.<sup>7,17</sup> Findings indicate that GF mice have decreased P1- and increased P2-HNF4 $\alpha$  compared to Conv mice, which suggests that development of liver disease, such as hepatocellular carcinoma, might be modulated by the gut microbiome. A previous study has reported that microbiota can decrease HNF4 $\alpha$  activity in the intestines, but did not include distinguished differentiation between the different isoforms of HNF4 $\alpha$  or examine the liver.<sup>18</sup> It is

likely that the effects observed by this previous study were also on P1-HNF4 $\alpha$ . Further studies should be conducted to define the effect of microbiota on liver health, specifically because the results of this study showcases that the hepatic expression of one of the master regulators of liver function and gene expression, HNF4 $\alpha$ , can be modulated by the microbiome.

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