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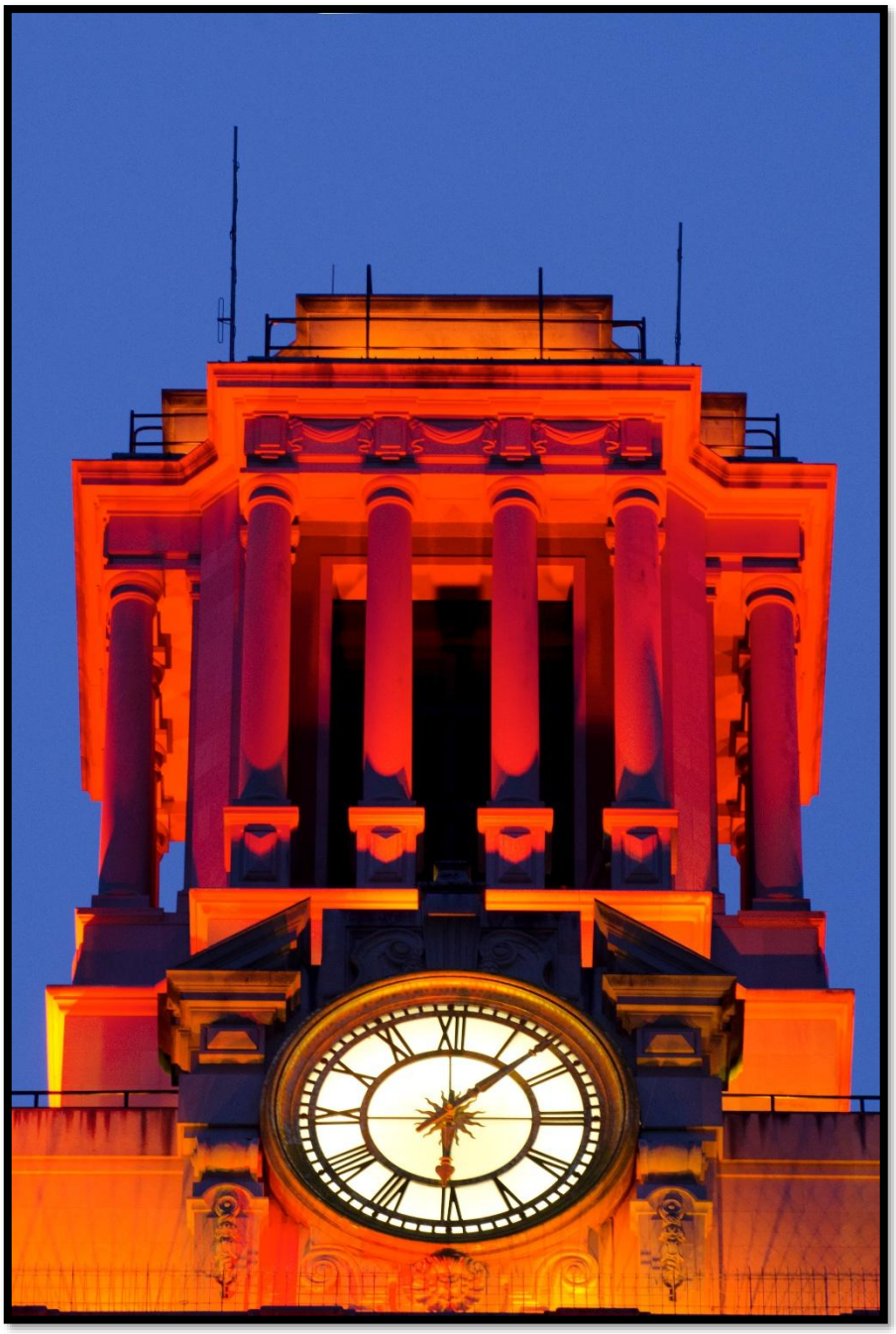

THE RONALD E. MCNAIR SCHOLARS RESEARCH JOURNAL

THE UNIVERSITY OF TEXAS AT AUSTIN

THE MCNAIR RESEARCH JOURNAL IS A MULTIDISCIPLINARY JOURNAL OF UNDERGRADUATE RESEARCH CONDUCTED BY THE MCNAIR SCHOLARS.

The University of Texas at Austin
Undergraduate College

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March 31, 2025

The Undergraduate College is honored to have the Ronald E. McNair Post-Baccalaureate Achievement Program as part of its portfolio and fully supports the program's goal of increasing the number of low-income and first-generation college students pursuing graduate education. This goal is consistent with the Undergraduate College's efforts to promote and support an intellectually vigorous environment at the University.

Therefore, it is my pleasure to offer a congratulatory introduction to this year's issue of the *McNair Scholars Research Journal of The University of Texas at Austin*. The students published in this issue are Scholars who were selected to participate in UT's rigorous McNair Summer Research Institute.

These student scholars work with faculty mentors who support their research interests and guide them through a research process that concludes in completing articles for publication in this, the *McNair Journal*. Not only does the journal offer a steppingstone for student scholars seeking out publication opportunities, it also provides a public venue for graduate programs to see impressive examples of the Scholars' academic work.

As is evident in this issue, the research interests of the 2023 cohort of McNair Scholars are as wide-ranging as the students themselves. This annual publication marks the culmination of each Scholar's dedication and determination in McNair. We are confident it also marks the launching of a fruitful pursuit of scholarly advancement, followed by a fulfilling professional career in research.

I have no doubt readers of this journal will recognize the value of McNair Scholars, and the opportunity it offers to participating undergraduates whose scholarly ambitions and accomplishments reflect what represents the best of The University of Texas at Austin.

Dr. Richard Reddick

Senior Vice Provost and Dean
Undergraduate College

THE UNIVERSITY OF TEXAS AT AUSTIN

McNair Scholars

I am honored to present this year's volume of the *McNair Scholars Research Journal at The University of Texas at Austin*. This journal represents the acuity and effort of our McNair Scholars during their Summer Research Institute. The scholarly research included here is a testament to the deep commitment and high capacity of our Scholars. This year, as every year, the *McNair Journal* showcases the persistent tradition of our Scholars achieving academic excellence.

Established at UT-Austin in 2007, McNair Scholars prepares high-priority students for doctoral studies through engagement with research methods, faculty mentoring, graduate studies colloquia, and other scholarly and communal activities. As we near our twentieth anniversary, our McNair program continues to prepare low-income and first-generation college students for success in earning and employing their Ph.D.'s.

This year's journal contains the work of Scholars who completed the 2022 Summer Research Institute. We are proud of their achievements in conducting and completing original research. It has been, and will continue to be, our privilege to support these Scholars with their future academic endeavors.

Many individuals across campus make McNair successful. First and foremost, I want to thank all the faculty mentors for their guidance and expertise working with our Scholars and helping them reach their fullest potential. McNair is also dependent on collaborations with our incredible staff, especially our Graduate Assistants, who work hard to ensure that each year we put forth a program that is meaningful for students and respected across higher education. I want to extend especial appreciation to Dr. Hillary Procknow, Assistant Dean in the Undergraduate College, and Dr. Richard Reddick, Senior Vice Provost and Dean of the Undergraduate College, both of whom offer unwavering commitment to McNair Scholars. Finally, I want to acknowledge Dr. Nabeeha Chaudhary, the McNair Senior Program Coordinator, whose dedication to and support of Scholars is unflagging. I am fortunate to have such a terrific colleague running the program with me.

With the support of these, and many other, professionals, alongside the perseverance and goodwill of the McNair Scholars themselves, we can continue to demonstrate that, in The University of Texas at Austin's McNair Scholars program, "What Starts Here Changes the World."

Thank you,
Eric Dieter

Director and Principal Investigator
UT-Austin McNair Scholars

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The Effects of a Nonverbal Autistic Child's Diagnosis on their Primary Caregivers

Chasity D. Webb

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Faculty Mentor: Mary Beth Schmitt, Ph.D.

Abstract

When a child is diagnosed with autism spectrum disorder (ASD), it doesn't just impact the child but also the family, especially the primary caregiver. While the diagnosis can explain why the child might develop differently or struggle with things like loud noises, it can also make the caregiver feel overwhelmed, guilty, and even depressed. I looked into how having a child aged 4 to 18 with nonverbal ASD affects four primary caregivers. I used different tests to understand how they felt mentally, physically, and emotionally. Based on the previous research with topics very similar to my research, the results clearly showed that these caregivers were dealing with a lot of stress, feeling strained, and often depressed due to their role. From what I learned in previous studies, caregivers of nonverbal autistic children go through a tough time. I also think that if these caregivers and their nonverbal child can communicate well, it can really help lower the stress. And not just that, having support from the community, enough money, and taking care of themselves will also reduce the stress. By looking at all these factors, my research aims to help us understand more and find ways to make things better for caregivers in this situation. I want to highlight how important it is for caregivers and their nonverbal autistic children to connect in the right way and also show that having support from the community and taking care of themselves can make a difference in their lives.

Introduction

Autism Spectrum Disorder (ASD) is a neurodivergent disorder characterized by a developmental disability caused by differences in the brain. While problems with social communication and interaction and restricted or repetitive behaviors or interests can be traits commonly observed in individuals diagnosed with ASD, there are many other traits for this disorder. (American Psychiatric Association [APA] 2013). The symptoms are thought to arise from a complex interplay of genetic, epigenetic, and environmental factors (Bhandari et al., 2020). ASD is measured on a spectrum that helps diagnose the severity of the disorder. Nonverbal Autism is characterized by a lack of or limited use of verbal communication. This can include difficulty using words to express needs or desires, speaking in single words or short phrases, and difficulty understanding language. Nonverbal autism can also manifest in other ways, such as through limited eye contact, poor nonverbal communication skills, and difficulties with social interaction." (Gurny, P. 2022) Parenting is an essential factor in child development, and the effects of parenting stress on the mental, emotional, and behavioral health of offspring has been receiving increasing attention (Holly et al., 2019). Parenting stress may be experienced when parents experience parenting demands inconsistent with expectations (self or other) or when their resources are inadequate (Holly et al. 2019). Parents' appraisals, environment, and life stressors may also contribute to parenting stress. This type of stress partially overlaps with marital or vocational stresses experienced by parents and may adversely affect parenting and parent-child relationships (Holly et al., 2019)

I chose the primary caregivers of nonverbal autistic children from the ages of 4-18 because A child diagnosed with autism spectrum disorder (ASD) who has not begun to verbalize by the age of four is considered to have nonverbal autism (Gunnery, 2019). An estimated 40% of individuals diagnosed with ASD are nonverbal. We are taught to explain to families that most children with nonverbal ASD past the age of four most likely will never speak. Among the many psychological hypotheses explaining the psychopathology of autism, the deficit in joint attention is a prominent one used to explain its social and communicative deficits. The study demonstrated that 2- and 3-year-olds with autism displayed deficits mainly in initiating joint attention, especially in high-level skills, compared to both typically developing and delayed comparison groups. The deficit in the frequency of communication acts was marked even compared with typically developing infants with younger mental age. (Chiang, Soong, Lin, Rogers, 2016)

Although there is a chance that most Nonverbal Autistic four years or older may never speak, that is not always the case. There has been research that provides proof that nonverbal Autistic children can overcome severe language delays. "The research shows that most of these children later acquired—language skills. Almost half (47%) became fluent speakers, and over 1/3 (70%) can speak simple phrases. Factors that may predict a severely language delayed child would eventually develop speech found that most kids had higher IQs (assessed with nonverbal tests) and lower social impairment. They also found that a child's repetitive behaviors and restricted interests did not affect the likelihood of language development. (Wodka, Mathy, & Kalb)

The diagnosis of ASD in a child affects family processes, increases parenting stress and marital conflicts, and may lead to parental psychopathology (Chan and Leung 2020; Hickey et al. 2020; Schnabel et al. 2020; Walton and Tiede 2020). Parents of children with ASD may experience elevated levels of stress compared to parents of children with other disabilities (e.g., intellectual disabilities, specific learning disorders, attention deficit hyperactivity disorder, cerebral palsy, etc.) as well as those of children with typical development (Lin et al. 2020; Miranda et al. 2019). Higher levels of parenting stress among parents of children with ASD were reported to be associated with adverse outcomes for couples (e.g., poor

marital relationships; Chan and Leung 2020), for the family unit (e.g., emotional climate; Hickey et al. 2019; McStay et al. 2014), for parents themselves (e.g., poor parental mental health, reduced quality of life, fatigue and burnout; Alnazly and Abojedi 2019; Alshekaili et al. 2019; Giallo et al. 2013; Huang et al. 2019) as well as for their children (e.g. elevated levels of externalizing and internalizing behavior problems, worse prognosis and effects on parent-child relationships; Reid et al. 2019; Rodriguez et al. 2019; ZaidmanZait et al. 2014).

The process of psychological stress may include four components, comprising a causal event, the cognitive appraisal of the event, coping mechanisms, and stress reactions (Lazarus 1993). According to this theory, parenting stress among parents of children with ASD may be caused by the severity of the children's autistic symptoms and/or co-occurring internalizing and externalizing behavioral problems (Miranda et al., 2019). Parental cognitive appraisals of their children's diagnosis may include concern, denial, confusion, and feeling overwhelmed by emotions at the initial stages. This may be followed by feelings of isolation and anger at the lack of acknowledgment of their children's disability by the wider public and their contributions to children's care and rehabilitation by the service providers. They also need to continuously negotiate knowledge on their children's diagnosis and management while balancing multiple roles

(Boshof et al. 2019). Perceived social support may be crucial in increasing those parents' resilience during ASD diagnosis and management (Drogomyretska et al. 2020).

The prolonged duration of stress takes its toll on the parents. Studies from various countries and using different methodologies suggest that caregiving burden, being the sole parent/caregiver in the family, unemployment, duration of care, lack of social support, ASD symptom severity, and internalized stigma may be significant predictors of parental psychopathology (Alnazly and Abojedi 2019; Alshekaili et al. 2019; Huang et al. 2019; Minichil et al. 2019; Zhou et al. 2019; Scherer et al. 2019). A recent meta-analysis of 31 studies and 9208 parents found that anxiety and depression were the most common disorders among those parents, with median rates of 33.0% and 31.0%, respectively (Schnabelet al. 2020)

Daniels et al. (2017) reported that parents of children with ASD from Albania, Bulgaria, Croatia, and Turkey all reported challenges in access to care and stigmatization. Gürbüz Özgür et al. (2018) found that the severity of autistic symptoms, presence of psychopathology in mothers/fathers, lack of attendance of children to specialized schools, time since diagnosis and the need to use medications to manage ASD symptoms and related problems were associated with lower quality of life among parents. Kuru and Piyal (2018) reported that elevated levels of social support and higher levels of paternal education with skilled paternal vocation were associated with higher quality of life. Baykal et al. (2019) found that the severity of ASD symptoms and caregiver depression was associated with a more significant caregiver burden, and the lack of functional speech was critical in this regard. (Kilincaslan et al. 2019) also reported that functional language and greater family income were associated with better functioning among a sample of Turkish children with ASD.

Therefore, the primary aims of this study were to determine:

1. Identify to what extent having a nonverbal child with ASD causes distress in the caregiver.

   My hypothesis for research question one is that primary caregivers with nonverbal autistic children will exemplify high levels of distress.

2. Identify the extent to which effective communication systems mitigate the primary caregiver's stress.

   My hypothesis for research question 2 is that effective communication between primary
   caregivers and their nonverbal Autistic child will significantly mitigate caregiver stress.

3. Identify the extent to which external factors (community, financial support, self-care) mitigate caregiver stress.

   My hypothesis for question three is that external factors such as community, financial support, and self-care will significantly mitigate caregiver stress.

These research aims and hypotheses are based on factors I believe could either increase or decrease the mental, physical, and emotional effects on primary caregivers of nonverbal autistic children. Based on my secondary research, there is research out there to show the mental, physical, and emotional effects of being a primary caregiver of an autistic child. However, none show these same effects on the primary caregivers of nonverbal autistic children. I also noticed that many previous studies had participants from both male and female primary caregivers, mainly the child's parents. However, unexpectedly, my study only had female primary caregivers, and these were the mothers of the children.

Method

This study surveyed 4 Primary Caregivers using the Caregiver Strain Questionnaire, Beck's Depression Inventory Zarit Burden Interview Assessing Caregiver Burden, and a questionnaire I created to measure external factors effects such as finances, community, and communication.

Caregiver Strain Questionnaire (CGSQ):

Caregiver Strain Questionnaire (formerly the Burden of Care Questionnaire) was developed by Brannan, Heflinger, and Bickman in 1994 for use with families of children and adolescents with emotional and behavioral disorders. The primary caregivers completed the CGSQ. The average of these scores was 53.5, proving that according to the CGSQ scores, these mothers exhibit high strain levels as an effect of being a primary caregiver to a nonverbal autistic child.

Beck Depression Inventory (BDI):

The BDI was developed by Beck in 1961 to evaluate symptoms of depression (Beck et al. 1988). This scale includes 21 Likert-type items evaluating motivational, cognitive, somatic, social, and vegetative symptoms of depression. Previous studies on parents of children with disabilities, including ASD, supported its reliability and validity (Baykal et al. 2019; Cifci-Tekinarslan 2013; Yıldız et al. 2016). Three of the primary

caregivers completed the BDI in the current study. The average of these scores was 38.33, therefore proving that according to the BDI scores, these mothers are exhibiting severe depression levels as an effect of being a primary caregiver to a nonverbal autistic child.

Zarit Burden Interview Assessing Caregiver Burden:

The Zarit Burden Interview Assessing Caregiver Burden was developed by Zarit, Reever, and Bach-Peterson in 1980. This scale originally included 29 Likert-type items that reflect how people sometimes feel when caring for another person, but I used the short 12-item version of this scale for this study. The average of these scares was 34.75, which suggests that the mothers in this study exhibit high levels of caregiver burden as an effect of being a primary caregiver to a nonverbal autistic child.

Chasity's questionnaire:

This questionnaire was created by me to assess other factors such as community, communication, financial support, and self-care. Based on the responses that I received from the participants about communication, financial support, and self-care, I did not find a significant difference between these factors and their correlation with mitigating caregiver stress at all because even a parent who has all or most of these factors individually had very high caregiver strain, depression, and caregiver burden scores.

Discussion

The diagnosis of Autism Spectrum Disorder SD in a child affects family processes, increases parenting stress and marital conflicts, and may lead to parental psychopathology (Kütük, M. Ö., et al., 2021). Having and raising children is challenging, expensive, and a huge responsibility for all families and caregivers; adding a special needs child into the mix and everything it takes to raise a child is even more stressful. Caring for children with ASD is very challenging, as parents have to balance parenting, caregiving, and teaching basic life skills. They also have to act as co-therapists in reinforcing positive behaviors while simultaneously trying to extinguish negative behaviors; educate friends, relatives, and peers on the nature of ASD; struggle with transportation between different service environments; and prepare for children's transition into adulthood. This leads them to experience elevated levels of stress and burnout (Giallo et al., 2022).

Aside from the daily task of raising a child, add the stress of a child who is not reaching their normal milestones, and not being able to explain why your child is not walking, talking, and developing as they should. This is the life of parents and caregivers with children with autism spectrum disorder. The signs of ASD can start as early as 12-18 months, and even with these signs, most children cannot be officially diagnosed with ASD until they are two years old or older.

Autism Spectrum Disorder is a developmental disorder of variable severity characterized by difficulty in social interaction and communication and restricted or repetitive patterns of thought and behavior. (Oxford.com). ASD is measured on a spectrum that helps diagnose the severity of the disorder. There is also Nonverbal Autism, which we focus on in my research. Nonverbal Autism is defined in this case as a child that may not talk at all or may talk very little. "Nonverbal autism is a form of autism characterized by a lack of or limited use of verbal communication. This can include difficulty using words to express needs or desires, speaking in single words or short phrases, and difficulty understanding language. Nonverbal autism can also manifest in other ways, such as through limited eye contact, poor nonverbal communication skills, and difficulties with social interaction." (Gurny, P. 2022)

Nonverbal Autism Effects on Children:

How does nonverbal autism affect a child? Even if an individual with autism is nonspeaking, they still use words in other ways. An example would be writing. They also may understand the words they overhear or are spoken to them. Symptoms of nonspeaking autism may include the inability to speak clearly or without interference. This could be because they have apraxia of speech, a disorder that affects specific brain pathways—interfering with an individual's ability to say what they want correctly. It could also be because the individual has not developed verbal communication skills. Some children lose verbal skills as symptoms of the condition worsen or become more noticeable. Some autistic children have echolalia, causing them to repeat words or phrases repeatedly (Holland, K. 2021).

Although there is a chance that most Nonverbal Autistic four years or older may never speak, that is not always the case. There has been research that provides proof that nonverbal Autistic children can overcome severe language delays. "The research shows that most of these children later acquired.

Among the many psychological hypotheses explaining the psychopathology of autism, the deficit in joint attention is a prominent one used to explain its social and communicative deficits. Joint Attention is defined as a behavior in which two people focus on an object or event to interact with each other. It is essential in communication

because it develops crucial social skills such as bonding and seeing another's point of view. The study demonstrated that 2- and 3-year-olds with autism displayed deficits mainly in initiating joint attention, especially in high-level skills, compared to both typically developing and delayed comparison groups. The deficit in the frequency of communication acts was marked even compared with typically developing infants with younger mental age. (Chiang et al., Soong, Lin, Rogers, 2016) language skills. Almost half (47%) became fluent speakers, and over 1/3 (70%) can speak simple phrases. Factors that may predict a severely language delayed child would eventually develop speech found that most kids had higher IQs (assessed with nonverbal tests) and lower social impairment. They also found that a child's repetitive behaviors and restricted interests did not affect the likelihood of language development. (Wodka et al., n.d. Mathy, & Kalb)

How are families and Caregivers financially affected by a child with a nonverbal ASD diagnosis:

There is a $42,000 difference between the cost of raising an autistic child and a child without ASD. On average, autism costs an estimated $60,000 a year through childhood, with the bulk of the costs in special services and lost wages related to increased demands on one or both parents. Costs increase with the occurrence of intellectual disability. (Autismspeaks.com, n.d.) On average, it costs $18,000 a year to raise a child without any special needs or disabilities. (Eyewitness News, 2022)

What are the effects on parents and caregivers from having a child diagnosed with ASD:

There was a commonality within the research I conducted in this area: high-stress levels, depression, anxiety, and burnout have been common in parents and caregivers with children with ASD. I am referring to this study that compared parents' caregiver burden of children with ASD to parents with caregiver burden of Down Syndrome and Type 1 Diabetes. This study confirmed that "mothers and fathers of children with ASD carry a huge caregiving burden in the form of objective difficulties, subjective distress, and symptoms of depression and anxiety" (Picardi et al., (2017). This study also concluded that "The parents of children with ASD reported higher objective and subjective burden, more frequent psychological distress, lower social support. Mothers reported greater subjective burden than fathers. Structural equation modeling showed that the most consistent positive and negative predictors of objective and subjective burden were ASD symptom severity and social support, respectively." (Picardi et al., (2017).

This study highlights the high burnout and depression levels of parents with children diagnosed with ASD. This study also showed that "They also have to act as co-therapists in reinforcing positive behaviors while simultaneously trying to extinguish negative behaviors; educate friends, relatives, and peers on the nature of ASD; struggle with transportation between different service environments; and prepare for children's transition into adulthood. This leads them to experience elevated levels of stress and burnout. Experiences of stigmatization may lead to self-blame and psychopathology (Giallo et al. [20]), while perceived social support may promote resilience (Drogomyretska et al. [17]).

In the ASD group, mothers reported higher burnout levels, while fathers reported higher depressive symptoms. Clinically significant depression levels based on BDI among mothers of children with ASD were associated with children's attendance in special services, children's mutism, and lower depressive symptoms among spouses. On the other hand, clinically significant depression levels in BDI among fathers were associated with moderate-severe ASD symptoms, children's attendance in special education services, mothers' clinically significant depression symptoms, and children's receiving psychopharmacological treatment." (Kütük et al., 2021).

During my research, I have also found that COVID-19 has impacted parents and caregivers of children with ASD. "Our study confirmed that COVID-19 restrictions resulted in an increase in the care burden for many caregivers. This finding is consistent with the explanation that improvement in maladaptive behavior behaviors may come at the expense of more care burden. Our finding corroborates that behavior either remained the same or improved in most autistic individuals whose caregivers reported an increased care burden. A point worthy of mention here is that more than half (58.6 %) caregivers reported no change or reduction in their care burden. All these caregivers looked after autistic individuals whose behavior remained the same or improved except one whose behavior worsened. This does contrast with our previous impression that COVID-19 restrictions resulted in reduced challenging behavior in autistic individuals at the expense of an increase in the level of care burden in their caregivers." (Khan et al.,2021)

Next, I have found researchers who identified caregiver strain as "disruptive behavior for objective strain, autism severity and disruptive behavior for subjective internalized strain, and oppositional behavior and hyperactivity for subjective externalized strain. Individualized interventions that attend to specific elements of parental strain may reduce strain and improve family well-being. Results from this study suggest that distinct types of caregiver strain are predicted by unique behavioral characteristics of children with ASD." (Bradshaw et al., 2021)

Aside from the mental toll it takes on a parent and caregivers to raise an autistic child, it can also take a physical toll on parents and caregivers. Although not statistically significant, it is noteworthy that fewer caregivers of children with ASD were considered overweight or obese (52%) compared with caregivers of TDC (67%) or national prevalence rates of overweight and obesity among US adults (70.7%) (National Center for Health Statistics, 2016). In univariate analyses, ASD caregivers consumed significantly fewer empty calories from solid fats and alcohol. They added sugars than TDC caregivers ($p = .03$). However, they did not differ significantly in any other dietary outcomes including nutrient adequacy ($p \geq .10$) and mean total HEI scores ($p = .20$). Caregivers of children with severe ASD scored significantly lower on the physical functioning, role-physical, social functioning, role-emotional, mental health subscales as well as the physical and mental component summary scores compared to caregivers of typically developing children.

Caregivers of children with moderate ASD, on the other hand, only significantly differed from caregivers of typically developing children in the mental health subscale (JSPN, 2018).

We have talked about the mental, physical, and emotional effects of having an autistic child on parents and caregivers. Now, we can talk about ways to help parents manage stress, anxiety, depression, and any other possible effects of this. Parents and caregivers have more resources than they know to help them with their children, but also to help them not forget about themselves and take care of themselves mentally, physically, and emotionally. Parents need to know how to access resources, seek out support, and promote positive child development, especially after receiving an ASD diagnosis (Hinman [24]). Using assessment tools to measure parent stress (general stress and stress related to the disability of the child), parent self-efficacy, and parent knowledge of ASD can help interventionists individualize family resources and supports (Shamash, E. R., & Hinman, J. A., 2022).

The main finding from this study was that a relatively brief parent-focused intervention for parents with a child newly diagnosed with ASD effectively reduced child-related parenting stress. This intervention was also more effective in increasing self-efficacy when parents were scoring low in self-efficacy prior to intervention. Parents receiving similar information via a self-directed DVD and activity sheet package were not similarly advantaged. High child-related stress scores indicated that children with ASD displayed qualities that parents found challenging and unacceptable, such as distractibility, negativity, and demanding behaviors (Abidin, 1995) (Keen et al.,2010).

Conclusion

In conclusion, the findings from this study shed light on the profound challenges faced by primary caregivers of nonverbal autistic children, highlighting the significant impact on their well-being and mental health. The utilization of established assessment tools, such as the Caregiver Strain Questionnaire (CGSQ), Beck Depression Inventory (BDI), and Zarit Burden Interview Assessing Caregiver Burden, revealed alarming levels of strain, depression, and caregiver burden experienced by these mothers.

Although a questionnaire was created to explore the potential mitigating effects of factors like community, communication, financial support, and self-care, the responses from participants revealed a different narrative. Even when caregivers possessed favorable circumstances in these individual factors, they still reported elevated levels of caregiver strain, depression, and burden. This suggests that these external factors alone may not significantly alleviate the substantial stressors associated with being a primary caregiver to nonverbal autistic children.

It is essential to underscore the significance of a notable aspect observed during this study—the limited sample size of participants. This observation sheds light on a critical aspect of the lives of primary caregivers of nonverbal autistic children. The fact that only four responses were received, despite reaching out to hundreds of thousands of potential participants through social media, serves as a poignant testament to these caregivers' immense demands and time constraints.

This finding provides a compelling insight into the challenging reality of these caregivers' lives. The scarcity of responses suggests that the primary caregivers of nonverbal autistic children are overwhelmingly occupied with the intensive demands of tending to their children's needs and managing familial responsibilities. The notable lack of time available to participate in a 30–45-minute survey underscores the pressing urgency of addressing the unique challenges faced by these caregivers.

This aspect of the study further strengthens the hypothesis that these caregivers have limited time for activities beyond their caregiving role. It also accentuates the need for tailored support systems that consider the time constraints and overwhelming responsibilities faced by primary caregivers of nonverbal autistic children. It highlights the necessity of recognizing their limited availability and the importance of developing interventions and resources that are both accessible and accommodating to their demanding schedules. In light of these findings, it is imperative to recognize the urgent need for tailored support systems, interventions, and resources that address the unique challenges faced by primary caregivers of nonverbal autistic children. This study

underscores the importance of holistic approaches that encompass the child's needs and the well-being and mental health of the caregivers who play a critical role in their lives.

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Comparative Analysis of Berlin Heart EXCOR Pediatric VAD and Heart Transplantation

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Abstract

Congenital heart defects (CHD) encompass a wide range of structural heart abnormalities, most commonly dilated cardiomyopathy, atrial septal defects, and cardiomyositis. These heart conditions manifest at birth and significantly contribute to morbidity and mortality in the pediatric population, whose ultimate goal is heart transplantation. The treatment options are also more limited for these pediatric patients, making timing more critical compared to adults. The development of ventricular assist devices (VAD) has been one of the key therapeutic advances in the treatment of severe heart failure in patients with CHD, either as a bridge to heart transplantation or as destination therapy. The Berlin Heart EXCOR Pediatric VAD, in particular, provides mechanical circulatory support tailored to the anatomical and physiological needs of children. Despite its success in aiding children with CHDs, current supply chain issues, increased demand, and regulatory hurdles have led to a shortage, affecting the timely treatment of pediatric patients with severe heart failure. This review explores the indications for VAD implantation, the associated risks, and the outcomes for children with CHDs requiring VAD support. Furthermore, it highlights the importance of comprehensive care, integrating advanced technological solutions like VADs with robust support systems for patients and their families. The overall objective is to raise awareness among families, policymakers, and stakeholders about the complex issues surrounding CHDs and the vital role of VADs in enhancing survival and quality of life for affected children.

Introduction

Cardiovascular diseases (CVD) stand amongst the nation's leading causes for medical bankruptcy and death toll. One of the most prevalent, congenital heart diseases (CHD) are a group of heart-related complications which are costly and highly inaccessible, not to mention the low rate of patients undergoing full recovery even after receiving treatments and surgeries. CHD are separated into two general subgroups: pediatric and adult CHDs. Pediatric congenital heart diseases are known to have significantly higher mortality rates than its adult counterpart. Patients who could surpass the childhood difficulty of suffering from CHDs continue their life living with higher risk of developing more severe cardiovascular diseases. For many patients, the ultimate treatment goal is to undergo a heart transplant. There has yet to be sufficient social welfare supporting families and patients suffering from these heart diseases.

In regards to the international landscape, there is major disparity. Advances in surgical techniques and medical care may have significantly improved survival rates in high-income countries (HICs). However, in low- and middle-income countries (LMICs), access to life-saving interventions is limited, resulting in high mortality and morbidity rates. Up to 90% of children born with CHD in these regions do not have access to the necessary cardiac care. This disparity highlights a critical global health issue that needs to be addressed through better resource allocation and international support(World Heart Federation, 2023)

Challenges to Understanding Cardiovascular Diseases

The first challenge to treating cardiovascular diseases relates to understanding the different types that heart diseases encompass. In his review paper, "Cardiovascular Disease", Lopez et al. (2023) analyzed and evaluated the various array of heart diseases and their treatments in the current medical setting, placing an emphasis on the specific etiology of each disease. These included cerebrovascular disease, peripheral artery disease, aortic atherosclerosis, coronary artery heart disease, and many more. Lopez claimed the utmost and most important factor to improve treatments was by executing early detection of these diseases. Since some cardiovascular diseases can show up as asymptomatic in clinical presentation, coupling with high cost of treatment, patients are less likely to detect and seek professional help before any complications show up.

The second challenge lies in the high cost and accessibility nature of medicine. Berry and colleagues provided a review of the current economic burden of heart failure across different regions of the world. Berry argued against the rapid inflation of costs of treatments for chronic heart failure specifically and CVD in general, stating that healthcare expenditures are spent

largely on hospitalization and rarely truly effective treatment. His data was collected from surveys and other research based on both state-funded (e.g. the UK) and private (e.g. US) healthcare systems, not excluding at-home treatment and nursing homes. This large sample size provides statistically significant results and therefore are reliable. Berry's report was useful due to the fact that he included costs for specific treatments and their limitations in order to reduce any bias.

The Current Landscape of the Heart Transplantation System

Matthew Movsesian, a cardiologist, claimed that the medical organ donation & transplantation system prioritized patients based on the disease' severity and the level of aggressive treatment they received (Movsesian, 2016) due to a higher number of donors with higher age groups, which created a big disparity and mismatch between donor and recipient and ultimately making transplantation match more scarce (Donné M., De Pauw M., 2021) . This created a dilemma where physicians are "encouraged" to unethically advise treatments against the best interest for the patient's welfare, but rather for the sake of becoming eligible for a transplant. There was evidence showing physicians were forced to "game the system" and how these incentives presented harm to their own patients, if not every other patient- also known as the zero-sum nature of the transplant list. However, according to the article, the governing head for this transplant system (the United Network for Organ Sharing (UNOS for short) was considering a new system for allocating donor hearts, involving more priority classifications, potentially using even more aggressive measures than the current ones. Although this might resolve the pressure to overtreatment of patients, Movsesian questions yet again its probability of succeeding. From this insight, it is clear that there needs to be a better system designed to accommodate and help patients without normalizing dishonesty and unethical medical practice. Most importantly, resolution lies within the capitalism of the heart transplantation system in addition to unfair resource distribution.

In conjunction with Movsesian's argument, Stevenson et. al (2016) argued that the most effective resolution to reduce the overtreatments and overwhelmingly abused system of heart transplant was to limit the number of patients who were listed to more closely approximate the number of hearts likely to become available. They also urged the United Network for Organ Sharing (UNOS) to incentivize a new consideration system, in which only patients with intra-aortic balloon pump (IABP) support through the subclavian artery to allow ambulation during long waiting times in hospital can be eligible for immediate heart transplant.

The following parts of this review will give a general overview of how pediatric VADs are indicated and used currently, and what the future may hold.

Indications for VADs implantation

In babies with a type of CHD known as Dilated Cardiomyopathy (DCM), the risk of developing heart failure is progressively related to a low survival rate. Characteristics of DCM include enlargement and dilation of one or both of the ventricles along with impaired contractility (Mahmaljy et al., 2023). This enlargement could lead to the weakening of the pumping system, causing decreased cardiac output and severe heart failure symptoms. For this reason, DCM is one of the most common reasons for severe heart failure in children and adults. It is a primary indication for the use of a ventricular assisting device (VAD).

Another common complication needing the assistance of a VAD are atrial septal defects (ASD). This phenomenon occurs when there are faulty valves indicating the presence of gaps or orifice, leading to miscommunication between the chambers as the blood flow becomes abnormal (Geva et al., 2014). In most cases, the blood flows penetrate the incorrect heart chamber when it has not been correctly oxygenated, causing the heart to pump more frequently than the normal rate. ASD are one of the most common congenital heart defects, but they do not usually lead to severe heart failure by themselves. However, in the context of complex congenital heart disease or other underlying cardiac conditions, ASDs can contribute to heart failure.

Recently, there has been an upward trend seen in early detection of cardiovascular diseases, especially in children. Due to the ever-growing advancement of medical equipment, more and more institutes are establishing specialized centers advocating for early disease detection. Accompanying congenital heart defects are also, oftentimes, genetic compilations. Early detection and appropriate management are crucial for optimizing clinical outcomes in affected children.

Ventricular Assist Device

The Berlin Heart EXCOR® Pediatric Ventricular Assist Device (VAD) represents a significant advancement in the field of pediatric mechanical circulatory support, particularly for managing complex cases of congenital heart diseases (CHDs) and severe heart failure. This device functions as a temporary mechanical substitute for the heart’s pumping action, utilizing an external pneumatic pump connected to the heart through cannulas surgically implanted into the left or right ventricle and the aorta. The EXCOR® VAD supports the circulation by actively

pumping blood from the ventricles to the aorta, ensuring continuous perfusion of vital organs and stabilization of hemodynamics in critically ill patients.

One of the key advantages of the Berlin Heart device is its ability to provide long-term support in a wide range of pediatric patients, from neonates to adolescents. This feature makes it a more versatile option compared to heart transplantations that require identical sizings. The system allows tailored adjustments in pump settings to meet the specific circulatory needs of individual patients, including those with complex cardiac anatomies such as single-ventricle physiology. This adaptability makes it particularly valuable as a bridge to heart transplantation, offering a crucial window for patients who might otherwise not survive the wait for a donor heart.

Moreover, the Berlin Heart EXCOR® can also serve as a bridge to recovery in cases where cardiac function is expected to improve, offering temporary support that allows the heart to rest and remodel. Clinical outcomes have demonstrated that the device significantly improves survival rates and quality of life for pediatric patients awaiting transplantation. The ongoing development and refinement of the EXCOR® system reflect the broader trend in pediatric cardiology towards using mechanical support to extend the lives of children with severe heart disease, highlighting its critical role in contemporary pediatric cardiac care.

Comparative review of VADs and Heart transplantation

The data used in this review were collated from two sources: Jaquiss et al., 2015 and the Mayo Clinic, 2024. The Jaquiss et al. report utilized a prospective, multicenter, single-arm methodology, whereby all participants underwent the same intervention without any contrasting treatment or placebo group for comparison. Such an approach is particularly valuable when it is neither feasible nor ethical to include a control group. This study focused on patients aged 16 years or younger who were undergoing heart transplantations (n=48) or were fitted with the Berlin VAD (n=247). Clinical data, spanning from February 1990 to August 2015, were meticulously gathered and analyzed with FDA approval. Additionally, the Mayo Clinic's report enriched this analysis by providing extended survival rates at five, ten, and twenty years post-intervention.

Findings

The comparative analysis of the Berlin Heart EXCOR® Pediatric Ventricular Assist Device (VAD) and heart transplantation provides key insights into managing pediatric end-stage heart failure. It evaluates survival rates and complications for both treatments. The Berlin Heart

EXCOR® VAD serves as a bridge-to-transplant for children, offering temporary support. However, heart transplantation is the definitive treatment for long-term survival and quality of life improvement. According to the data below, the 1-year survival rate was 89.6% for patients undergoing heart transplants (Fig.1) , which was noticeably higher than patients undergoing the treatment using the Berlin VAD (76.9%) (Fig. 2) . This analysis highlights the need for personalized treatment strategies and careful decision-making in pediatric cardiac care. Further research and longitudinal studies are essential to refine protocols and enhance outcomes for this patient population.

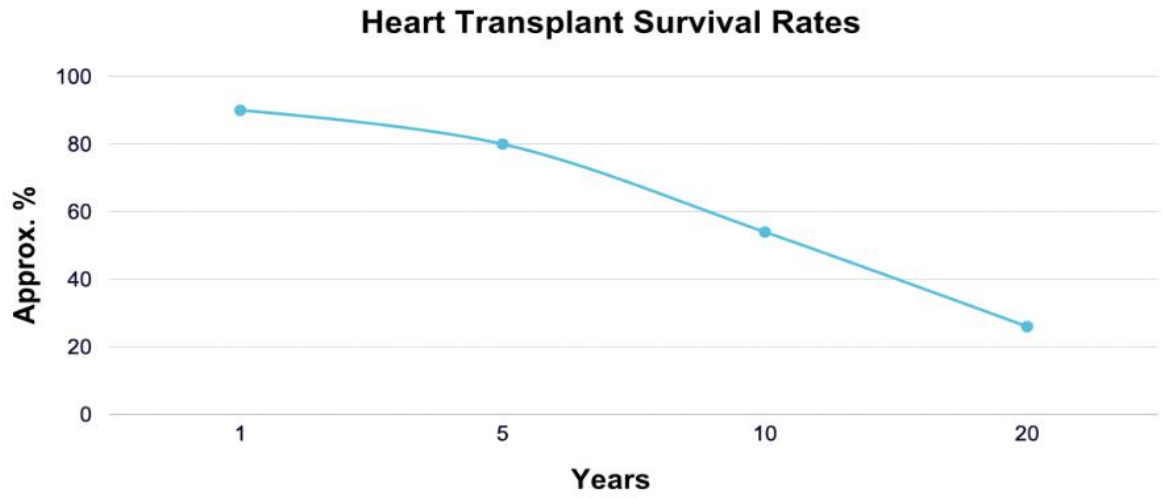

Figure 1. Heart Transplant Survival Rates over 20 years for pediatric patients (n=48). The 1-year survival rate was 89.6%.

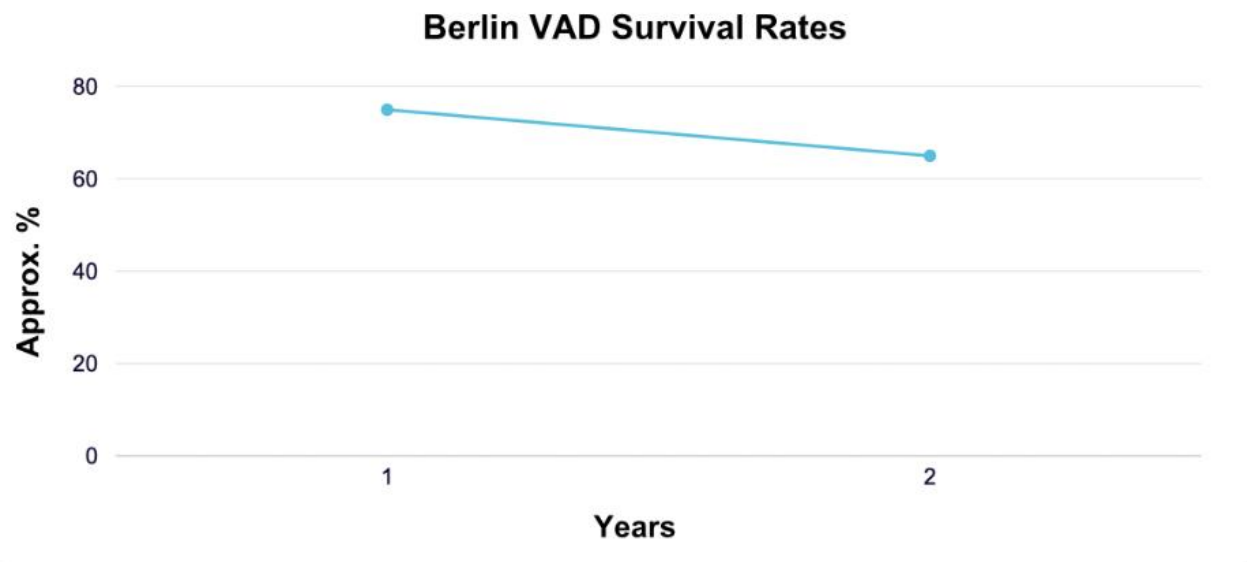

Figure 2. Berlin VAD survival rate over 2 years for pediatric patients(n=48). Data on Berlin VAD survival rates is more limited due to the recent popularity of this treatment option. 1-year survival rate was 76.9%.

Discussion

Intra-aortic balloon pumps (IABP) and other transcatheter aortic valve implantation (TAVI) procedures, such as those discussed by Brandt and Pibarot (2021), are emerging as advanced treatment options for patients with severe cardiovascular diseases, including aortic stenosis. These minimally invasive interventions have demonstrated improved patient outcomes, particularly in high-risk individuals who may not be suitable candidates for open-heart surgery. However, despite their clinical benefits, the financial burden associated with these procedures is substantial, often placing them out of reach for many families. The high costs of TAVI devices, coupled with hospital charges, specialist fees, and post-procedural care, can lead to significant financial strain. As a result, families who pursue these treatments frequently face overwhelming medical bills, which can push them into severe financial distress or even bankruptcy.

The Berlin Heart EXCOR® Pediatric Ventricular Assist Device (VAD) represents a groundbreaking advancement in pediatric cardiac care, poised to transform the treatment landscape for children with severe heart failure and congenital heart diseases (CHDs) for years to come. Despite its powerful impact, current data still underscores that heart transplantation remains the definitive treatment for many patients with CHDs, particularly those with advanced heart failure or complex cardiac anomalies. The Berlin Heart EXCOR® serves as a vital interim

solution, supporting patients who are either awaiting a suitable donor organ or whose condition necessitates immediate circulatory support. However, while it significantly improves survival rates and quality of life during the critical waiting period, it does not provide a permanent solution to heart failure. Long-term outcomes still hinge on successful transplantation, which remains the gold standard in achieving durable results and improved life expectancy for these patients.

Moreover, while the Berlin Heart EXCOR® has reduced mortality rates and eased the strain on the heart transplantation system, challenges such as device-related complications, including thromboembolic events, infections, and the need for careful anticoagulation management, highlight the ongoing need for heart transplantation as the ultimate curative approach. Continued advancements in VAD technology, coupled with improvements in post-implantation care, may further optimize outcomes, but the pursuit of transplantation remains essential for achieving optimal, long-term cardiac function in pediatric patients. This highlights the complementary roles of VADs and transplantation, with each playing a critical part in the broader continuum of care for children with life-threatening heart conditions.

In addition, the financial implications of opting for high-cost cardiovascular treatments are compounded by the complexities of the healthcare system. Weber (2022) highlights this issue through the narratives of parents who have lost children to heart disease, exposing a healthcare system fraught with billing inaccuracies and insurance practices that often leave families bewildered and unprotected. Weber's storytelling approach sheds light on how systemic issues, including hidden costs, denial of coverage, and convoluted billing practices, create additional emotional and financial burdens for patients and their families. These challenges are indicative of broader systemic problems that disproportionately impact those undergoing advanced medical interventions.

The stories presented by Weber and others reveal the stark reality of navigating a healthcare system that, while capable of providing life-saving treatments, also presents significant financial barriers. The need for greater transparency in medical billing, improved insurance coverage policies, and a more patient-centered approach to healthcare financing is underscored by these accounts, calling for reforms that can make advanced cardiovascular treatments more accessible and affordable.

Conclusion

Congenital heart defects (CHDs) pose a significant challenge in pediatric healthcare, contributing to high morbidity and mortality rates. Not only complex and severe, these

conditions are also limited with the treatment options available for children. This dilemma calls for more innovative therapeutic approaches. Ventricular assist devices (VADs), such as the Berlin Heart EXCOR® Pediatric VAD, represent a critical advancement, providing life-saving mechanical circulatory support as either a bridge to heart transplantation or as destination therapy. While these devices have significantly improved survival rates and quality of life for pediatric patients, they are not without limitations, including device-related complications and the ongoing need for careful management.

This review highlights the importance of integrating VADs into comprehensive care strategies for children with CHDs. However, the current reliance on VADs underscores broader systemic challenges, including supply chain issues, regulatory barriers, and the financial burdens placed on families. Additionally, the disparities in access to advanced cardiac care between high-income and low- and middle-income countries further complicate the global landscape of CHD management, emphasizing the urgent need for equitable resource allocation and policy reform.

Despite the promising role of VADs, heart transplantation remains the definitive solution for many children with advanced heart failure, offering the best long-term outcomes. VADs serve as complementary interim solutions that have alleviated some pressure on the transplantation system. Nevertheless, it highlights the need for ongoing technological improvements, better patient selection criteria, and enhanced support for families navigating the complexities of care.

Ultimately, addressing the challenges associated with CHDs requires a multidisciplinary approach that combines technological innovation, patient-centered care, and policy advocacy. By raising awareness among healthcare providers, policymakers, and stakeholders, and by improving access to both VADs and transplantation, we can strive to enhance survival rates and quality of life for children affected by these devastating heart conditions. Continued research, investment in medical advancements, and systemic reforms are essential to closing the gaps in care and ensuring that all children with CHDs have the best possible chance at a healthy life.

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Identification of the Membrane Uptake Domain of Antibacterial Microcin EN112

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Abstract

New antibacterial drugs are urgently required to treat drug-resistant bacterial infections. The development of treatments for Gram-negative bacteria is particularly challenging due to the need to breach the impermeable outer membrane, which restricts the diffusion of most molecules larger than 600 Da. Class IIa microcins, an underexplored class of bacteriocins, present a promising approach to addressing this problem by utilizing outer membrane proteins to cross the membrane. Understanding the mechanism by which microcins traverse the outer membrane could be useful in developing antibiotics targeting Gram-negative bacteria. Here, we located the uptake domain of Enterobacteriaceae 112 (EN112), a class II microcin that targets the OmpF outer membrane receptor for uptake and is active against a diverse range of bacterial strains. Additionally, we determined how OmpF L3 loop affects EN112's translocation and whether the microcin requires energy for its activity.

Keywords: microcin; bacteriocin; Gram-negative; OmpF; outer membrane; membrane transport

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Introduction

Antibiotics are considered one of the cornerstones of modern medicine. Their discovery and distribution have significantly reduced the morbidity and mortality of bacterial infections, leading to improved health and longer lifespans. (Rossolini et al., 2014) However, the efficacy of antibiotic is on a steady decline due to the emergence of antibiotic resistance bacteria. The lack of novel antibiotic discoveries in recent decades to outpace this emergence has turn this issue into a global health concern. (Aslam et al., 2018) The main challenge in undercovering new antibiotics is the presence of the outer membrane (OM) that prevent the drug from reaching its target within the cell. (Lehman & Grabowicz, 2019) This is especially a problem in Gram-negative bacteria which has an asymmetric lipid bilayer in the OM that resists the diffusion of most molecules >600 Da. (Horne et al., 2020) Therefore, identifying biomolecules that can bypass the OM by utilizing the bacteria's native transport system could be instrumental for novel antibiotic development. Class IIa microcins, an understudied class of bacteriocins, may offer valuable insights.

Class IIa microcins are small antibacterial proteins (<10 kDa, ~100 residues) produced by Gram-negative bacteria to kill other Gram-negative bacteria. (Parker & Davies, 2022) To reach their target and exert their cytotoxic effect, these proteins utilize outer membrane receptors, such as CirA and FhuA, to bypass the OM. (Parker et al., in review) This ability to 'trick' the cell for their uptake suggests that a specific section of their sequence is dedicated for that purpose. This has been the case for microcin V (MccV) which has an uptake domain specific for CirA. (Azpiroz & Laviña, 2007) The implication is that the uptake domain may serve as a valuable tool for novel antibiotic design by facilitating the bypass of the OM. However, MccV remains the only class IIa microcin with an identified uptake sequence. To fully explore the therapeutic potential of class IIa microcins' uptake domains, identification of more uptake domain is necessary for finding new outer membrane targets or increased target specificity (i.e. against a specific strain). To address this, the study will focus on identifying and characterizing the uptake domain of Enterobacteriaceae 112 (EN112), one of the 22 class IIa microcins discovered in our previous study. (Parker et al., in review)

EN112 exhibits broad antimicrobial activity against various bacterial strains, including *Escherichia coli, Shigella flexneri, Enterobacter hormaechei*, and *Citrobacter braakii.* (Parker et al., in review) Previous data indicate that EN112 utilizes the outer membrane receptor OmpF for its activity. The ability to target a wide range of pathogenic bacterial strains with varying OmpF compositions makes this microcin's uptake domain an especially interesting subject for analysis.

As a class IIa microcin, EN112 has a precursor form consisting of an N-terminal signal domain and a C-terminal core, which encompasses both the toxic and uptake domains. The signal sequence of EN112, comprising the first 18 residues from the N-terminal, can be quickly identified by the 'double-glycine' cleavage site motif. (Parker & Davies, 2022) The remaining 94 residues are the toxic and uptake domains, but no rule exists to precisely define the boundary between these two regions. Based on MccV's uptake domain, it is likely that the uptake sequence for EN112 is located at the C-terminal. (Azpiroz & Laviña, 2007) Using this assumption, here, we roughly identify the uptake domain of EN112. Furthermore, we tested EN112 against two characterized mutations at OmpF L3 loop (G119D and R132A) to understand its mechanism of translocation. Lastly, we analyzed whether EN112 activity required energy for its activity to assess its potential use against persistor bacteria.

Results

Identification of Microcin EN112 Uptake Domain Sequence

To define the boundary between EN112 toxic and uptake domain, a self-inhibition growth curve assay was used. This assay is based on two principles: (1) Microcin-secreting bacteria require the corresponding immunity protein(s) to survive and effectively secrete microcins, and (2) the uptake domain is located at the C-terminal of the core microcin. (Parker et al., in review; Azpiroz & Laviña, 2007) Using these two principles, *E. coli* susceptible to EN112 were transformed with pBAD18Km containing the construct to secrete EN112 directly into the periplasm instead of outside the cell **(Fig. 1A)**. This is possible by replacing the native secretion signal (the first 18 residues from the N-terminal) with TorA secretion signal sequence to use the Tat secretion system **(Fig. 1B)**. (Thomas et al., 2001) With this construct, called TorA-EN112, the *E. coli* would experience 'self-inhibition' due to the production and export of toxic proteins into its own periplasm. (Parker et al., in review)

Using TorA-EN112 as the template, various truncations starting from the C-terminal were made and transformed into *E. coli.* The purpose of these truncations is to determine at which truncation site the microcin loses its antibacterial activity because that would indicate that the truncation has removed not only the uptake domain, but also the toxic domain **(Fig. 1B)**. With the assumption that the toxic and uptake domain have clearly defined boundary like MccV, this boundary would be located between the two truncation sites: one where full inhibition occurs (like the template) and the other where

inhibition is decreased. The uptake domain will be identified once this boundary is uncovered.

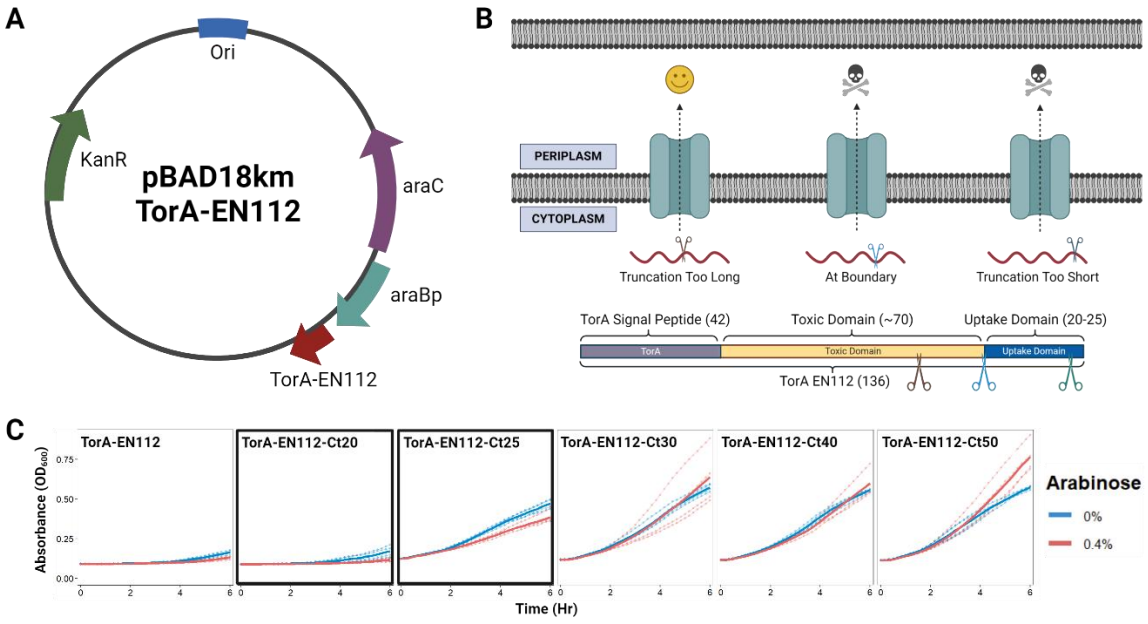

Figure 1. Identification of EN112 uptake domain. (a) Plasmid design for the expression and secretion of TorA-EN112. This is the template that the truncated TorA-EN112 is also based off on. (b) A visual representation of the self-inhibition growth curve assay. TorA-EN112 and its truncated variants are secreted into the periplasm to test for antibacterial activity. If the truncation involves the toxic domain, the antibacterial activity would be affected. (c) The growth curve plots of the different truncations tested in the assay. For example, Ct20 means 20 amino acids are truncated starting from the C-terminal and Ct30 is 30 amino acids. The arabinose legend indicates the level of arabinose added to induce the pBAD18km expressing TorA-EN112 and its truncated variants. For 0% induction, the equivalent volume of water was added instead. Bolded plots show that EN112 uptake domain is between these two truncations as seen in the change of antibacterial activity.

To determine the uptake domain, we tested TorA-EN112, TorA-EN112-Ct20, TorA-EN112-Ct25, TorA-EN112-Ct30, TorA-EN112-Ct40, and TorA-EN112-Ct50 **(Fig. 1C)**. Ct indicates how many residues were removed starting from the C-terminal. The plot of the growth curve showed that the expression of TorA-EN112 and TorA-EN112-Ct20 led to severe inhibition of *E. coli* across the 6 hours with the uninduced (0%)

pBAD18km being only slightly better off than when induced with 0.2% arabinose. The fact that uninduced E. coli has similar growth characteristics as induced ones indicates the strong activity of EN112 and sensitivity of this assay. There doesn't seem to be any sign of inhibition, induced or uninduced, for *E. coli* expressing TorA-EN112-Ct30, Ct40, and Ct50 as indicated by the similar OD_{600} values across the 6 hours. This showed that the toxic domain has been completely compromised by the truncation. Interestingly, the growth curve for TorA-EN112-Ct25 showed when the plasmid is induced, antibacterial activity is significantly impaired, but not fully as the average OD_{600} is slightly less than induced TorA-EN112-Ct30, Ct40, and Ct50. Based on the transition from full to decreased inhibition between TorA-EN112-Ct20 and Ct25, the boundary between toxic and uptake domain is between 20-25 amino acids from the C-terminal.

OmpF L3 Loop Is Critical for EN112 Translocation

In our previous study, it was shown that EN112 requires the OmpF porin for translocation through the OM to reach its antibacterial target. (Parker et al., in review) However, the exact mechanism by which EN112 interacts with OmpF to facilitate its uptake remains unclear. Understanding this process is crucial for exploring EN112 uptake domain's potential in novel antibiotic design. In particular, it's important to determine whether EN112 enters the periplasm through OmpF or anchor into OmpF to 'fish' for its target like colicins. (Budiardjo et al., 2022) For this purpose, EN112's activity will be tested against two characterized W3110 OmpF loop 3 (L3) mutants: G119D and R132A. (Jeanteur et al., 1994; Simonet et al., 2000)

OmpF L3 mutants are of particular interest because this loop forms the channel constriction region (CR) which affect what molecules can pass through the porin by size. (Acharya et al., 2023) In OmpF G119D mutants, the size of the lumen is constricted due to the subdivision of the pore into two sub-compartments that are 3-4 Å in diameter by the aspartate side chain **(Fig 2A)**. (Jeanteur et al., 1994) This mutation lead to a decreased sensitivity toward cephalosporins and colicin N. (Simonet et al., 2000) The other OmpF L3 mutant tested is OmpF R132A where the substitution for alanine result in a slight increase in pore opening. (Simonet et al., 2000) This substitution also eliminates the residue's interaction with Y102 which separates it from the positively charged cluster. OmpF R132A mutants have increased sensitivity toward cephalosporins and no change in colicin N activity. (Simonet et al., 2000)

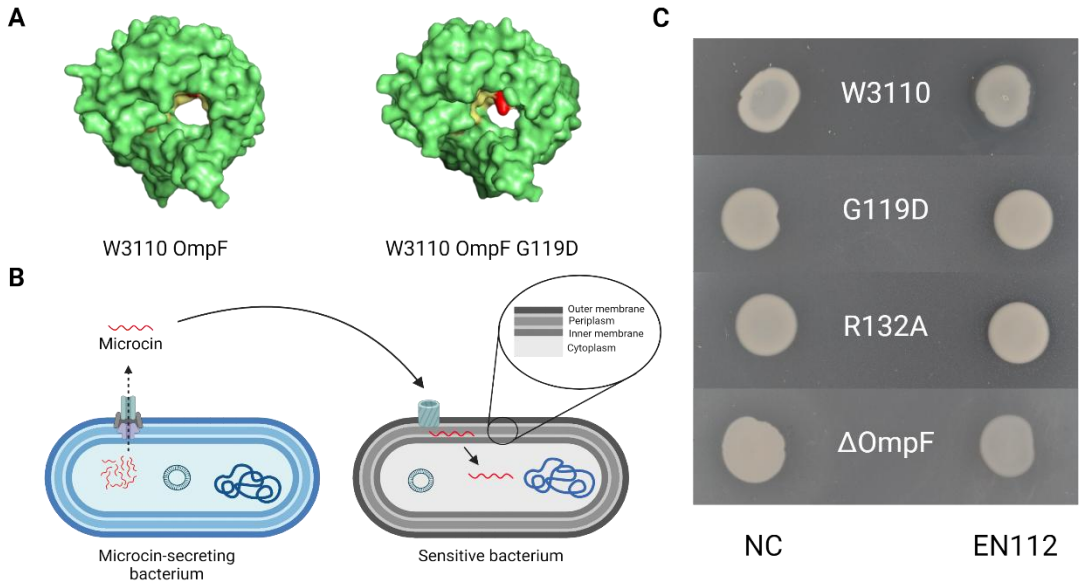

Figure 2. Effect of OmpF G119D and R132A mutations on EN112 translocation. (a) Crystal structures of *E. coli* W3110 OmpF wild type (WT) and OmpF G119D mutant generated in Pymol. (Protein Data Bank (PDB) code: 2OMF & 1MFP, respectively) R132A structure isn't included as a PDB file isn't available. (b) A visual representation of the ZOI (Zone of Inhibition) assay used to test EN112 against OmpF mutants. In this assay, the secreting bacteria export microcins (EN112) out of the cell and into indicator strains that express either the wild-type (WT) OmpF or OmpF mutants. If the EN112 activity is not obstructed by the OmpF mutants, a clear halo will appear around the indicator strain, indicating inhibition of the indicator. (c) ZOI result of EN112 against BW25113 ΔOmpF containing pBADamp to express only the W3110 WT OmpF, OmpF G119D, or OmpF R132A. BW25113 ΔOmpF that expresses no OmpF serves as the negative control.

To understand EN112's mechanism of translocation through OmpF, a zone of inhibition (ZOI) assay was used to determine if its activity is affected by these mutants in the same ways as antibiotics or colicins, or if it exhibits unique behavior **(Fig. 2B)**. In this assay, the indicator bacterial strain (BW25113 ΔOmpF) transformed with pBADamp expressing the specified OmpF variants (WT, G119D, or R132) is grown as a lawn on the plate. The secretor strain which produces and export EN112, is then spotted onto the lawn. A halo around the spot indicates inhibition by EN112.

The assay revealed that OmpF G119D and R132A mutants acquired significant resistance toward EN112 compared to the WT as seen in the reduced ZOI **(Fig. 2C).**

Since previous studies have shown that the OmpF variants G119D and R132A are still functional, the assay results suggest that EN112 cannot enter into the periplasm through OmpF as effectively as it did with the WT OmpF. (Jeanteur et al., 1994; Simonet et al., 2000) These results indicate that EN112's translocation through OmpF may differ from that of antibiotics and colicins. Specifically, while the pore opening size is crucial for the uptake of cephalosporins, colicin N, and EN112, EN112 may require more specific interactions with OmpF for its translocation as an enlarged pore (OmpF R132A) may interfere with this process. Interestingly, OmpF G119D mutants have a higher resistance toward EN112 than OmpF R119D mutants **(Fig. 2C)**. This suggests that the size of porin opening is more important for EN112 translocation than receptor interaction.

Determining if EN112 Activity is Energy-Dependent

To determine whether EN112 require energy for its activity, an energy assay was conducted using carbonyl cyanide 3-chlorophenylhydrazone (CCCP) as a protonmotive dissipator. (Kwan et al., 2013) In this assay, BW25113 + pBADamp *E. coli* are treated with CCCP or left untreated for 3 hours before being co-cultured with a secretor strain transformed with pBAD18km, which either secretes EN112, MccV, or carries an empty vector. MccV will serve as a model for energy-dependent microcins as previous study have found that ΔTonB mutants are resistant to MccV. (Chehade & Braun, 1988; Parker et al., in review)

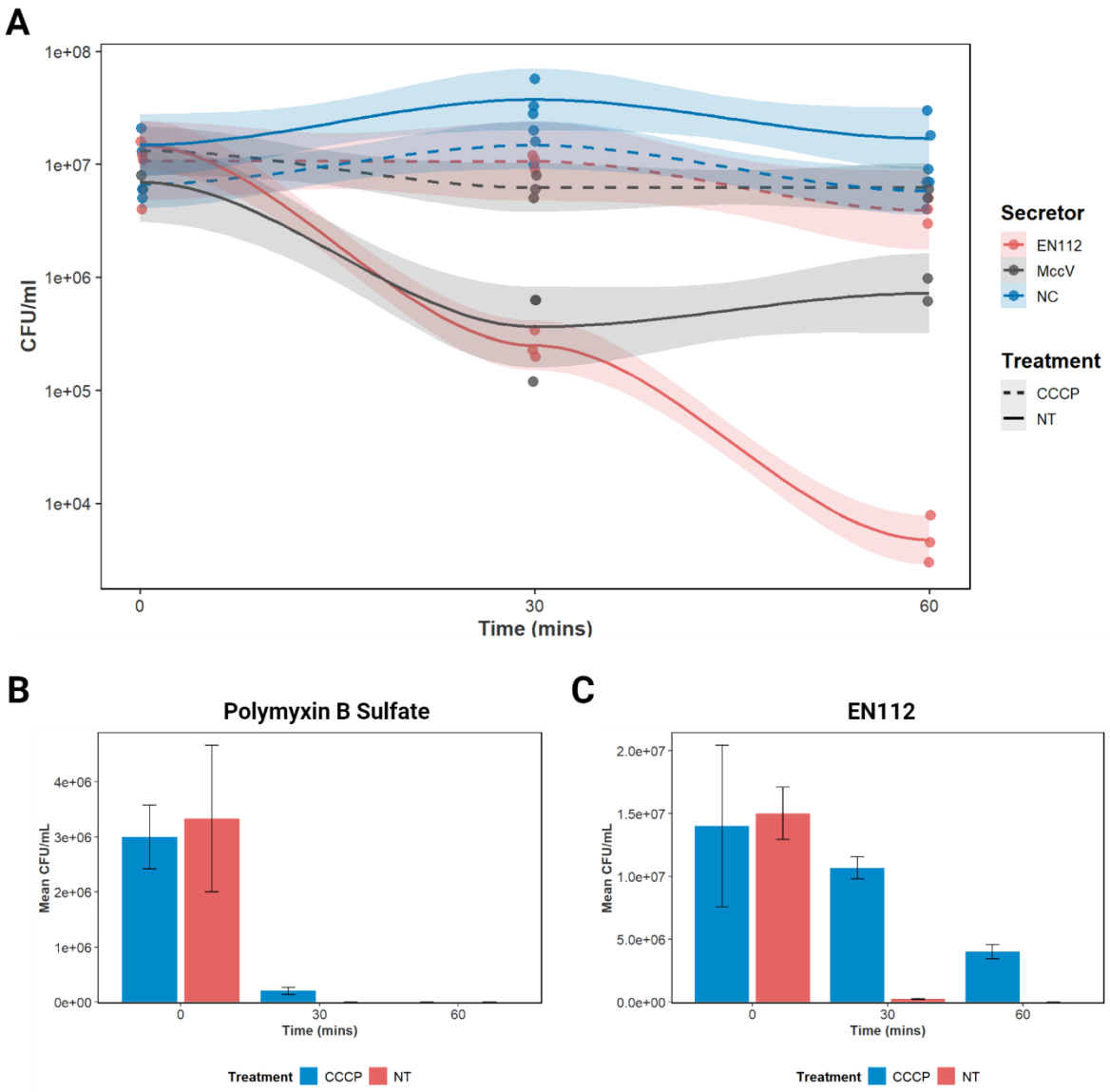

Figure 3. Determining whether EN112 activity is energy-dependent. (a) Energy assay results across 1 hour for CCCP-treated and untreated (NT) BW25113 pBADamp against secretors transformed with pBAD18km to produce EN112, MccV, or no microcin (NC). Prior to co-culture, BW25113 cells are treated with CCCP or left untreated for 3 hours, then adjusted to an OD_{600} of 0.03. The secretors were set to OD_{600} of 0.4 and was induced with arabinose at 0.2% for 2 hours before co-culture. CFU is colony forming unit. (b) Energy assay result across 1 hours for CCCP-treated and no treatment (NT) BW25113 pBADamp against polymyxin B sulfate at 10 µg/mL. (c) Data of EN112 from part a is represented in a grouped barplot.

The energy assay revealed that like MccV, CCCP-treated bacteria that were co-culture with EN112-secreting cell didn't experience a significant decrease in number of colonies over a period of 30 and 60 minutes **(Fig. 3A)**. Bacteria that were not treated with CCCP did experienced a significant decrease in number of colonies from 10^7 to 10^3 mean CFU/mL in the same time period for EN112 **(Fig. 3A)**. Therefore, the lack of decline in CFU/mL by EN112 on CCCP-treated BW25113 suggests that for EN112 to inhibit its target, energy is needed.

To confirm that the ineffectiveness of EN112 on CCCP-treated BW25113 wasn't due to an unintended effect of CCCP, the same energy assay was performed using polymyxin B sulfate at 10 μg/mL. This antibiotic doesn't require cellular energy to inhibit cell growth, therefore, CCCP-treated BW25113 *E. coli* should experience severe inhibition. This behavior is confirmed in **Fig. 3B**, where the presence of polymyxin B sulfate results in dramatic decrease in CFU/mL for CCCP-treated and untreated BW25113. Conversely, CCCP-treated BW25113 didn't experience significant inhibition at 30 minutes for EN112 **(Fig. 3C)**. The decrease in mean CFU/mL at 60 minutes by EN112 can be attributed to BW25113 recovering from CCCP, which subsequently makes them sensitive to EN112 again. (Kwan et al., 2013) Overall, the energy assay showed that EN112 toxic and/or uptake required energy.

Discussion

As bacteria develop resistance to existing drugs, the need for new antibiotics becomes increasingly urgent. That said, a major challenge in finding new antibiotics is the need to overcome the protective OM of bacteria to reach its antibacterial target. A potential solution may be found in the uptake domain of class IIa microcins which may serve as a valuable tool in novel antibiotic design by facilitating the bypass of the OM. However, the only identified uptake domain for class IIa microcin is MccV. Here, we roughly identified the uptake domain of EN112 to be around 20-25 residues from the C-terminus. Future experiments will be to test TorA-EN112-Ct21, Ct22, Ct-23, and Ct-24 to pinpoint the boundary between toxic and uptake domains for EN112. In doing so, the minimal uptake domain may be identified. An interesting note is that the uptake domain of MccV is 32 residues long which is not that far off from EN112. (Azpiroz & Laviña, 2007) This may suggest that other class IIa microcins' uptake domains may be in the 20-30+ residues range regardless of what outer membrane receptor it targets.

Through ZOI assays, we investigated how OmpF L3 mutants (G119D and R132A) affect EN112 activity to gain insights into its translocation mechanism. The decreased inhibition by EN112 on these mutants, which is unlike colicin N and cephalosporins, suggests that EN112 may interact with OmpF differently. Previous

studies indicate that the G119D mutation disrupts colicin N binding, a process that can be bypassed under low-salt conditions when OmpF act purely as a translocator. (Fourel et al., 1993) This led to two possible explanations for EN112 resistance for OmpF G119D mutants: (1) EN112 may require specific interactions with L3 for effective translocation, similar to colicin N; or (2) EN112 experience steric hindrance due to constriction of the lumen which impede EN112 passage. The fact that OmpF R132A mutant shows resistance to EN112 but not to colicin N implied that their binding or translocation pathway differs which would weaken the former argument. To further understand EN112's mechanism of translocation, future study involving translocation tracking is necessary. A possible experiment would be the split-luciferase assay **(Fig. 4)**. (Wagstaff et al., 2020) This assay can help answer whether EN112 fully translocate into the periplasm to reach its target or relies on specific binding interactions at OmpF L3 like colicin N. Knowing how EN112 translocate is important for using its uptake domain for novel antibiotic development.

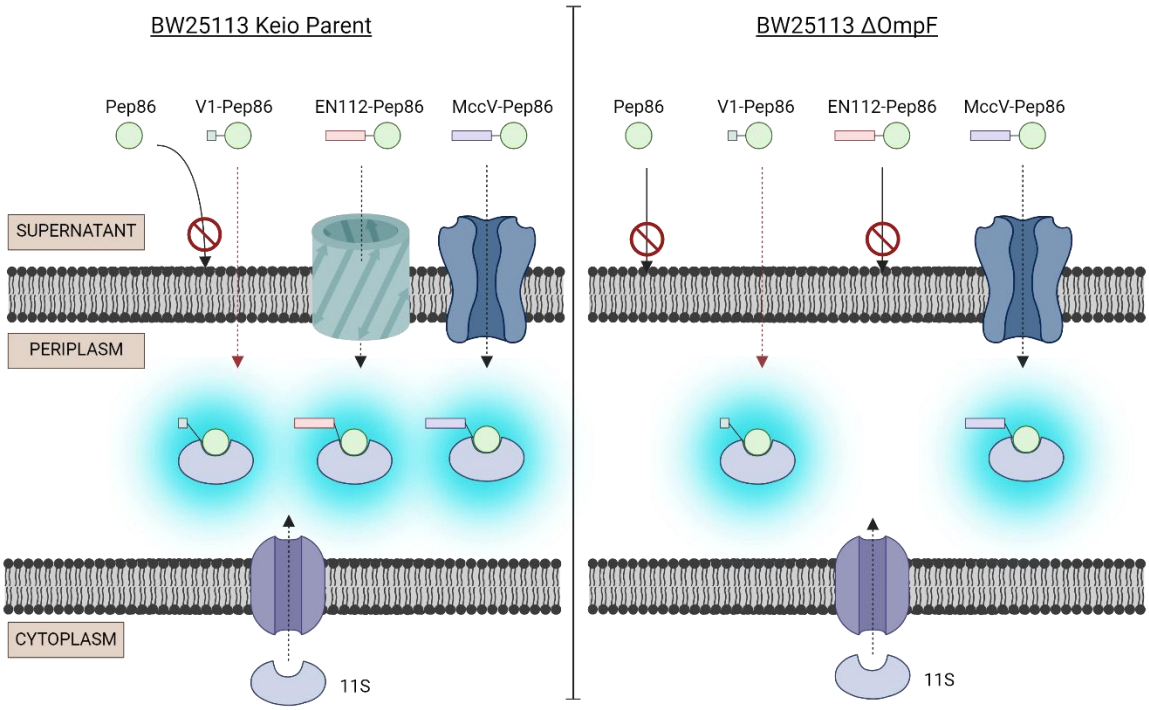

Figure 4. A proposed split-luciferase assay. This assay is based on the principle that luciferase can be split into 11S and pep86 temporarily. To determine whether EN112 fully translocate into periplasm, the uptake domain of EN112 will be fused to pep86. This peptide will then be added to BW25113 *E. coli* that secretes 11S into periplasm.

Luminescence would indicate that EN112 uptake alone is sufficient for import into periplasm. As negative control, there should be no luminescence if the same experiment is performed on 11S-secreting BW25113 ΔOmpF.

Finally, we investigated whether EN112 activity requires energy. The energy assay results indicate that energy is necessary for EN112 to eliminate its target. However, it remains unclear whether this energy is needed for the uptake of EN112, the function of its toxic domain, or both. For the purpose of using EN112 uptake domain as a tool for novel antibiotic designs, it is crucial to determine whether ATP is required for its uptake. If ATP is not necessary for translocation, this would suggest that the EN112 uptake domain could target persistor bacteria, which are difficult to treat. A possible experiment to test the energy-dependency of EN112 uptake domain would be to fuse the uptake domain to a fluorescent tag and perform flow cytometry.

Materials and Methods

Self-Inhibition Growth Curves

TorA-EN112 construct was created by replacing the native signal sequence of EN112 with TorA signal sequence. Truncations are made starting from the C-terminal. The constructs are cloned into pBADKm through Azenta and transform using *E. coli* DH5α. Once transformed, two colonies were selected and grown in LB and kanamycin overnight. To screen for self-inhibition, cells were seeded (OD600 = 0.05) into M9 minimal medium, loaded (200 µL/well) into a 96-well plate, and treated with 0.4% arabinose to induce microcin expression (induced) or water (uninduced). For each truncation constructs, replicates were performed for each colony and treatment (n = 2 colonies x 2 replicates x 2 treatments = 8 wells per truncation construct). The 96-well plate were incubated at 37C with shaking at 800 rpm using BioTek LogPhase 600 Microbiology Reader (Agilent) and OD600 was measured every 10 min for 6 hrs.

Site-Directed Mutagenesis

To create pBADamp OmpF G119D and R132A, the W3110 WT OmpF gene was cloned into pBADamp using primers designed for the OmpF gene in *E. coli* W3110. After verifying the insert sequence and confirming functionality of WT OmpF through a zone of inhibition (ZOI) assay, the Agilent QuikChange II kit was used to introduce single amino acid substitutions for G119D and R132A. The primer designs, PCR process, and transformation procedures specified in the kit were followed.

Zone of Inhibition Soft Agar Overlay Assays

The zone of inhibition (ZOI) assay was used to evaluate the activity of EN112 and its mutants against ΔOmpF *E. coli* pBAD18Km that expressed various types of OmpF. The plates for ZOI were created by overlaying molten M9 minimal soft agar (0.75%) containing the indicator bacteria at OD600 = 0.01 over M9 minimal agar (1.5%). The secretor which hosts pMMB67EH was induced at 1 mM IPTG and the indicator with pBAD18Km at 0.1% arabinose. The induction for the indicator has been optimized at this level as induction higher than 0.1% creates burden for the indicator cells. Culture for secretors were grown overnight at 37C and centrifuged at 4000 xg for 5 min. The supernatant is then removed, and the pellet is resuspended in M9 medium to OD600 = 50. On the soft agar, 10 µl spots were applied of the secretors and negative control (NC) which is an empty vector secretor. The plates were then incubated at 37C overnight before the pictures were taken.

Energy Assay Using CCCP

The energy assay was conducted to determine whether EN112 uses ATP for its activity. The indicator (BW25113 + pBAD18Amp) and secretors (*E. coli* DH5α + pBAD18Km + pACYC184) are cultured overnight at 37C. Overnight secretors are centrifuged at 4 xg for 5 min and the supernatant is poured off. To ensure antibiotic are removed, the pellet is resuspended with 1 mL M9 medium and centrifuged again at 4 xg for 5 min. The pellet is then resuspended in M9 medium at OD = 0.4 and induced with arabinose at 0.2% for 2 hr at 37C shaking incubator.

Overnight BW25113 culture is diluted with LB to an OD600 = 0.03 before being exposed to CCCP (50 µg/ml) for 3 hr with shaking. The OD shouldn't increase doing this period. The cultures are then spun down in the same fashion as the secretors and finally resuspended using M9 medium at 20 µl/mL of original culture volume. For untreated BW25113 (NC), the same procedure from above is applied, and the OD should match that of the CCCP-treated BW25113 after the 3 hr period. Afterward, 100 µL of the CCCP-treated or NC is added to each of the M9 culture containing the secretors. For each secretor, replicates for each treatment is performed (n = 2 replicated x 2 treatment = 4 cultures per secretors) The co-culture are collected at 0, 30, and 60 minutes, and the number of surviving BW25113 cells were determined by serially diluting the culture in 0.85% NaCl solution and plating 100 µL spots on LB + carb plates. For the confirmation test using polymyxin B sulfate, 10 µg/ml was added to the M9 culture rather than adding a secretor.

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Transitioning from Safety Nets to Economic Empowerment: Redefining American Social Welfare through Social Investment Policies

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Abstract

This research paper critically examines the limitations of the current means-tested social welfare programs in the United States, which are characterized by their fragmented structure, restrictive eligibility criteria, short-term assistance, and a predominantly reactive approach to addressing financial hardship. These deficiencies undermine the effectiveness of robust social safety nets for those experiencing poverty. The primary aim of this study is to explore how adopting the social investment policy paradigm could reshape the U.S. social policy landscape. Through an extensive review of relevant literature, this research will assess the existing conditions of the American social welfare system, the role of social work in poverty alleviation, and the application of anti-poverty policies through a social investment framework. The study will highlight the advantages of integrating social investment strategies to establish a more equitable social infrastructure in the United States. Ultimately, the findings will underscore the imperative for social workers to advocate for a shift toward social investment approaches and offer policy recommendations to enhance the efficacy of social welfare programs.

Introduction

This paper critiques the current social assistance paradigm, emphasizing how means-tested programs frequently fail to support individuals' self-sufficiency and instead perpetuate dependency among welfare recipients. It also examines the challenges social workers encounter due to the limitations of the existing social welfare model founded on principles of work and financial independence. Utilizing the social investment policy theory as a framework, this paper explores alternative anti-poverty strategies focused on investing in human capital and enhancing individuals' capabilities. These proposals include universal pre-k, universal community college, and guaranteed income. Ultimately, this research underscores the importance of creating opportunities for all individuals to thrive in a knowledge-based economy.

The Shortcomings of Means-Tested Social Welfare in a Capitalist Economy

The capitalist system has permeated every aspect of life in the United States, from social institutions to labor market dynamics and the shaping of policies and laws. Over the past several decades, the gap between the rich and the poor has widened, driven by technological advancements from globalization and a significant shift toward higher-skilled, higher-paying jobs, leaving many low-skilled workers trapped in low-wage positions. (Wolcott, 2021). This economic system has both created and perpetuated inequalities in employment, education, and income distribution. For example, in 2021, annual earnings for the top one percent of earners increased by 9.4 percent, and for the top 0.1 percent, they surged by 18.5 percent. Contrastingly, annual earnings for the bottom 90 percent of workers fell by 0.2 percent (Economic Policy Institute, 2021). Additionally, in 2023, the top ten percent of earners controlled 66.9 percent of the nation's wealth, while the bottom 50 percent owned just 2.5 percent (Statista, 2024). This data highlights the widening divide between the wealthy and the rest of the population, emphasizing the concentration of wealth and power among the top earners and the economic stagnation or decline experienced by most American workers. In response to these stark disparities, the social welfare system has been used as a mechanism to address the inequalities rooted in a capitalist market.

Means-tested programs, which provide assistance only to those below a certain income threshold, are at the core of U.S. anti-poverty strategy. The primary means-tested programs include the Supplemental Nutrition and Assistance Program (SNAP), Temporary Assistance for Needy Families (TANF), Medicaid, the Earned Income Tax Credit (EITC), and programs for assistance with housing and child care. In the U.S.,

social assistance is highly fragmented, consisting of over 80 local, state, and federal programs, each with its own set of rules and regulations. These programs cover various household expenses, including food, healthcare, housing, and child care, but operate independently of one another (Tanner, 2012; Rector, 2018). The absence of a unified social welfare system forces many low-income recipients to depend on multiple programs to meet their basic needs, complicating the social welfare process and making the path out of poverty increasingly complex and difficult to navigate (Han, 2020).

Additionally, the fragmentation of social benefits often leads to significant gaps in service delivery that hinder enrollment in safety-net programs. Applicants undergo rigorous scrutiny and navigate extensive paperwork in hopes of qualifying for social assistance, often resulting in many eligible individuals being denied assistance despite needing it (Fox, et al., 2012). For example, in 2015, only 23 out of every 100 families in poverty received TANF cash assistance, despite meeting the income eligibility criteria (Pavetti et al., 2021). Moreover, the stigmatization of welfare recipients, driven by the notion of the "deserving" versus "undeserving" poor, has shaped public perceptions of poverty and imposed social repercussions on those in need, often discouraging individuals from applying for benefits (Stuber et al., 2006). This stigmatization, combined with excessive bureaucratic requirements and low eligibility thresholds, causes means-tested programs to frequently fail in reaching all those who need social assistance.

The current social welfare model often fails to enhance recipients' self-sufficiency and instead perpetuates cycles of dependency (Joseph, 2021). Research shows that low-income households that receive one or more federal means-tested benefits will be less likely to attain and maintain economic self-sufficiency compared to those who do not engage in federal welfare programs (Joseph et al., 2018). In fact, involvement in these programs often correlates with reduced labor participation, which is a critical factor in achieving economic self-sufficiency. Ultimately, while American social welfare programs provide short-term relief, they do not address the root causes of poverty, thereby perpetuating dependency rather than offering a path to economic prosperity.

Rising inequality within the U.S. capitalist market is a pressing concern that poses significant threats to economic stability and social well-being. As the income gap between the wealthy and the poor widens, more individuals are at risk of falling into poverty, exacerbating social tensions and undermining economic progress. The growing number of Americans facing economic risks is the result of societal trends impacting all working individuals. These include weakened job security, a rising number of people without healthcare, increased income volatility and downward mobility, record-high

consumer debt, a severely eroded social safety net, and as previously stated, widening income and wealth inequality (Sandoval et al., 2009). It is imperative to address the limitations of the current social welfare model and consider a more comprehensive approach to provide robust social protection and equip all individuals with the tools needed to thrive in a knowledge-based economy.

The shortcomings of the current social welfare model create significant challenges for social work. American social assistance is reactive and targeted, providing limited, short-term benefits only after proof of financial hardship is provided. As the social safety net has eroded, social welfare programs have become more restrictive, conditional, and stigmatized, further diminishing their effectiveness. Social workers deal directly with the consequences of a system that does not adequately equip all individuals with the resources and skills needed for economic prosperity. As more individuals struggle to survive without the proper means to achieve self-sufficiency, the demand for social work services rises. The demand for social workers is anticipated to increase by about 7.8 percent from 2022 to 2032 (Rieley, 2024). This increased need places a heavy burden on the profession, leaving social workers to navigate complex cases with limited resources. Unless policy changes are made to provide adequate opportunities for low-income individuals to achieve financial stability, this burden will persist, making it difficult for social workers to meet the growing demands for their services effectively.

Examining the social landscape of economic inequality and the current social protection mechanisms underscores the critical need to address significant flaws in the American social welfare model. Rethinking social welfare requires a thorough analysis of the system's structure, including the ideologies—such as American individualism and an emphasis on self-sufficiency—that have shaped its development. Understanding how social welfare interacts with the capitalist system and the skills needed to succeed in an economy that relies on human capital is essential. Additionally, it is necessary to critique whether recipients are truly provided with opportunities to escape poverty and how these opportunities are structured throughout their lifetime. Addressing these issues is vital for developing a more equitable and effective social infrastructure.

The Impact of Economic Disparities on Education and Human Capital Development

The reliance of American social welfare on labor market participation is grounded in the concept of human capital. Human capital refers to the value of an individual's skills, knowledge, experiences, and other attributes. It closely correlates with an individual's economic output as human capital enhances productivity, earnings, and

employment opportunities, thus making human capital a vital component of the modern economy. Human capital ultimately shapes societal labor market outcomes and significantly influences individual achievement.

Today, the education system is the primary source of human capital formation due to its pivotal role in providing individuals with the knowledge, skills, and credentials needed to obtain employment opportunities that align with economic demands. Studies show that individuals with higher levels of education and specialized job training are more likely to secure stable, well-paying jobs (Autor et al., 2013). As workers' educational attainment rises, their employment rates and earnings increase. For example, in 2023, the unemployment rate among full-time workers aged 25 and over with less than a high school diploma was 5.6 percent, compared to 2.2 percent for those with a bachelor's degree. Those without a high school degree also had the lowest median weekly earnings in 2023 among all education levels. The median weekly earnings for workers without a high school diploma were $708, whereas those with a high school diploma earned $899 weekly. This difference becomes even more pronounced with higher education: those with some college education earned an average of $992, and bachelor's degree holders earned $1,493 weekly (Bureau of Labor Statistics, 2024). It is crucial to recognize that opportunities for human capital development through education are not equally accessible to all, even less so for those from lower socioeconomic backgrounds.

Welfare recipients often have some of the most limited opportunities for human capital development, especially regarding the quality and quantity of education they receive (Beverly, 1997). It is common for children from low socioeconomic backgrounds to attend underfunded schools that lack essential resources, such as a lack of experienced teachers, up-to-date class materials, advanced coursework, extracurricular activities, and limited access to technology, all of which negatively impact the quality of education students receive (Darling-Hammond, 2010). Moreover, the environment outside of school can also hinder educational attainment. Families with low incomes often do not have the means to provide their children with proper at-home educational support, time, or guidance as families with higher incomes typically can.

Furthermore, the lack of resources throughout an individual's lifetime due to a low socioeconomic background severely restricts access to higher education. Financial constraints and the necessity of working to support themselves or family members can negatively affect low-income students' ability to afford and focus on their studies (Goldrick-Rab et al., 2016). These challenges often lead to lower college enrollment and completion rates among disadvantaged students, resulting in limited educational

attainment and economic instability. Low educational attainment perpetuates cycles of poverty as job opportunities are limited in an increasingly competitive labor market.

The Role of Labor Market Participation in the American Social Welfare State

The American social welfare state, characterized by its welfare-to-work approach, inadequately provides opportunities for economic stability. In the context of this discourse, attention is directed towards TANF, the Temporary Assistance to Needy Families program, as the primary federal means-tested program designed to encourage employment among low-income families. Welfare-to-work initiatives, notably stemming from the 1996 Personal Responsibility and Work Opportunity Reconciliation Act (PRWORA), exemplified by TANF, have increased employment rates amongst welfare recipients (Lim et al., 2009). Nevertheless, focusing on immediate job placement, these programs frequently secure low-wage positions without essential benefits like health coverage and paid time off, thereby perpetuating persistent economic vulnerability.

Strict work requirements and time limits under TANF push recipients into the labor market with little hope of obtaining high-paying jobs with benefits. It is required for the vast majority of families receiving TANF cash assistance to be engaged in a work activity for at least 30 hours a week (20 hours a week for single parents with children under age six) (Center on Budget and Policy Priorities, 2021). Additionally, structural barriers, such as limited access to affordable child care, reliable transportation, or mental and physical healthcare services, create significant obstacles for welfare recipients transitioning into stable employment (Lee et al., 2007). Marginalized groups, including women, particularly single mothers and people of color, face additional challenges like racial discrimination and gender wage gaps, making economic stability even more challenging to attain (Monnat, 2008). Programs frequently fail to provide adequate support for financial prosperity, such as job training, education, or financial counseling, resulting in many individuals cycling back into welfare dependency. Long-term studies indicate that these programs do not break the cycle of intergenerational poverty, as children of welfare recipients often face similar economic hardships (Hartley et al., 2022).

Moreover, the 60-month lifetime limit on TANF benefits at the federal level does not account for different families' varying needs and challenges. Once the time limit is reached, families lose their benefits and are stripped of their safety net, regardless of whether they have achieved economic stability. Hence, time limits can have dire consequences for affected recipients. Failing to meet work requirements most often results in a series of increasing sanctions, resulting in reduced and if not cured,

terminated benefits. These penalties can exacerbate financial hardship, making it even harder for families to achieve stability (Fording et al., 2013).

Consequently, many individuals on welfare rolls find themselves in dead-end jobs with little or no opportunities for advancement. The issue of "benefits cliffs" further exacerbates this problem as recipients begin to earn a higher income and their welfare benefits are reduced or terminated. Families do not have an income low enough to qualify for benefits, yet it is not high enough to cover the costs of vital needs such as food, housing, medical care, and child care. Ultimately, benefit cliffs disincentivize recipients from pursuing higher-paying jobs or additional work hours due to the risk of being financially worse off. Punitive policies and the neglect of essential support measures reveal a systemic failure to provide families with the necessary opportunities for economic self-sufficiency.

Rethinking U.S. Social Welfare Through a Social Investment Lens

Merely reforming a flawed social welfare system by introducing additional work requirements or more means-tested programs is not a viable solution. Such efforts would exacerbate an already overburdened system by further fragmenting the existing social welfare landscape, increasing complexity in a system that is already challenging for recipients to navigate. A reactive approach to social assistance has historically failed to achieve economic prosperity for individuals and their families; it would be illogical to continue allocating public funds to an ineffective system. Instead, proactive policies that address the root causes of poverty must be implemented.

The social investment policy theory (SIT) is particularly relevant for its potential applications in the United States. SIT is a policy framework that advocates for strategically allocating resources to enhance individuals' skills, health, and overall capabilities, aiming for long-term economic and social benefits. This framework emphasizes preventing social issues before they arise rather than addressing them afterward. SIT invests in education, training, and social services to build human capital and promote sustainable social inclusion (Hemerijck et al., 2023). According to SIT, social investment policies can create a "life-course" multiplier effect, generating a cycle of well-being through skill development, employment, productivity, gender equality, and poverty reduction throughout an individual's life. This cycle starts with early childhood investments, which lead to improved educational outcomes. These outcomes, combined with subsequent training, result in more productive employment and, ultimately, a better quality of life.

The concept of social investment suffers from a lack of an exact definition. Giddens (2008), a leading pioneer of the social investment state, viewed social investment as a future-oriented approach in which the state would function as an entrepreneur making human capital investments focused on creating "positive welfare." Other early contributors, such as James Midgley (1996), described the model's appeal as an alternative strategy for a new economy that continues to grow in the developed world. The vast majority of social investment literature points to a consensus that this model arose out of a need to respond to a radically changed economic and social order (Mendes et al., 2014).

Moreover, a social investment approach offers a promising opportunity to align social policy with economic development by creating and implementing programs that contribute to long-term economic growth. Investment implies returns, and within the framework of social investment, programs are expected to yield positive outcomes not only for individuals but also for the broader economy. This approach emphasizes the strategic targeting of government spending in areas that generate the highest returns, enhancing the cost-effectiveness of social welfare policies. While the central objective of social investment is to improve life outcomes and opportunities—particularly for marginalized groups—there is a broad consensus that sustainable economic growth strengthens the justification for social spending and reinforces the importance of an active governmental role. Although short-term economic gains are not the primary focus, aligning social policy with economic goals supports the long-term viability and effectiveness of such policies.

The Implications of a Knowledge-Based Economy for Social Investment Theory

A globalized knowledge economy has led to success being dependent on the existence of a highly adaptable, skilled, and educated workforce. Notably, a knowledge-based economy has generated new social risks, particularly the lack of access to knowledge and an absence of required skills. Rather than providing citizens with direct security through redistribution mechanisms, social investment equips citizens to negotiate their own integration into the market, secure economic competitiveness, and participate fully in society. The social investment model still considers the free market as the central organizing principle in society. However, it no longer sees an unregulated market as the most effective or fair way to structure society. Instead, it recognizes the need for government intervention and direction of market forces to achieve better economic and social outcomes.

One major implication of the new knowledge-based economy is the proliferation of entrepreneurship rather than mere employment. The social investment state can drive innovation by improving human capital, skill development, and risk-taking behaviors (Bell et al., 2018). Nordic countries, known for their extensive welfare systems and social investment policies, are often recognized as highly innovative. In contrast, the lack of social safety nets in the US, especially regarding health insurance, is a significant barrier to entrepreneurship (Fairlie et al., 2011). Research indicates that higher inequality, such as the one prevalent within the United States, tends to hinder innovation (Gutierrez-Romero et al., 2017). Social investment highlights how providing resources and social protections improves citizens' quality of life and creates a conducive environment for innovation and economic growth (Choi et al., 2020). Providing sufficient resources for economic prosperity enables individuals to take risks, develop their skills, and contribute to the economy without the constant fear of economic insecurity.

Social Investment Strategies: Theoretical Approaches in the U.S. Context

Investing in a robust stock of human capital requires a multifaceted approach that enhances all individuals' skills, education, and capabilities. Key strategies include prioritizing high-quality education by introducing universal pre-kindergarten, which provides a strong foundation for human capital development and shapes future success. Additionally, offering universal access to community college ensures that adults have sufficient opportunities to gain valuable skills and education throughout their lives. Beyond education, addressing poverty-related challenges is crucial and can be achieved by providing a guaranteed income for all children under five. A guaranteed income initiative would directly support families and improve child well-being and development while alleviating the burden of child care costs and other essential expenses. These three strategies—focusing on universal pre-kindergarten, expanding access to community college, and offering a guaranteed income for young children—are essential for investing in all people and reducing economic inequality.

The Need for Further Investing in Early Childhood Education and Care (ECEC)

The United States does not have a federally established universal child care or pre-school education system. Despite this gap, there has been a growing acknowledgment within the policy and research fields regarding the critical role of early childhood education and care (ECEC) in providing essential educational and

developmental opportunities for all children, irrespective of their socioeconomic background (Pianta et al., 2009). State and federal policymakers are increasingly acknowledging the importance of ECEC in fostering fundamental abilities such as language, literacy, self-regulation, social interactions, and early mathematics. These proficiencies acquired within the initial five years of life establish the basis for future learning by cultivating crucial cognitive, social, emotional, and behavioral skills that impact higher education success and later workforce engagement (Robson et al., 2020). This increased awareness has led to a surge in investments in different ECEC programs, supported by a mounting body of research that underscores the crucial role these skills play not only in children's development but also in the broader economic and social prosperity of communities.

ECEC in the United States includes three critical programs: Head Start, formal child care services, and state-specific pre-kindergarten programs. Head Start, a federally funded initiative, offers pre-school education and additional support services for low-income children. Formal child care, supervised by licensed providers, delivers organized care and educational support. Lastly, state-specific pre-kindergarten programs, customized to meet local needs, concentrate on school readiness for children who meet eligibility criteria. These distinct initiatives play a vital role in preparing American children for academic success and social development. The diversity in these programs has led to a range of school preparedness levels for children under five.

Furthermore, research indicates that children who exhibit poor academic performance before beginning kindergarten are more prone to adverse outcomes later in life, including an increased likelihood of becoming teenage parents, involvement with the justice system, and facing unemployment or underemployment as adults (Rouse et al., 2005). These negative trajectories have long-lasting implications, leading to higher government expenditures on special education, increased reliance on social welfare programs, and elevated crime rates. The financial repercussions of these trends are substantial when considered over an individual's lifetime. For instance, estimates show that a high school dropout can cost society between $243,000 and $388,000 throughout their life, while the societal expenses linked to a typical criminal career range from $1.3 to $1.5 million (Karoly et al., 2005). These figures underscore the importance of early interventions like ECEC in mitigating such costs and fostering positive outcomes for individuals and communities in the long run.

Despite investments and efforts to expand and improve ECEC programs, a significant proportion of children, particularly those from economically disadvantaged backgrounds, start kindergarten significantly behind their peers regarding knowledge and social competencies required for children to perform at even the most basic levels.

Significant disparities are apparent among disadvantaged children in reading and math proficiency, behavioral issues, and readiness for learning. For instance, while 18 percent of all children lack basic print and writing understanding, this percentage jumps to 32 percent for children with mothers with less than a high school education (Karoly, 2005). Moreover, although there has been a rise in ECEC program enrollments, only a fraction of eligible low-income children actually benefit from such initiatives (Magnuson et al., 2010). Disparities in enrollment for pre-school education from low-income families highlight a concerning trend where those who stand to gain the most from early education are often the least likely to access it. Additionally, the skill gap experienced by disadvantaged children is further shaped by significant differences in the quality of education across communities. Research reveals that state pre-school programs in low-income and high-minority areas are often rated lower in classroom quality (Bassok, 2015). Consequently, even disadvantaged children participating in pre-school may lag in skill development if the program lacks quality. Without a coherent and unified social support system for children, the potential benefits of ECEC are diminished, and the ability to address the diverse needs of all children remains limited.

The observed skill gap at school entry amongst disadvantaged and low-income children cannot be effectively addressed solely by increasing child care subsidies or program enrollment under currently fragmented state and federal policies. Universal pre-kindergarten (pre-K) stands out as a significant poverty alleviation strategy, offering exceptional efficiency and equity benefits. High-quality universal pre-school education for children ages three to five can address educational disparities by providing all children with a strong developmental foundation during a key stage of brain growth. Early access to high-quality pre-school ensures that children from all backgrounds develop essential cognitive, social, and emotional skills, leveling the playing field before they enter kindergarten.

Investing in high-quality pre-school education for all children can deliver significant educational, social, and economic benefits, especially when focused on programs with effective teaching. The quality of education is crucial, and social investment policies should prioritize programs that offer substantial returns relative to their costs. Research indicates that high-quality pre-school education can lead to lasting improvements in the life outcomes of economically disadvantaged children while offsetting government program costs (Duncan, 2007; Reynolds, 2008; Watts et al., 2018).

A recent analysis by the Washington Center for Equitable Growth (2018) delved into the expenses and advantages of public investment in a voluntary, high-quality universal pre-kindergarten program for all three- and four-year-old children nationwide.

The program would cost $5,832 per participant, enrolling about seven million children, averaging $40.6 billion annually. The study demonstrates that implementing such a universal initiative would yield yearly benefits that would outweigh the program's annual costs within eight years. The expenses of the program would differ by state, ranging from just under one dollar in South Carolina to seven dollars in Vermont and the District of Columbia. By 2050, the federal government is projected to achieve a budget surplus of $34.8 billion from pre-kindergarten investments. Furthermore, the overall yearly benefits in terms of budgetary gains, earnings, health improvements, and crime reduction would total $304.7 billion, comprising $81.6 billion in governmental budgetary advantages, $108.4 billion in augmented worker compensation, and $114.7 billion in decreased individual costs due to improved health and reduced crime rates.

A universal pre-kindergarten initiative is an opportunity for robust development, overall well-being, and active engagement in the labor market over a lifetime. Given their high demand by employers, it is imperative to establish a unified system that nurtures essential skills such as literacy, numeracy, and communication. Such skills provide a solid foundation for future specialization in vocational expertise, especially in the era of rapid technological progress. This program would not only enhance educational achievements but also pave the way for higher-quality employment opportunities, ultimately elevating living standards and decreasing reliance on social welfare by reducing the prevalence of poverty. The returns on investments in early childhood education are substantial and offer policymakers a compelling rationale for prioritizing such initiatives.

Institutional Stocks of Human Capital: The Strategic Importance of Community Colleges

With escalating tuition fees making post-secondary education increasingly unattainable, the significance of higher education in the 21st-century job market is growing. This educational level unlocks pathways to career progression beyond low-paying jobs. However, the financial hurdles associated with pursuing advanced education often limit individuals from lower and middle-income backgrounds from accessing these opportunities. President Obama's America's College Promise proposal, a key element of his domestic policy agenda, sought to provide two tuition-free years of community college education, enabling students to earn credits towards a bachelor's degree and gain essential workforce skills without financial burden (Holland, 2015). Although this initiative was not enacted, it ignited nationwide discussions regarding making higher education more accessible across party lines. While there is a strong case for a tuition-free four-year university, concentrating a widespread federal initiative

specifically on universal community colleges carries economic and political considerations that could enhance the policy's viability for a broader segment of the American population (Ison et al., 2020).

In the American higher education sector, community college students represent the largest proportion of students, with over 38% enrolled in 2-year public institutions in the 2015-2016 academic year (Grinder et al., 2018). Community colleges play a crucial role in bridging educational divides by allowing students to pursue higher education, irrespective of their academic history or family background. These institutions cater to a more significant proportion of underrepresented students than other sectors of the American education system, with most attendees falling into the "nontraditional" students category. Community colleges boast greater racial and ethnic diversity than traditional four-year colleges and universities. They also tend to enroll individuals who are employed full-time and who represent the first generation in their families to pursue higher education. Offering a cost-effective, inclusive, and academically varied post-secondary pathway, community colleges prioritize accessibility, making them an appealing option for a population at risk of higher academic achievement.

Moreover, community colleges play a vital role in equipping individuals with the education and skills employers seek. These institutions offer diverse programs spanning various fields, encompassing associate degrees and certificates that pave the way for higher education pursuits (Van Noy, 2023). Furthermore, collaboration between educational institutions and local employers is essential for linking labor supply with local demand. In this capacity, community colleges act as institutional human capital stocks, establishing the frameworks necessary to facilitate job placements and ensure the smooth functioning of the labor market. Enhanced access to community college would facilitate the acquisition of advanced skills, resulting in increased economic prosperity within the labor market. This heightened investment in human capital can decrease income inequality by boosting labor force participation rates.

Universal community college education serves as a vital social investment by providing individuals, regardless of background, with the necessary human capital for the job market. Community colleges enhance employability and economic mobility through diverse programs tailored to industry needs, closing socio-economic gaps. By equipping graduates with in-demand skills and fostering collaboration with local industries, these institutions prepare individuals for success and drive workforce readiness and societal progress.

Redefining Welfare: Guaranteed Income as a Tool for Social Investment

The idea of cash assistance has gained significant attention in policy discussions worldwide (Laenen, 2023). Numerous cash transfer experiments conducted both globally and within the United States have aimed to alleviate poverty and improve economic security by implementing varying cash assistance programs (Castro, 2022). Guaranteed income (GI) programs are unconditional cash transfers that provide unrestricted, reliable monetary support to those experiencing income insecurity. Since 2018, nearly 100 guaranteed income programs have been implemented across state, county, and city levels, largely in response to the cost-of-living crisis, concerns about job losses due to automation, and the economic instability brought on by the COVID-19 pandemic (Ipwr, 2023). Participants of GI programs receive a specific amount of money on a scheduled basis for a specific period, which they can spend as they please. Participants are not required to meet certain conditions in exchange for program benefits, yet participants are required to meet eligibility requirements based on income, geographic, and demographic characteristics.

A common concern about guaranteed income is that it might discourage people from working and increase dependency on government support. However, pilot studies on guaranteed income have shown the opposite effect. One of the first guaranteed basic income pilots launched in the US includes the Stockton Economic Empowerment Demonstration (SEED) project in Stockton, California. SEED aimed to test the effects of a 500-dollar-per-month guaranteed income for two years on health and financial outcomes (Ghuman, 2022). Recipients reported lower rates of income volatility, lower mental distress, better energy and physical functioning, increased ability to secure full-time employment, and increased ability to cover emergency expenses (West et al., 2023; Elliot et al., 2023). Another notable pilot study, The City of Saint Paul's CollegeBound Boost, aimed to investigate the impact of addressing immediate basic needs versus future security and growth needs in the lives of individuals in poverty. Participants in the study prioritized their spending and saving according to a hierarchy of needs: first covering survival essentials such as food and bills, then saving for security, and finally for growth needs like education and retirement, reflected by actual spending data (Elliot et al., 2023). The study also demonstrates that consistent with financial needs theory and contrary to typical views of irresponsible spending, those receiving guaranteed income allocate part of their 500-dollar payment toward savings for future security and growth once their immediate survival needs are met. Overall, studies provide compelling evidence of positive health and financial outcomes for recipients of guaranteed income.

The benefits observed in Guaranteed Income pilot studies underscore the potential of guaranteed income as an effective anti-poverty strategy. A national GI initiative aimed at families with children under five represents a robust social investment approach with the potential to address economic inequality in multiple ways. By providing meaningful direct financial support, this policy can help low-income families manage essential expenses, support their children's development, and enable parents to seek better job opportunities, education, or training.

As previously addressed in this paper, one of the critical issues with means-tested programs is the existence of benefit cliffs, where individuals face a sharp reduction or complete loss of benefits as their labor earnings increase. This system inherently discourages self-sufficiency by creating a financial penalty for those seeking to improve their economic situation. In contrast, guaranteed income offers an unconditional financial base that remains stable regardless of income, providing continuous security without the threat of benefit withdrawal. For low-income families, this predictability is crucial, as it allows them to pursue employment and economic mobility without encountering the disincentives embedded within means-tested frameworks. As guaranteed income initiatives demonstrate that these programs do not create a widespread disincentive to work, with recipients often remaining active in the labor market, they present a more effective and dignified alternative to means-tested assistance in promoting long-term self-sufficiency.

In an increasingly costly economy, a 500-dollar stipend can ease financial pressures, allowing families to afford better nutrition, healthcare, and early childhood care—vital elements during a child's formative years. The social investment theory supports that such early investments lead to improved educational outcomes, better health, and higher lifetime earnings. By offering a stipend to all families, regardless of income, this policy can promote equitable access to resources that foster a child's healthy development and can help reduce inequality. Although modest, the stipend can disproportionately positively impact lower-income families, creating supportive opportunities for children from diverse socio-economic backgrounds. Providing children with a strong foundation for future success can ultimately break intergenerational cycles of poverty in the long-term. Most notably, GI benefits offer recipients more free time by reducing financial stress, which can lead to increased engagement in creative pursuits, entrepreneurship, caregiving, and exploration of new opportunities—ultimately enhancing individual and community well-being.

In 2023, there were approximately 23.4 million children aged zero to five in the United States (Child Stats). If the federal government were to provide $500 monthly stipends to all families with children under five, the cost of a nationwide guaranteed

income (GI) would amount to approximately $138 billion. Although the financial burden would be significant, the consequences of failing to address growing social issues, such as increasing income inequality and the looming threat of mass unemployment, could be far more detrimental (Laenen, 2023). However, any large-scale GI initiative would likely be economically unfeasible if implemented alongside existing welfare programs (Murray, 2008). Therefore, funding for a GI program would need to be sourced from current social assistance programs and the bureaucracies that manage them, given the financial constraints.

Guaranteed income has gained traction not only among those on the political left, who view it as a tool for social justice but also among libertarians, who see it as an efficient method for the government to redistribute wealth with minimal disruption (Murray, 2016). Although still a relatively new concept, more progress is needed to demonstrate the value and impact of cash assistance programs with varying levels of assistance. Advancing this idea requires an emphasis on educating the public and policymakers about the larger economic and social risks of growing inequality and the importance of protective social policies. Moving forward, it is essential to identify which existing institutions and influential actors can effectively lead the way toward such transformative change.

Anti-Poverty Strategies in Social Work

Throughout its evolution, the social work profession has continuously adapted to meet the evolving needs of society, confronting pressing social issues through persistent advocacy, anti-oppressive practices, and advancements in research and methodologies. Social workers were highly involved in expanding civil rights and advocating for reforms in areas such as child welfare, health care, juvenile justice, and income support. Considering the conditions of growing economic inequality, the social work profession must take a leading role in advocating for economic opportunities for individuals from disadvantaged socioeconomic backgrounds through a social investment approach.

The Preamble to the NASW Code of Ethics establishes the primary mission of the social work profession is to “enhance human well-being and help meet the basic human needs of all people, with particular attention to the needs and empowerment of people who are vulnerable, oppressed, and living in poverty” (NASW, 2021). Given this foundational mission, the critical role of social work in developing effective anti-poverty strategies for the well-being of clients and society within the contemporary social welfare framework emerges as a paramount consideration.

In the realm of social work practice in the United States, a historical reliance on casework and crisis management has been prevalent, with a primary focus on facilitating client access to existing social welfare resources and programs. This conventional approach has not only been fundamental to the profession but has also significantly influenced its evolution, serving as a cornerstone for providing immediate stability and support to those in need. However, as a profession committed to fostering social and economic justice, it is imperative to also scrutinize the prevailing power structures and systems that perpetuate oppression. The current system, which has long been integral to social work practice, undoubtedly perpetuates inequality. Therefore, it becomes incumbent upon social workers to challenge the prevailing paradigms of social work and seek alternative solutions. Critical questions must be raised regarding the efficacy of social work strategies to address the immediate needs of individuals in poverty and whether these strategies inadvertently reinforce the existing societal status quo. Scholars such as Reisch and Jani (2012) argue that despite the profession's rhetoric of change, social work increasingly aligns with institutional goals and assumptions, thereby endorsing the prevailing societal norms. This belief is exemplified by the recent trend towards the marketization of social welfare and an emphasis on client resilience rather than resistance.

A commitment to social justice requires social workers to not only focus on providing direct social services through case management but also on enhancing the effectiveness of current systems and programs. Recognizing social work's dual role as both supportive and transformative, it becomes crucial for the profession to adopt efficient anti-poverty strategies that encompass both dimensions (Boone et al., 2018). Supportive strategies aim to address inequalities within existing social structures without fundamentally altering the underlying system, while transformative strategies strive to reshape the fundamental organization of society. In this context, the adoption of social investment policy theory by social workers becomes critical. SIT emphasizes long-term societal benefits gained through investments in human capital, viewing these investments as pivotal for economic growth, social stability, and individual well-being. By embracing this approach, social workers can shift their focus from short-term relief to long-term empowerment, aligning with the transformative aspects of social work.

Implications

While this paper explored a range of social investment policy proposals in the expansion of American education and cash assistance, there are numerous other social investment strategies that also warrant consideration. The social investment policy framework has gained considerable attention in international circles for compelling reasons. Policymakers have a duty to represent the needs of their constituents by

crafting effective policies that yield positive social and economic outcomes. Social policy development must be evidence-based, and accurately assessing the success or shortcomings of these policies requires rigorous analysis and scholarly insight across academic disciplines. Furthermore, social workers must leverage their roles as advocates to advance social investment strategies that empower individuals to gain economic control over their lives. As actors who witness the injustice and inequities created by the existing social welfare system, social workers should strive to shed light on the lived experiences of those experiencing poverty in order to spark critical dialogue amongst the public realm on the dangers and implications of an eroding safety net. Ultimately, American social infrastructure should progressively align with a social investment paradigm to better meet the demands of a knowledge-based economy and address the persistent economic inequality that limits individual capabilities, freedoms, and opportunities for success.

The current American social welfare system is undeniably failing on multiple fronts. As inequality deepens and social tensions rise, it is crucial to reconsider our approach to social policy. Conversations must be ignited about the profound struggles faced by Americans trapped in poverty, navigating a fragmented and overburdened system that is inadequate in ensuring their success. It is imperative to bring these private hardships into the public arena, challenging the status quo and driving the transformation of American society

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Balancing Health(care): Traditional Chinese Medicine Use, Beliefs, and Health-Promoting Behaviors in San Gabriel Valley, California

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Abstract

Certain practices serve to promote the health and well-being of groups or communities of people. Interviews were conducted with Asian Americans in San Gabriel Valley, California. In exploring the routine health practices, especially those that are health-promoting, we can have a better understanding of the individual health behaviors that support long life expectancy and ability to age well. The purpose of this study was to gain insight into the relationship between the target group's use of traditional Chinese medicine and conventional Western medicine, perceptions of healthcare and personal health, taking into account the beliefs, utilities, and health outcomes of the different modes of healing.

Keywords: Traditional Chinese medicine, acupuncture, herbs, Asian American, San Gabriel Valley

Background

Complementary and Alternative Medicine

The idea that Western medicine can be accompanied by cultural forms of medicine like traditional Chinese medicine is recognized as “complementary and alternative medicine.” The National Center of Complementary and Alternative Medicine established in October 1998 defined complementary and alternative medicine, or CAM, as “a group of diverse medical and health care systems, practices, and products that are not generally considered part of conventional medicine.” With the added emphasis on contextualizing these healing practices, this definition remains appropriate today. Conventional medicine, Western medicine and allopathic medicine are various ways to refer to the medicine that is practiced by M.D. (medical doctor) and D.O. (doctor of osteopathy) degree holders, as well as allied health professionals (Sanadhya et al., 2015).

Traditional Chinese medicine, or TCM, is utilized by many Asian Americans today and is rising in popularity among non-Asian populations who seek alternatives to conventional Western medicine. TCM is a prehistoric tradition of healing that includes acupuncture, herbal medicine, qigong, tai chi, massage, and nutritional therapy. TCM falls under the umbrella of traditional complementary and alternative medicine (TCAM), which refers to several healthcare practices with origins outside of scientific Western practices (Zhao et al., 2021). Higher rates of TCAM use were seen among Chinese Americans and Korean Americans compared to other sub-ethnic groups. Additionally, old age was a significant predictor of TCAM use for Chinese Americans (Yi et al., 2024).

Traditional Chinese medicine knowledge spread from China to other countries, such as Japan, Korea, and Vietnam between the seventh and ninth centuries AD. Today, TCM is recognized and used across Western nations. Over 70% of the export market can be accounted for by Japan, Hong Kong, the United States, and South Korea (Cooper et al., 2017). TCM sustained China’s medical care for thousands of years, and it wasn’t until the nineteenth century during the Opium War that Western medicine began to take off (Dong, 2013). With a growing interest in the field, acupuncture and herbal remedies have become widely studied and gained popularity in the growing field of integrative medicine.

Motivations for CAM use have included having a chronic medical condition, experiencing pain, and poor self-rated health. For Asian Americans, additional

motivators include a lack of access to healthcare, being uninsured, and having an immediate need for care (Yi et al., 2024). Consistent with Haug et al's definition of self-care, it is a concept for maintaining one's health by reacting to different health needs and symptoms of illness (Høy et al., 2007). According to the early, influential Chinese classic of medical herbs, *Shen-Nong Materia Medica*, herbs could be divided into three different grades with distinct medicinal properties. Herbs in the upper grades were used to strengthen physical health while herbs in the lower grades were used to treat diseases. The use of upper grade herbs represents active involvement in improving current health conditions even within healthy individuals (Cooper et al., 2017).

Migration and Demographic History

Asians, in large numbers, have migrated to the United States since the 1840s, crossing the Pacific Ocean in search of better economic opportunities. By the 1930s, nearly a million individuals from China, Japan, Korea, the Philippines, and India immigrated to the United States and Hawaii. In 1882, Congress passed the Chinese Exclusion Act, renewed in 1892, and prohibited immigration to the U.S. by Chinese individuals until the Act was repealed in 1943. Chinatowns were introduced as places where Chinese immigrants could seek refuge; this allowed them to preserve their identities with very little assimilation. Reinforcement of their ethnicity, cultural practices and beliefs resulted (Ling, 2023). Now, the Chinese represent a significant portion of the population. Chinese individuals make up the majority of Asian-origin individuals in the United States. In addition, the largest ethnic Chinese population outside of Asia is found in the United States (Zhou, 2009).

In 2019, the overall life expectancy in the United States was 79.1 years with Asian and Pacific Islanders being the ethnic group with the highest life expectancy at 85.7 years (Dwyer-Lindgren et al., 2022). Between 1989 and 2001, immigrants in the United States had a higher life expectancy than those who were born in the U.S. (Singh et al., 2013). Among the foreign-born population in the United States, the Asian-born population had a higher proportion of people 65 years and older than other regions of birth (Azari et al, 2024). Moreover, the life expectancy of Asians in the United States is higher than that of any Asian country (Acciai et al., 2015). This presents Asians with a significant mortality advantage over other racial and ethnic groups in the U.S.

Setting and Sample

East of Los Angeles, California is the suburban region San Gabriel Valley. San Gabriel Valley is made up of 31 municipalities, including the Chinese ethnoburb known as Monterey Park. An ethnoburb refers to “suburban ethnic clusters of people and businesses,” a term coined by the Chinese American geographer Wei Li (Ling, 2023). California experiences the greatest amount of Asian suburban clustering than anywhere in the United States. The Asian majority of San Gabriel Valley, California and large influx of new immigrants to the region makes this region an ideal site to research Asian American experiences (Nicolaides & Zarsadiaz, 2017). That is why I have selected it as my field site, interviewing Asian Americans on their health and healthcare-seeking behaviors, self-care practices, and beliefs about traditional Chinese medicine and conventional Western medicine.

San Gabriel Valley in Los Angeles, California represents a community of immigrants with a heightened Chinese presence in areas like Monterey Park (Nicolaides & Zarsadiaz, 2017). It has been shown previously that the lack of health insurance in these populations motivated Chinese immigrant workers to take caution in their daily lives. They couldn’t risk getting injured or becoming sick. Otherwise, they wouldn’t be able to afford health care at a clinic or hospital, as it would be above their financial means to do so (Pih et al., 2012). As a result, they led healthier lives for themselves and their families. Possessing heightened awareness around one’s health, this population was ideal for a study exploring health and health-conscious beliefs and health-promoting practices.

The target population was underserved Asian American adults residing in Los Angeles, receiving their care at a federally qualified health center, a dental office, or private clinic in San Gabriel Valley. Participants were oftentimes multilingual with various levels of English proficiency ranging from “non-English speaking,” “poor,” “fair,” “good,” or “excellent.” Most often, English proficiency levels were self-rated as “poor.” As a result, an interpreter assisted in translating during some in-person interviews while other interviews took place in English or a mix of English and another language, including Mandarin Chinese and Burmese.

Methods

All self-identified Asian Americans whose age was 18 and older, received medical care in San Gabriel Valley, and had used and/or were using traditional Chinese

medicine (e.g., using TCM herbs, seeing an acupuncturist, practicing qi gong) were eligible. Participants were first asked to fill out a demographic survey to assess eligibility for the study, answer a few demographic questions, and provide consent to participate in the study. All participants happened to be insured, and participants were born in different regions of the world, including the United States, China, Indonesia, Malaysia, Mauritius, Taiwan, and Myanmar. Participants were English-speaking, Mandarin-speaking, Cantonese-speaking, and/or Burmese-speaking. A Mandarin Chinese interpreter and Burmese interpreter were available to help interpret interviews conducted in Mandarin Chinese, Burmese, or Mandarin Chinese and English depending on the interviewee's proficiency levels and preferences. Remaining interviews were conducted in English.

Semi-structured interviews with research participants were used to obtain information on health status, self-rated health, the use of remedies, traditional Chinese medicine, and conventional Western medicine. During interviews, participants were expected to spend about a quarter of an hour to half an hour answering questions. In reality, the length of the interviews ranged from about a quarter of an hour to an hour depending on how often the interviewee provided expanded narratives or digressed from the topic. All face-to-face interviews took place in San Gabriel Valley and took place in July 2024.

Interview questions covered self-care, maintaining good health, preventing disease and illness, recovering from illness, and treatment by TCM and conventional Western medicine practitioners. I conducted 6 face-to-face interviews and 4 telephone-based interviews. Informed consent was obtained by all participants prior to starting any interview. Face-to-face interviews were conducted in English, Mandarin Chinese, or both, while phone call interviews were done in English, with the exception of one Burmese interview with a Burmese interpreter. Interviews took place in private conference rooms. All interviews were audio-recorded using a tape recorder. After interviews were conducted, audio recordings were translated and transcribed into English for data analysis.

Usage and Perceptions of Traditional Chinese Medicine

Asians are the biggest consumers of traditional Chinese medicine (Cooper et al., 2017). After pouring through interviewee responses, situations for which TCM was sought included being sick, dealing with a headache, dealing with a stomach ache, general feelings of disease or being unwell, trouble sleeping, irregular menstruation, chronic bronchitis, frozen shoulder, and more generally for situations that did not

improve using conventional Western medicine. Another reason for seeking out Chinese medicine was to “dehumidify [the] body.” This refers to a concept in Chinese medicine known as dampness, which is treated using bitter substances, such as herbs (Yang et al., 2013). In a similar vein to general feelings of unease or unwellness, discomfort due to qi imbalances was another reason to seek out TCM. According to Participant 2, TCM was useful “when I feel uncomfortable because of my qi.” Qi is the vital energy that is essential for maintaining balance in the body. When experiencing imbalances in yin and yang within an individual, one may start to experience symptoms, but qi can help to restore the body’s balance and harmony (Aung et al., 2013).

Problems that conventional Western medicine couldn’t help with but Chinese medicine could include stomach ache and cough. Participant 1 stated, “When I had a stomach ache before, I saw a Western doctor, but it didn’t help a lot, so I took Chinese medicine instead.” Similarly, Participant 5 expressed, “If I had stomach sick, I would choose to see a Chinese medicine practitioner.” For cough, Participant 6 voiced, “In the United States, I usually go to Western medicine first, but sometimes Western medicine can't cure my illness, such as cough; at this time, I go to Chinese medicine and it is cured soon.” Repeatedly, stomach ache and cough appeared as instances in which traditional Chinese medicine was more effective than conventional Western medicine for study participants.

During questions directed at one’s use of traditional Chinese medicine, TCM was commonly associated with herbs:

> “When I was referring to Chinese medicine, I was referring to herbs, so I’m not taking herbs anymore. I will receive acupuncture if I have some other issues.” - Participant 9

TCM herbs were taken for their medicinal properties and ability to restore balance within one’s body. However, TCM herbs would also be used in cooking. For example, Participant 3 stated, “I usually use Chinese medicine to cook. It also gives the food flavor.” Of significance, with regard to perception of TCM, it was common for study participants to initially report not using TCM. However, after following up about use of acupuncture, they would report having used acupuncture in the past or currently using acupuncture. This is an interesting conclusion that acupuncture was not commonly associated with TCM, yet herbs were.

Moreover, perception of TCM was referenced in multiple interviews as not a medicine. Participant 1 remarked, “For me, Chinese herbal medicine is not medicine. I usually use it for daily body conditioning, like daily supplements.” Participant 3 similarly expressed, “Sometimes Chinese medicine is not medicine, it maintains your body.”

Here, the blurring of TCM and everyday health management surfaces. Its similar methodology to the patient's own strategy is what made traditional Chinese medicine so unordinary as to not be considered a medicine (Venit Shelton, 2013). Perhaps, the common label ascribed to TCM by study participants as "natural" could also be indicative of why it was explicitly not recognized as a medicine by some participants.

TCM, in practice for over 5,000 years, has been ascribed as a "natural, safe, and effective treatment" (Cooper et al., 2017, p. 126). Frequent identification of TCM as "natural" can influence perceptions and beliefs around TCM. Participant 7, in response to being asked about whether he uses traditional Chinese medicine, said, "Yes, I use it very often because Chinese medicine is natural. Traditional Chinese medicine is used for most physical conditions, but Western medicine is still used in emergencies like inflammation." Several aspects of TCM have gained wider acceptance in the medical community. Massage treatment has gained acknowledgement from modern medicine as effective in improving blood flow and circulation (addressing 'stagnant qi'), increasing metabolism, and helping to recover from soft tissue injuries (Dong, 2013).

Acupuncture, a treatment used in TCM practice, was employed by multiple study participants. Acupuncture was commonly used for pain management and TMJ disorders. TMJ refers to the temporomandibular joint, which is essential for allowing the mouth to open, swallow, breathe, phonate, suck, and perform different facial expressions (Bordoni, 2023). Acupuncture can be employed to effectively manage a patient's TMJ pain: at weekly visits, acupuncture needles are inserted to particular acupoints until pain symptoms are relieved (Chidambaranathan, 2021). Although here it was used as the main treatment for painful symptoms in participants with TMJ disorders, it can also be used as a complement to other kinds of therapy (Zotelli et al., 2018). Other than herbs and acupuncture, TCM encompasses dietary therapy, massage, and other forms of treatment.

Participants mentioned mixed responses on the availability of TCM resources in San Gabriel Valley. Participant 7 lives in Covina where "There are very few traditional Chinese medicine resources there. San Gabriel has more traditional Chinese medicine resources." TCM resources in San Gabriel Valley mentioned by study participants included private clinics offering acupuncture and massage, Tak Shing Hong and other herb stores. Additionally, for Participant 1, it wasn't easy for him to find a Chinese medicine doctor, but at times, he had to resort to it: "When I couldn't make an appointment with Western medicine and I thought Chinese medicine might be helpful, I would go to Chinatown to find a Chinese medicine doctor, but it was still hard to find." When seeking out Chinese medicine doctors, private clinics typically offered acupuncture and massage, but these services were not covered under insurance plans. Increased out-of-pocket expenses reduces Chinese medicine's accessibility in the San

Gabriel Valley region. However, a significant number of participants expressed a lack of knowledge around the TCM resources offered in the areas in which they live.

Usage of Conventional Western Medicine

Conventional Western medicine was often considered a first choice for seeking care. Even for a Chinese-born individual like Participant 1 whose first language is Cantonese and speaks Cantonese and Mandarin at home, he claims “After I came to the U.S., I went to Western medicine more often.” He also admits to “use Western medicine or see a Western doctor first every time, and I see the Chinese doctor only when I think Western medicine does not help.” Conventional Western medicine was used during specific instances by study participants, such as when experiencing intense discomfort, gastrointestinal problems, kidney problems, prostate problems, back pain, emergencies, regular check-ups, cardiovascular problems (e.g., congestive heart failure), infection, high blood pressure, and “something more than a cold.”

Perceived severity was an influencing factor for seeking Western medicine occasionally, or ”If it’s more serious than a simple cold.” Emergency situations fed into the decision to see a Western doctor. Participant 5 recalled, “I didn't treat it at home because my headache was sudden, so I went to the doctor right away.” Similarly, Participant 3 proclaimed Western medicine was used sometimes, during an emergency: “I cannot wait for Chinese medicine, it’s too slow. Western medicine immediately saves your life.” Conventional Western medicine can be fast acting in comparison to TCM herbs, for example, that may take multiple days of brewing and consumption to see a noticeable impact on one’s health. Likewise, acupuncture is likely to require frequent sessions for optimal results. Like TCM, conventional Western medicine was also utilized when feeling sick and dealing with a headache.

Comparative Analysis

Cultural tradition and expression is far-reaching, as new beliefs and practices become widely recognized, and familiarity grows among individuals who were born in the U.S. to U.S.-born parents. Tomas Jiménez refers to them as “established” individuals, who have roots to the U.S. that extend at least three generations (Jiménez, 2017). The adoption of CAM, including TCM, by established individuals and institutions is indicative of the influence of ethnic culture on mainstream medicine. Born in the U.S.,

Participant 10 shared, “I think Western medicine would be my first option, I would try, although I will say in particular the acupuncture for my frozen shoulder and for my TMJ, I think that the acupuncture was the most effective thing I’ve ever done.” TCM was frequently complementary to Western medicine, reinforcing the definition of CAM. Traditional Chinese medicine and Western medicine are sought out for various reasons concerning one’s health, from curing or overcoming disease to maintaining and preserving health.

Both forms of medicine – TCM and Western medicine – are offered at community health centers across San Gabriel Valley and neighboring areas. Centers with TCM and Western medicine services under one roof primarily offer TCM services in the form of acupuncture. Acupuncture is the practice of using thin, metallic needles to stimulate anatomical points on the body and promote health (Wang & Wang, 2024). The combined use of acupuncture and Western medicine has proven therapeutically effective for anxiety disorders, knee osteoarthritis, pediatric care, and cancer, offering improvements in physical and emotional symptoms, quality of life, and patient well-being (Tan et al., 2020; García-Escamilla & Rodríguez-Martín, 2017). Preferences for one medical system over another are exemplified by participants like Jones, who stated, “The anti-inflammatory drugs and antibiotics given by Western medicine sometimes do not affect me. I have seen both Chinese medicine and Western medicine, and I think Chinese medicine is more helpful to me. Chinese medicine can regulate my body.”

Self-Care and Recovery from Illness

Medical care accounts for 10 to 20 percent of overall health. Other factors like health-related behaviors, socioeconomic factors, and environmental factors account for the other 80 to 90 percent (Magnan, 2017). Apart from TCM and Western medicine, homeopathy was sought by a study participant. In the following excerpt, Participant 7 highlights his use of complementary and alternative medicine besides TCM:

I have been exposed to "homeopathy". This is a system from Europe. Homeopathy mainly studies energy and can test the energy in you and the energy in the surrounding environment. Radiation from mobile phones and computers, water and air pollution, and toxins in food all have a great impact on the human body.

Furthermore, when asked about self-care practices, all study participants said that they exercised. Their exercise took place in the form of daily walking/jogging, hiking with friends once a week, attending the gym several times a week, or having an occupation that is physically demanding (e.g., being a dog trainer). Participant 10 experienced kidney failure on her right side, having to follow a strict diet, specifically a renal diet, following the advice of her specialists. Cooking and consuming healthy foods and taking daily supplements (e.g., B-complex, fish oil, vitamin D) and probiotics were the second most reported self-care practices. Lastly, massage, stretching, using herbal patches, drinking adequate water, sweeping the floor, maintaining a skin care routine, controlling one's emotions, and getting enough sunshine were other self-care activities reported by study participants. These self-care practices largely resemble health-promoting behaviors.

While there was some overlap with self-care practices, when asked about recovering from illness, some new ideas popped up. As with reported self-care practices, drinking plenty of fluids including water and massage were ways study participants recovered from illness. Considerably, half of all study participants reported resting and getting enough sleep as important for recovering from illness. Participants reported visiting the pharmacy to purchase nonprescription drugs, taking over-the-counter cough medicine, making chicken soup, visiting the doctor ("if serious"), going to the Chinese doctor for stomach sickness, taking TCM herbs, getting acupuncture, and using natural remedies, such as salt water and honey for sore throat. Participant 8 described at-home remedies as typically lasting 3 days before illness went away.

Taking traditional Chinese medicine herbs was reported as a way to recover from illness. Table 1 shows a list of commonly used Chinese medicine herbs, and the number of participants that reported using that herb. From the table, ginger root was the most used herb among study participants; poria and reishi were least commonly used. A significant number of participants also reported having used/using goji berry, chrysanthemum flower, dried tangerine peel, hawthorn berry, and turmeric. Herbs were commonly used in cooking, as with traditional dishes like four herbs (sì shén tāng) soup and ginseng soup. A former Chinese medicine doctor, Participant 7 displayed extensive knowledge on select, individual herbs:

> I take Chinese medicine. I use Chinese medicine as dietary therapy. Chrysanthemum flower, especially for the summer, I use it to clean liver fire. Ginger as food. Ginger is a warm food that can neutralize the coldness of vegetables. Yin and Yang balance. Dried lily bulb can treat upper respiratory tract inflammation and clear lung heat. Dried tangerine peel relieves cough and

reduces phlegm. Goji berries are good for the eyes and liver… Hawthorn berry is good for the cardiovascular system and can clear blood vessels. The curcumin in turmeric is good stuff. Purified curcumin consumed with black pepper is very good for cardiovascular health.

Table 1: List of common traditional Chinese medicine herbs and frequency of use

English name	Chinese name	Frequency (No.)
angelica root	dang gui	4
astragalus root	huang qi	5
atractylodes root	bai zhu	4
black peppercorn	hei hu jiao	6
black sesame seed	hei zhi ma	6
chysanthemum flower	ju hua	8
cinammon bark	rou gui	5
codonopsis root	dang shen	4
coix seed	yi yi ren	6
dried lily bulb	bai he	5
dried tangerine peel	chen pi	7
ginger root	jiang	10
gingko nut	bai guo	5
goji berry	gou ji* zi	9
hawthorn berry	shan zha	7
jujube date	zao	6
licorice	gan cao	4
lotus seed	lian zi	5
poria	fu ling	3
reishi	ling zhi	3
turmeric	jiang huang	7

*Some sources will refer to it with a “q,” as with “gou qi zi.”

Discussion

By referring to traditional Chinese medicine as a “daily conditioning of the body,” it almost seems TCM pervades all of life. Even in TCM’s introduction to the United States in the mid-19th century, Chinese herbalists played several, very distinct roles. This included functioning as Chinatown’s postmaster, banker, translator, labor broker,

and defender in addition to serving in their primary role as herbalist. Their role was not limited to medical services. As a result of their leadership and ubiquitous presence in the lives of Chinese individuals, Chinese herbalists were able to help sustain these underserved communities (Venit Shelton, 2020). It was not strictly about caring for their health but ensuring their survival. As such, they put their communities on their shoulders, taking care of community members in all aspects of their livelihood. In their attempt to overcome the isolation and lack of social assets present within their communities, Chinese herbalists were able to promote self-sufficiency.

Perhaps, some individuals did not consider acupuncture as a form of TCM because physicians consolidating the traditional medicine marginalized external treatments, including those of acupuncture and eye surgery. These procedures were seen as superstitious and technical (Lo & Stanley-Baker, 2018). Specifically, when asked about the use of acupuncture, participants either said they had used acupuncture in the past and/or they were currently using acupuncture. A second argument for TCM's dissociation with acupuncture can be made. It is possible that acupuncture has become more and more regarded as falling under the purview of conventional Western medicine. The application of acupuncture has extended into Western medicine procedures, including transcutaneous electrical nerve stimulation, subcutaneous nerve injection therapy, peripheral nerve stimulation therapy, and dry needling. In the last 50 years, the United States has introduced certification processes for M.D.s wishing to get certified as medical acupuncturists (Gong, 2020).

Western medicine was easily recognized by participants as part of their healthcare regimen, whereas this was not always the case for using traditional Chinese medicine. It is important to recognize that the posing of the interview question could have played a direct role in this. The question, "Do you use traditional Chinese medicine?" could be interpreted as using traditional Chinese medicine as a self-medicating practice rather than a form of healthcare. Perhaps, asking the question, "Do you visit a traditional Chinese medicine doctor?" could have been clearer to the interviewee. Due to the physical and sociocultural contexts, conventional Western medicine as the predominant medical practice of the United States can distinguish itself from traditional Chinese medicine despite not being entirely mutually exclusive. One example is that various Western medicines, including antibiotics and anticancer medicines, have been derived from Chinese herbs.

Among all participants, there was a weighty decision that needed to be made using good judgment about which kind of provider to seek out and whether they are trained in conventional Western medicine, traditional Chinese medicine, and/or other schools of thought. This occurred on a case-by-case basis where past experiences, cultural preferences, and beliefs around efficacy contributed to the decision-making

process. For some, conventional Western medicine was viewed as being more advanced, and hence, more desirable. Others recognized the strengths of each form of medicine individually and applied their past experiences of conventional Western medicine and TCM to delineate the kind of healthcare they would seek in the future based on their medical problems; as such, this process is highly contextualized.

Culture is a dynamic force that may help to form individuals' health behaviors and healthcare-seeking attitudes. Behaviors and attitudes related to health and healthcare-seeking seemed to change over time for study participants. The majority of participants were born abroad and moved to the U.S. a few decades ago. After interacting with the healthcare system, some started to develop new conceptions of Western medicine as "more advanced." When the minority-culture individual takes the dominant form of medicine in the host country as the default, new behavior(s) becomes adopted. This adoption of behavior is now a regular, routine part of one's actions and thought processes (Fox et al., 2017).

The extent to which the host culture leads to a minority-culture individual's internalization of host cultural orientation depends on sociocultural context (Fox et al., 2017). Acculturation shapes one's worldview in the new environment they are in. The influence of the group can play a key role in shaping behavior, which extends this discussion to the fields of sociology and social psychology (Edberg, 2020). Through trial and error, participants who were conventional Western medicine-leaning were able to distinguish situations for which Western medicine was effective and ineffective. For the latter circumstance, participants typically relied on TCM, natural remedies, and self-care strategies to improve health and restore their quality of life.

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Analyzing the Prevalence of Racial Disparity in Pain Perception and Pain Treatment in Patient-Provider Relations

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Abstract

The United States healthcare system offers one of the highest quality accesses to pain management, however there is substantial evidence of racial disparity in pain perception due to racial bias in patient-provider relations. The fundamentals of this relationship influence the interpretation of pain assessment, quality care, and improvement of pain management. America's identity as a melting pot of diverse races and ethnicities is a significant factor of health disparity because of false racial biases surrounding pain and pain treatment of racial minorities. Evidence shows that racial minorities are misrepresented in pain intensity scores, receive less quality treatment and attention, and are hindered by limited access to pain care. As a result, this disparity causes aversive physiological consequences, distrust in patient-provider relations, continuous misinformation fueling biases, and increased minority disappointment with healthcare. This paper reviews the racial biases in pain perception in the U.S. healthcare system and aims to provide an insight into its effect on racial minority relations and treatment in the hospital.

Keywords: pain perception, pain management, pain treatment, racial bias, racial minorities, patient-provider

Introduction

Pain, as defined by the International Association for the Study of Pain, encompasses "an unpleasant sensory and emotional experience associated with, or resembling that associated with, actual or potential tissue damage" (Raja et al., 2020). It is commonly classified into three primary types: acute (lasting less than six months), episodic (occurring at intervals), and chronic (persisting for over three months) ("Pain," 2024). Importantly, pain transcends physiological conditions, often stemming from cognitive, social, and psychological factors, yet it remains inherently variable among individuals. This inherent variability underscores the complexity of patient-provider interactions, where understanding of pain can become distorted.

Consider a scenario where a patient presents with intense, persistent stomach pain to a local physician, expecting a thorough assessment and tailored treatment plan. However, during the interaction, the physician dismisses the patient's pain concerns, opting for common pain relievers without addressing the underlying issue. Such encounters not only fail to alleviate the patient's pain but also engender feelings of disregard and erode trust in the healthcare provider.

Unfortunately, such experiences are all too common among minority communities, indicative of the systemic challenges they face in accessing equitable care (Plater, 2021). Historically, minorities have been disproportionately ignored and undertreated, particularly in the realm of pain assessment and management (Campbell & Edwards, 2012). This disparity perpetuates a structure of bias and discrimination, fueled by the blind spots of structural racism within the healthcare system.

Racial bias significantly influences adverse health outcomes, contributing to heightened rates of serious injuries and mortality. For instance, hospitals serving predominantly black trauma patients exhibit a 45% higher mortality rate compared to those with lower concentrations of black patients (Glance et al., 2013). Similarly, pregnancy-related mortality ratios for Black and American Indian/Alaskan women over 30 are four times higher than those for White women (Petersen, 2019). These disparities extend to cancer-related pain management, where minorities are undermedicated due to a lack of guideline-recommended prescriptions compared to non-minority patients (Cleeland et al., 1997). These instances underscore the pervasive nature of pain mistreatment and the evident racial disparities across all healthcare sectors.

Racial biases in pain perception and treatment recommendations often stem from entrenched sociocultural and historical beliefs. This review endeavors to provide a

comprehensive analysis of racial and cognitive biases in pain, exploring themes of patient-provider mistrust and misconceptions, and elucidating the systemic and cultural influences on pain management. By shedding light on these factors, this study aims to inform targeted interventions aimed at mitigating racial disparities in healthcare delivery.

Historical Background of Racial Bias in Medicine

Throughout history, racial biases against minorities, particularly in the context of pain management, have been deeply entrenched and perpetuated by scientific, medical, and societal beliefs (Hoffman et al., 2016). As medical science advanced, prevailing beliefs in the inherent biological differences between minority and white populations influenced research experiments and medical practices. These erroneous notions often served to justify inhumane treatment and malpractices against minority communities (Guillory, 1968).

One notorious example of such racial bias is the Tuskegee Syphilis Study, conducted from 1932 to 1972. In this unethical experiment, African American men with syphilis were deliberately left untreated, despite the availability of effective treatments such as penicillin. The study aimed to observe the progression of untreated syphilis, leading to severe health consequences and needless suffering among the participants. This type of exploitation of vulnerable minority populations highlights the extent to which racial biases permeated medical research and practice (Washington, 2006).

Furthermore, the prevailing belief that minority bodies were inherently more accustomed to pain often led to the exploitation of minority groups in medical experiments (Washington, 2006). For instance, African Americans and other minorities were subjected to painful and invasive procedures without their consent or adequate medical care. These experiments, conducted under the guise of scientific inquiry, inflicted unnecessary suffering and perpetuated racial biases in pain management (Trawalter and Hoffman, 2015).

Pain Perception

The concept of pain perception examined within this review pertains to healthcare providers' assessment of a patient's pain intensity, influenced by stereotypes related to the individual's race, ethnicity, gender, and other factors. Notably, racial biases play a significant role in shaping these perceptions (Campbell & Edwards, 2012). In the context of this discussion, racial bias against minority groups, including American Indian or Alaskan Native, Asian, Black or African American, and Native Hawaiian or Other Pacific Islander populations, is not rooted in overt racism but rather in erroneous

beliefs and biases regarding biological differences between non-white and white individuals (Budget, 1997). This bias extends to the treatment, recommendations, and management of pain among minority populations.

Historically, erroneous beliefs, such as the misconception that Black individuals possess higher pain tolerance due to thicker skin, have perpetuated racial biases in pain perception (Meints et al., 2019). Within the framework of pain-related theories, these biases have led to the unfounded assumption that minorities exhibit greater resilience, resistance, and desensitization to pain compared to their white counterparts, thereby justifying past injustices such as slavery and segregation, as well as contemporary racial disparities in healthcare (Trawalter and Hoffman, 2015).

Central to this flawed belief is the misconception that pain resilience equates to the absence or reduction of pain. Pain resilience, however, is a multifaceted construct encompassing dispositional resources and coping mechanisms that can enhance pain management, but it does not negate vulnerability or the presence of pathology (Bartley et al., 2017). While some research suggests that minorities may exhibit higher levels of pain resilience overall, it is crucial to recognize that their perceived pain intensity can match or exceed that of white individuals (C. Evans et al., 2017). Indeed, studies have demonstrated that minorities often report higher levels of pain intensity (Yang et al., 2021).

Effective pain care and treatments should not be predicated solely on unilateral judgments and assessments by healthcare providers, particularly given the potential influence of implicit racial biases (Chapman et al., 2013). Inadequate communication with patients may lead to the undervaluing and dismissal of their pain experiences, exacerbating health disparities and hindering efforts to foster inclusive healthcare practices and research (Hausmann et al., 2011). This perpetuates a power imbalance in healthcare, where providers impose their perceptions of what is appropriate, fueled by implicit racial biases, thereby perpetuating racial disparities and exacerbating inequities in pain treatment between white and non-white populations (Gowda et al., 2019).

Patient-Provider Relations

Successful patient-provider connection with favorable health outcomes often is from the basis of a collaborative relationship (Harbishettar et al., 2019). This starts with the manner of communication as a primary method of influence in the nature of the relationship and key indicator of racial bias (Meints et al., 2019). Deflecting (passive) conservations are characterized by which the provider uses avoidant or dismissive words that brush over the patient's health issues. This can cause the patient to question

their perception, distance themselves, or avoid voicing their concerns, expectations, and request for more information (Ha & Longnecker, 2010). Misinterpreted conversations also prove to be a hinderance to proper care. Due to cultural barriers, certain words or descriptions may be understood differently causing both parties to misunderstand each other in terms of pain assessment, influencing the type of pain treatment recommended (Meints et al., 2019). Another type of communication is the physician-centered conversation by which the provider tends to emphasize his or her beliefs against the patient's opinions or expression (Berman & Chutka, 2016). This discourages dialogue, lowers the chance of utilizing valuable information for better management, and reduces collaborative effort.

The quality patient-provider relationships are sometimes predetermined from previous experiences and notions determine how providers may act towards the patients regardless of the provider's race (Chapman et al., 2013). The same also applies for some patients. Patients who have experienced racial bias or are exposed to it may become tense, fearful, or uneasy during a healthcare interaction. Assessments to gauge pain intensity may be dismissed as a mere formality or a guise for unwanted medical criticism from the preconceived judgement of healthcare providers minority patients hold (Harbishettar et al., 2019). These beliefs are not unwarranted as there is much evidence and cases of providers adversely responding and behaving from a seemingly racially biased approach (Tait et al., 2009). Because of patient distrust in the healthcare system, it creates a distance by which providers may disregard or not perceive patient frigidity limiting the possibility of effective treatment. Frustration can ensue for both parties as patients may become increasingly non-compliant and unsatisfied with healthcare whilst providers become notably accustomed to feeling less motivated to be diligent with patients (Dyrbye, 2022). Low patient-centered interaction increases the likelihood of provider avoidance subsequently directing them to utilize complementary and alternative medicine potentially distancing them from healthcare system (Faith et al., 2015). These conflicting characteristics are factors that further amplify health disparities amongst racial groups, especially in the prevalence, treatment, and outcomes of pain-related conditions.

Lack of proper education about minority patients and their culture is one of the most prominent constituents of a patient-provider relationship (Madeira et al., 2022). If healthcare providers were properly trained on the upstream and downstream determinants of health and social and cultural issues of minorities, they would be able to tailor their pain management care accurately (Franks & Fiscella, 2008). For example, a provider might assume a Hispanic man is facing back pain because they work too much and does not care to rest. However, Hispanic populations tend to live in small, clustered homes with substandard beds, work in conditions not suitable for adequate back health,

and may not have enough knowledge about sustainable health management. If the provider took initiative to research about health and cultural factors affecting the Hispanic population, he could tailor the pain treatment to provide him affordable bed options, health resources for back pain alleviation, and educate and reassure him about taking prescribed medications. These types of mindful approaches reinforce the cohesiveness and consistency of a patient provider relationship (Smith & Martini, 2023). It replaces the standard view of the patient as a system to the patient as a person.

Mechanisms Influencing Disparities

The reliance on "Western, Educated, Industrialized, Rich, and Democratic" (WEIRD) populations in medical contexts and studies can exacerbate disparities and perpetuate biases, particularly in the context of pain management (Burkhard et al., 2021). This term, commonly employed in social science research, underscores the homogeneity of certain demographic groups. However, studies predominantly conducted on WEIRD populations may fail to capture the diverse experiences and perspectives of individuals from non-WEIRD backgrounds, particularly racial minorities, in the realm of pain management (Lillas & Marchel, 2015). This research gap significantly contributes to the proliferation of false beliefs rooted in three key underlying mechanisms that perpetuate disparities in pain treatment: biological, societal, and structural (Campbell & Edwards, 2012).

Biological

From a biological standpoint there are many prevalent false beliefs about biological peculiarities of minority and white populations. This dates backs to the early centuries such as during the start of African slave trades where scientist, physicians, philosophers, and regular owners endorsed these beliefs (Cartwright, 1851). Some of them have become a staple in the medical field which has influenced the consistency of racial bias even up until date (Feagin & Bennefield, 2014).

A key false biological misbelief comes from the idea that minorities, especially black individuals, have higher pain tolerance. This pain tolerance is attributed to minor occurrences like small cuts to more life-threatening circumstances like surgery or heavy bleeding (Mende-Siedlecki et al., 2022). Even in the instance of bleeding, it is believed that their blood coagulates more than whites, so they have a higher chance of withstanding (Hoffman et al., 2016).

As previously mentioned, minorities are believed to feel less pain than white counterparts so when they complain it is seen as an exaggeration or feigning of pain

(Trawalter et al., 2019). False narratives such as minorities have less sensitive nerve endings, are most biologically prone to develop drug addictions, or have fewer need for higher dosages of anesthesia for surgery has consequently led to the under-prescribing of pain medication and misevaluation of pain intensity.

From a mental illness standpoint, minorities are believed to have less chronic onset and symptoms because they have stronger mental capacity. However, this is far from the case as they are found to have more severe and debilitating forms of mental illness when properly assessed. The reality is most minorities are either underdiagnosed or misdiagnosed.

These biological beliefs are not hidden from the minority populations by which to a degree has also been transmitted as standard to them. Some minorities have taken on these unfounded claims and made it their reality therefore causing them to think their pain is insignificant and they can take more on closing them off from seeking proper treatment or even expressing the true reality of their pain.

Societal

Implicit bias in the healthcare system is also caused by sociocultural beliefs that affect the treatment and quality of care minorities receive in terms of alleviating their pain. Implicit biases of the pejorative stereotypical minority personality traits engrained in society's conceptions of each race have adversely influenced healthcare providers' behavior towards minority populations.

The belief that minorities are more aggressive than their White counterpart factors into how healthcare providers assess them (Chapman et al., 2013). Healthcare providers may assume that because minority individuals are unfeeling, they are exaggerating their pain sensitivity or seeking emotional validation (Trawalter et al., 2019). Because of this false assumption they may make the patient visitation abrupt or depersonalized to distance themselves from stirring up possible drama or unwanted conflict. When the patient is perceived to seemingly undismayed by their pain the provider may equate it to toughness thereby interpreting their pain level as maintainable (Kuntsman et al., 2023). This gap in patient-centered attention increases as providers unconsciously spend more time with White patients and are more inclined to perform comprehensive health check-ups whilst undervaluing time with minority patients.

Society has conferred the feeling of distrust with minorities because they may lie or manipulate to get a certain result. For example, African Americans have less access to certain analgesics because they of the belief that they may misuse it for inappropriate means (Ng et al., 1996). Some providers may think that if a minority has a high score on

their pain assessment, they may have manipulated their true scores because of a crafty motive behind it.

Structural

The healthcare system was not designed to serve and improve customized care for minorities but to cater to White patients in terms of health measures, treatments, and care (Feagin & Bennefield, 2014). The healthcare system is still consistently adapting and transforming, to advance minority centered care and research. Nevertheless, racial divide still exists and affects the outcomes of pain treatment and management (Meghani et al., 2012).

Minorities tend to have less unequal access to pain analgesics and difficulties receiving appropriate prescriptions. In low-income minority neighborhoods drug stores, pharmacies, pain specialists, etc are limited in supply or in service to properly serve the unique needs of the patients (Campbell & Edwards, 2012). This is evidence shows how racial bias extends to locations and thus proximity to resources. Minority patients feel excluded from the quality access and thereby resolve their issue by settling for general over the counter medicines (Anderson et al., 2009). Patients express more hesitance and avoidance to pain medications; they still express the need of having it available when necessary (Meints et al., 2019). Lack of access to proper healthcare also comes from doctor recommendations of low care treatment plans, medications, or facilities to minority populations leaving them prone to settling with ineffective interventions. When these populations do have substantial access to healthcare facilities, they are limited in their care.

In terms of timeliness of care, racial bias is present in priority care. Some healthcare facilities unconsciously cause minority patients to wait longer for pain treatment. Minorities patients are more likely to be placed on a longer wait time for curing painful ailments like chemotherapy, booking doctor’s appointments, and receiving timely medical assistance (Soares et al., 2019). Negligent actions like these are related to racial biases that minorities can endure pain longer or because of high resilience, urgency is not a primary factor. Because most minorities tend to be uninsured or have low quality insurance their treatments tend to be episodic or discontinuous (Hadley, 2007).

In healthcare, pain assessments are general and may not capture the true intensity or causes of the pain due to implications such as language, interpretation, or scope of question (Adeboye et al., 2021). It is uncertain how well administered pain assessment tools and measurements capture a patient’s true experience of pain since they were not

designed to encourage further consideration of influencing factors like racial backgrounds, social economic status, and individuality (Rambachan et al., 2023). General pain evaluations may only provide downstream solutions (proximal factors) whereas comprehensive assessment tools can also consider upstream solutions (distal factors) which can better address individual and social issues and possibly prevent the prevalence of racial bias in pain treatment (Letzen et al., 2022).

Historically, healthcare treatments have predominantly catered to the needs of the White population, often overlooking the diverse approaches necessary for addressing the healthcare needs of different racial groups (Meints et al., 2019). For example, imagine a scenario where a working-class black man and a full-time white-collar worker both experience heel pain. The recommended treatment for both individuals may involve minimizing physical activity for a month. However, this approach fails to account for the socioeconomic disparities that exist between the two individuals. While the white-collar worker may have the option to work remotely or limit physical activity, the black man, who works in a factory performing heavy labor, may face significant challenges in adhering to these recommendations. This disparity highlights the need for healthcare providers to adopt a more inclusive and culturally sensitive approach to treatment, one that considers the unique circumstances and challenges faced by individuals from diverse racial backgrounds (Anderson et al., 2009).

Confounds of Racial Bias

While it is crucial to recognize the presence of racial bias in healthcare, it is equally important to acknowledge that not all adverse actions stem from provider biases. A significant contributing factor can be the desensitization doctors experience due to the high volume of cases they handle throughout their careers (Tertemiz, 2019). This desensitization may lead them to inadvertently underplay the concerns of all patients, regardless of race. Consequently, patients who feel targeted may mistakenly attribute such actions to racial bias. Moreover, patients may enter medical settings with preconceived notions, particularly concerning interactions with White doctors, suspecting them of being judgmental rather than collaborative. This suspicion can lead patients to withhold expressing their true level of pain, hindering the development of a trusting patient-provider relationship (Green et al., 2003).

Cultural beliefs also influence how patients express and assess their pain, interact with healthcare providers, and engage in pain management processes (Lasch, 2000). Providers' interactions may be influenced by the patient's cultural nuances which could cause them to unknowingly project adverse reactions, communication, and decision-making processes onto the patient (Lasch, 2000). Such scenarios can falsely create

perceptions of racial bias. For example, in certain communities like the Black community, there may be a cultural endorsement of enduring pain as a mark of resilience. Consequently, individuals may accept higher levels of pain without complaint, skewing pain assessment scores and potentially leading to erroneous beliefs about bias.

Furthermore, a lack of education among minority populations regarding pain assessment and management can exacerbate these issues (Meints et al., 2019). Patients may not possess the knowledge to accurately articulate their pain experiences, leading providers to either underestimate or overemphasize their pain level. For instance, misconceptions about racial thresholds for pain severity may lead minority individuals to believe that only extreme pain intensities warrant serious consideration while low-mild forms of pain are dismissed by which thereby they riskily they may restrict themselves to only report higher pain severities (Deska et al., 2020). Additionally, systemic issues such as healthcare providers being trained to prioritize efficiency over patient rapport can further exacerbate these disparities, leading to a lack of foundational trust between providers and patients (Harbishettar et al., 2019).

Conclusion

In conclusion, the paper delves into the multifaceted nature of pain perception, assessment, and management, particularly focusing on the influence of racial biases within the healthcare system. Pain, often experienced differently among individuals, is shaped not only by physiological factors but also by cognitive, social, and psychological dimensions. However, systemic challenges, historical biases, and cultural beliefs have perpetuated disparities in pain assessment and treatment, especially among minority communities. The impact of racial bias on patient-provider interactions, where erroneous beliefs about pain tolerance and resilience among minority populations have led to undertreatment and dismissal of pain concerns. These biases have historical roots and are perpetuated by societal stereotypes, leading to adverse health outcomes and perpetuating health disparities.

Furthermore, the paper underscores the importance of patient-provider relationships in pain management. Successful relationships are built on effective communication, mutual trust, and understanding. However, racial biases can hinder these relationships, leading to misinterpretations, dismissals, and lack of appropriate care. Potential mechanisms through which racial biases manifest in pain treatment, including biological, sociocultural, and structural factors. These biases not only influence pain perception but also affect the timeliness and quality of care received by minority populations.

There is a significant need for targeted interventions aimed at mitigating racial disparities in pain assessment and management. Addressing implicit biases, improving cultural competence among healthcare providers, and fostering patient-centered care are crucial steps toward achieving equitable healthcare delivery and reducing health disparities among diverse populations.

Future Directions

Recognition and Acknowledgment of Racial Bias

Moving forward, it is imperative for the healthcare community to recognize and acknowledge the existence of racial bias within the medical system (White et al., 2017). This acknowledgment must extend to all levels of healthcare, from frontline providers to policymakers and medical educators. By openly acknowledging the reality of racial bias, steps can be taken to address and mitigate its impact on pain perception, assessment, treatment, and management (Morais et al., 2022).

Integration of Anti-Bias Training in Medical Education

To combat racial bias effectively, anti-bias training should be integrated into medical education curricula. Early provider training programs should include comprehensive modules on cultural humility, implicit bias recognition, and strategies for promoting equitable care (Morais et al., 2022). By instilling these principles early in medical training, future healthcare providers can develop the necessary skills and awareness to provide patient-centered care that is not constrained by race, gender, ethnicity, or other demographic factors.

Transformation Towards Patient-Centered Care

The healthcare system must undergo a transformation towards true patient-centered care. This involves shifting the focus from provider-centric models to approaches that prioritize the individual needs and experiences of patients (Meints et al., 2019). Healthcare delivery should be tailored to consider the unique cultural, social, and psychological factors that influence pain perception and management for each patient. By embracing diversity and inclusivity, healthcare providers can foster trust, enhance communication, and improve health outcomes for their patients (Ghoshal et al., 2020).

Promotion of Diversity and Representation

Increasing diversity and representation within the healthcare workforce is essential for addressing racial bias and promoting equitable care. Efforts should be made to recruit and retain healthcare professionals from diverse backgrounds, including underrepresented racial and ethnic groups (Ghoshal et al., 2020). Additionally, healthcare organizations should strive to create inclusive and supportive work environments that empower individuals from all backgrounds to thrive in their careers. By promoting diversity and representation, the healthcare workforce can better reflect the communities it serves and provide culturally competent care.

Continued Research and Evaluation

Finally, ongoing research and evaluation are crucial for identifying and addressing racial bias in healthcare. This includes conducting studies to better understand the underlying mechanisms of bias, evaluating the effectiveness of interventions aimed at reducing bias, and monitoring progress towards equitable care outcomes (Campbell & Edwards, 2012). By continuously monitoring and evaluating practices, policies, and outcomes, healthcare organizations can identify areas for improvement and implement evidence-based strategies to promote health equity for all patients.

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Looking for Ted: Black Trips, ‘Psychedelic’ Humanism, and Silence

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Abstract

Within Stanislav Grof and Joan Halifax’s The Human Encounter with Death is a claim that anxiety towards death can be relieved. As the book itself maps out Grof’s research with terminally-ill cancer patients and their respective psychedelic experiences. Within the bounds of this research is Ted, the only black patient with a qualitative report in the text. His very position within the text serves as a challenge to Grof and Halifax’s argument. Thinking alongside the violent history of racialization and coloniality, I ponder the position of Ted within what was then an emerging discourse of psychedelic science.

It is near impossible to piece together a definitive archival narrative of Ted from the fragments of his life curated by Grof and Halifax. But rather than dwelling on this impossibility, I argue that Grof and Halifax’s narrative ambivalence is crucial to imagining the place of blackness within psychedelic science. My paper asks what happens if the normative tripping that liberal humanism expects cannot be written about through qualitative reports? What if the tripping of Ted rests in alternative ways of knowing that resist the objectification of blackness?

I look for Ted. A black existence in an archive that has failed him. But I read not just for failure, I read for Ted. To meet him, even in all this failure, but I wonder if I will fail him. This paper will focus on blackness within the psychedelic archive, particularly Stanislav Grof and Joan Halifax's The Human Encounter with Death. Published in 1977, this book was a product of Grof's research in the early 70s at Spring Grove Clinic. His work was centered on the emergent scientific inquiry into the effects of psychedelics on "human" test subjects. Grof and Halifax sought to understand how psychedelics could overcome terminally-ill patients' anxiety towards death. The cultural production of death became critical to their argument while little cultural scrutiny was given to race, ability, or class. Yet the historicity and cultural contextualization of death ran central to their alternative model of engaging what mortality was through psychedelics. My paper thus sits with the cultural specificity afforded to psychedelics in conversation with death and imagines the limitations of said specificity when blackness and Otherness are brought into the conversation. I ask how we might read subjectivity within the psychedelic archive when it comes to a black psychedelic subject? To answer this question, I examine the implicit anthropological models that ground patient biographies and dissect what I term the psychedelic humanist model. Furthermore, in mapping the ways that Grof and Halifax narrativize Ted, I ponder how an archival engagement with Ted might push against the objectifying trends of psychedelic science. Finally, I piece together what my search for Ted sought to recuperate, as I instead found the destabilizing residue of the black trip within the psychedelic archive.

To understand the subject of the text, we must first situate the frame of the text. In particular, what do Grof and Halifax mean by the titular "human encounter with death"? Who is the human we are reading about? In posing such questions, I take Halifax's training as an anthropologist as a critical point of departure: how is a subject historically rendered in anthropology? Who then is afforded this human subjectivity? How does anthropology valorize these normative models through its anthropological subject? As Sylvia Wynter notes, the very crux of the political and scientific emergence of Man (which as a term stands in for the normative mode of being human) was predicated on the concept's construction by scholars "including centrally those of anthropology" who would force "non-Europeans" to be the "physical referent" (in the episteme of Man) of the "irrational or subrational Human Other" (266). This Man would then evolve due to what Wynter terms the Darwinian Leap, in purely secular and thus in "biologized terms, it was to be the peoples of Black African descent who would be constructed as the ultimate referent of the 'racially inferior' Human Other" to Man (266). This is to say the normative script that Halifax's anthropological methods are working through are sutured to a disciplinary history that can only represent this normative model of human, unless they grapple with and deconstruct the racialized history of humanity as is normatively known. Halifax and Grof fail to do so, instead abiding by the unquestioned script of Man.

Thus the desired anthropological subject is one that can approach the normative discourse of humanity. In order to have their subjects occupy this normative humanity, Grof and Halifax's study wash away blackness and the Otherness inextricable to their anthropological subject. As test subject, Ted, then, is afforded his humanity (from which he is normatively excluded) on the terms that a psychedelic experience grants him entry into the position. Thus the washing away of blackness and Otherness is important to their study, for it pieces together their universalist model, as the humanity of Ted seemingly emerges from psychedelic use. This is to say, a new universal model of thought comes into being once blackness and similarly Otherized positions are seemingly erased from the ontological model. We can see this in the way that Ted is "cured" of his Otherness and, through this "cure," shifts into the cured position of the psychedelic subject. But, the architecture of this racially sanitized and seemingly curative subjectivity is built upon a shaky foundation of a universalizable engagement with death.

This desire to undo the normative, empirical prescriptions of death is hinged on the fabrication of our current empirical mappings of death. For Grof and Halifax, death is a problem because of the way it is normatively presumed to be an end point of the state of being in the West. This illuminates why their reading is interested in "compar[ing] the situation of a person facing death in contemporary Western civilization with that of individuals in ancient cultures or from preindustrial countries… [since m]ost non-Western cultures have religious and philosophical systems, cosmologies, ritual practices, and certain elements of social organization that makes it easier for their members to accept and experience death" (2). Though an arguably reductionary comparison, they seek to mobilize disparate cultural definitions of death to challenge the normative prescription of death in the West. This, to Grof and Halifax, would aid in the overcoming of the anxiety around death, which is arguably a product of Western empiricism that holds no answer to what death is. This gap then leaves their work as a means of rethinking what death is through non-normative means: psychedelics. "Psychedelics drugs" they maintain, "have made it possible to develop a new understanding of the symbolic death-rebirth process that occurs in shamanic initiation, rites of passage, temple mysteries, and some schizophrenia episodes. Deeper phenomenological analysis shows that the extended map of the human unconscious derived from LSD research is indeed applicable to all these situations" (Grof, 175). Though a tenuous line, Grof and Halifax are theorizing that psychedelic science might provide the empirical foundation towards a cosmology beyond our normative empiricist life-death order. In other words, death is not the end, but merely an entryway into another way of being human—a seemingly better way due to its curative qualities. Yet their work suggests that these "ancient" claims must be "reformulated in modern terms" through the empiricism of phenomenological analysis of psychedelic experiences (175).

Thus the terminally-ill patient turns into the psychedelic subject that will (re)map these potential cosmologies that speak towards a here-after. Critical in this mapping then is also an apparent redescribing of the human, which is evident in the Grof and Halifax's rigorous contestation of the purely secular model of Man that defines the present human within their work. Instead, they turn towards empiricism (or better termed, Reason)1 yet yearn to sit with the spiritual as well, hence their common collapsing of 'non-normative spiritual experiments' by patients into Buddhist or Hindu readings (67, 79, 83, 93, 101). Though this common practice in their work did not limit their test subjects into a particularly Buddhist or Hindu narrative, it did foreclose their non-normative spiritual explorations—as seemingly arrived at through psychedelic experiences—entirely into Grof and Halifax's 'new' humanism. In effect, this 'new' human model they yearned towards was a reasoned yet spiritual model of the human—a psychedelic humanism. A movement of the human away from the normative precepts of contemporary Western, purely materialist empiricism towards an apparently unstable humanism that dared to accept not just a material way of being but also a spiritual way of being human, which became accessible through psychedelic experiences.

For all its seeming opposition to normative Western narratives, this model dittos the secular-religious model of Man that structures liberal humanism: what Sylvia Wynter would term a "hybridly religio-secular" (277) description of Man whose "choice [was] that of either growing downwards into the lower natures of brutes, or responding to the Creator's call to grow 'upward' to 'higher' and 'divine' natures" (287). Though not an exact model, the similar model speaks towards psychedelic humanism's curative yearning for a metaphysical frontier found through the material plane of being. This is to say that psychedelic humanism reflects the Wynterian "hybridly religio-secular" in its use of psychedelic subjects to map out a spiritual being while simultaneously instrumentalizing the material plane as a tool for accessing this spiritual elsewhere. This elsewhere becomes central to Grof and Halifax's project, for this is where marginalized subjects (particularly disabled patients) serve their use-value for the benefit of science, especially Ted. Thus their apparently human condition afforded through psychedelic use continues the practice of the Other becoming the defining lack for the human of the text. Their entry into 'humanity' is a mere ploy to map out the limits of the psychedelic

[1] For a more thorough engagement with antiblackness, empricism and Reason, please see La Marr Jurelle Bruce's *How To Go Mad Without Losing Your Mind: Madness and Black Radical Creativity*. Bruce maps out the rampant epistemic violence that empiricist and Reasoned epistemologies sustain, especially through their intentioned exclusion of black and Otherized existences (Bruce, 4).

experience and thus prove that psychedelics can be an empirically viable cure to anxiety towards death.

With this humanist-defining mechanism in place, it is imperative we understand the archival position that this research occupies, for it speaks toward a genealogical necessity in the research of psychedelic science. I must acknowledge Grof and Halifax's work is not at the forefront of psychedelic science and its present discourse. Yet the very discipline, as it is known today, is built off of the research done at Spring Grove Clinic, just as it is built off the backs of so many other psychedelic science institutions and projects. In speaking about the Spring Grove Clinic, Richard Yensen and Donna Dryer argue that psychedelic scientists "must use the insights available from past efforts in this culture and others to develop" contemporary research efforts (21). The research at Spring Grove Clinic's cultural influence carries sway since Rick Doblin was trained and influenced by Stanislav Grof (Endwell). To note, the Multidisciplinary Association of Psychedelic Studies (MAPS), where Doblin is executive director and its founder, doled out $12 million in the 2019-2020 fiscal year for psychedelic research (Christiansen). However, this is small compared to the companies that are investing hundreds of millions into research and development of psychedelic drugs ("Psychedelic Bulletin"). MAPS has been a cultural attaché for psychedelic research and therapist training. In thinking through these terms, it is critical to view the work done in The Human Encounter with Death not through a vacuum, but rather as a lived cultural artifact that is imbricated in the ongoing discourse of psychedelic science/therapy. This is easily seen in contemporary studies that have inherited the same central questions on the influence psychedelic experiences can have on anxiety towards death that Grof and Halifax originally focused on.2 A direct line even being acknowledged by Moreton et al. as they state how their work "echoes the claims of many early psychedelic researchers such as Walter Pahnke" (28). Pahnke directed the Spring Grove Clinic from 1967 to 1971 and worked alongside Grof on studies related to anxiety towards death.3 Subsequently, while Grof and Halifax's text is not contemporary psychedelic science, it speaks to the discursive focus of a particularized time that continues to influence the field, and it manages to explicitly fold blackness (through the figure of Ted) into the complex conversation of psychedelics. Thus in acknowledging the psychedelic archive, we are not viewing these texts as the end all be all, but rather as emergent conversations that

[2] See Sweeney, et al., 2022; Griffiths et al., 2016. Griffiths also cites Grof's 1973 qualitative study on psychedelics and anxiety towards death, continuing to validate the influence of this research to contemporary psychedelic science.

[3] See Pahnke, et al., 1970; Kurland et al., 1972.

establish and set foundational discursive norms. As such, the archive provides us a mode of thinking with these emergent discourses without acting like they came out of thin-air; we are provided the space to think with particular ruptures and movements that give rise to approaches or the lack of approaches that have been inherited over time.

With this archival acknowledgement in mind, I turn to Ted to examine his own influence within our conversation around psychedelics. What we come to find is that Ted occupies a particularized role that "functions to index the limit of science" due to his "blackness" (Warren, 110-111). According to Calvin Warren, it is through nothingness that science can even function, but it is by avoiding/overcoming nothingness (thus blackness) that science fundamentally works (110). As the very "function of black(ness) is to give form to a terrifying formlessness (nothing)" (5). Better said, the very idea of nothing within our epistemic structure, as organized through humanism, requires a lacking position to structure what it means to be and who is afforded being. Arguably, Warren's nothing is the space of the Other that Wynter is discussing, it is a lacking space that is not being (human). Through these theoretical frameworks, we find that Ted is not himself, but instead the narrative scripts of his seeming inhumanity and the reparative ground that psychedelics offered him to become human. This tale is curated through the careful mediation of Grof and Halifax as scientific and anthropological facilitators.

Ted was "a twenty-six-year-old Afro-American suffering from an in-operable cancer of the colon" (69). Grof and Halifax described him as having a limited education and "fairly open to a religious world-view. Communication in his family was disoriented and complicated, and required much psychological work" (63-64). He had a wife named Lilly and three kids. His entire childhood was characterized by severe emotional deprivation and outright physical abuse. He lost both parents at the age of three and spent several years in various orphanages. Finally he ended up in the house of his uncle and aunt, who became his foster parents. In their home he suffered much rejection and cruel emotional and physical abuse. During his childhood and adolescence Ted was involved in minor antisocial activities, had frequent fistfights with individuals in the framework of street-gang skirmishes, and liked rough entertainment. Later he enjoyed his involvement in the war, where his aggressive tendencies found a socially approved channel. In marriage he was extremely jealous of Lilly, but had strong tendencies towards extramarital affairs himself (71).

This is Grof and Halifax's humanizing description of Ted which implicitly sutures him to blackness through his violence, broken home, hypersexuality, and vulnerability. It is an exclusionary description that posits his inhumanity as an anthropological subject of study, but it will be through psychedelics that his humanity can possibly emerge and reimagine death.

Ted becomes a site of excess for Grof and Halifax, for if he can be brought into humanity, then perhaps these curative and exploratory models they are formulating have a use. In this, Grof and Halifax's descriptions of other patients begin to paint the "Chain of Being" (Wynter, 300). Ted is compared to the "full aware[ness]" other text subjects such as Matthew, who was "a physician," or Susanne the "attractive, sensitive, and intelligent woman… [who] was involved in the study of psychology," or Joan who "was a forty-year old housewife and mother of four children" (63, 83, 93). Respectively, each of these people are abiding by the same narrative scripts that bind Ted. Each of these patients that are not Ted display a site that can be returned to, as their lives were seemingly success stories until stifled by disability and mortality. As their narratives speak to the overrepresented chain of humanity that defines this text. Each biography is interlinked in the other to imagine the potential ways psychedelics can cure anxiety towards death. This chain thus places Ted at the very bottom ring. It is also important to note that none of the other patients are denoted as 'white' or 'European-American' or any other racialized group—for this is the expected normative position they would occupy. Ted is also described "in many respects on the opposite side of the spectrum" when compared to Matthew (63). Though a seemingly insignificant fact, these omissions perpetuate the greater script that the text is working towards. If Ted can be cured, then everyone else can be cured, for he is the bottom ring of the chain of humanity.

Interestingly, another test subject, Jesse, is mobilized in close approximation to Ted, Jesse's presence speaks to a criticism of class; he is described as an "almost illiterate" (64) man who "changed jobs several times, and because of his limited education did not reach high positions in any of them" (80). To read Ted also requires us to sit with Jesse, as an object of class analysis that psychedelic science, in this instance, intends to cure. Thus the precarious racial and/or class conversation of Grof and Halifax's text reflect an ontic presumption of inability, which is further perpetuated by the embodied disability of inoperable cancers. The terminal conditions that these psychedelic subjects occupy speaks to the precarity of this exploration, in which only expendable bodies are put into the position of explorer within the emergent scientific discourses of the time. While many folks were using psychedelics in the 70s, the scientific model was being constructed as curative towards madness, addiction, and anxiety towards death (as seen in Spring Grove). Thus these structurally negated people on the ontological margin, with Ted's blackness situated as this margin's limit, structure the project in the space of the Other. Through careful tabulation, deciphering, and integration, this map of death can be set into an abstracted model of humanism that Grof and Halifax work through. Thus, this emphasizes the potential for an utter abstraction from materiality, which these psychedelic biographies ground.

The narrative structure at work in Grof and Halifax's text cared for nothing more than the use-value that Ted afforded through his blackness, but what of my search? Ultimately I ran up short: not because Ted never existed, but because the search for Ted was the search for nothing (as Warren would argue). Thus the normative options when faced with this archival lack (or nothingness), according to Tavia Nyong'o, often pushes critics to piece together the "exploitation" that defines the text or towards the hope of "restor[ing]" the subject of the text to some form of being (48-49). In other words, these models postulate totalizing narratives that within themselves seemingly foreclose the frame as utterly denying the subject, but what if the subject within the frame is not totally denied? What of the eruptive capacity of what might rest within the frame itself? As Keguro Macharia warns, "recovery and representation can never be easy: minefields abound, and one tries to minimise the damage one will cause" (Macharia, 501). As this incessant desire to either move past the violated subject or romanticize them is a dangerous game, for we run the risk of unthinking the damage done and instantiating foreclosure on new, seemingly ethical terms. Instead, as Macharia suggests, even in the violent potentials of careful analysis, an attempt must be made to piece together a relation through the incommensurability and ineffability of the archive. Thus in my reading of Ted, I tarry with the very real potential of my own mode of objectifying. As the desire for restoration only makes another object out of a supposedly restored subject. "Instead of the search for an object that leads to a subject, the scholar's search should be for a subject effect: a ghostly afterlife or a space of absence that is not empty but filled" (Young, 3). Working in the vein of Anjali Arondekar's archival theorizations, Hershini Bhana Young argues towards an opaque model of reading that moves against reinventing violence towards a model that can grapple with "the disjunctures, chasms, and nodes of connection between different historically located fields of knowledge that can help us more fully flesh out the afterlife of black diasporic subjects" (3-4). Which extends into a greater conversation within psychedelic science when thinking of blackness, otherness, and structural violence, as we must move away from simply removing or adding these devalued narratives. The former being the psychedelic humanist model that Grof and Halifax employed, while the latter serves as the recent impetus in psychedelic scholarship towards inclusion4 which has inherited a similar

[4] See George et al., 2019; Morales et al., 2022; Williams and Labate, 2019 for recent discursive examples of inclusivity within the psychedelic science archive. To note, this paper is not arguing against treating black patients with psychedelic drugs, rather the paper is suggesting that normative methodological approaches within psychedelic science should expand past recuperative models of inclusion towards engaging the structural history of silence that black patients have and will continue to endure. It is in

valence of violence to the former—as argued by Macharia, Nyong'o, and Young. An opaque mode of reading moves against these subject-restoring projects, instead engaging silence for what it offers. It dares researchers to meet blackness within the object position that is so normatively avoided due to the messy history of colonial violence that has long defined modernity. It is within this meeting space that we can bear witness to how Ted's blackness is a mere afterthought, his otherness is but a useful tool for psychedelic research, his humanity is wholly predicated on faulty universalisms. If we begin here, we begin to realize the violent model of reading the trip that is normative for blackness as it simultaneously erases blackness. A model that bars this existence and merely uses it like one might a psychedelic substance.5

In reading against this normative trip, I offer up the black trip as a reparative alternative within the psychedelic archive: a model that dares to sit with nothingness, difference, incommensurability, excess, and all the other forms that we might read blackness through. But this particular model, situated within the psychedelic archive, positions blackness not as a thing to be overcome, as Grof and Halifax implicitly argue towards, but instead a position to be sat with and reimagined even in its violated place within the archive. The black trip destabilizes the normative terrain it occupies through its devalued presence. In effect, via the denied position that Ted is placed, we cannot necessarily reread him into being in the way that suits us. Instead, our reading must accept this violence and move with it. It must accept the gap of his being and position nothingness as a generative site of thought. Thus the black trip puts into question the very normative framing of psychedelic science through its humanist model and

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reckoning with this silence that we can come to remap the normative methodological terrain that defines the meeting of blackness with the psychedelic.

[5] This argument of blackness as being consumed like a psychedelic substance is in direct lineage to Patrick F. Walter's notion of 'ontological envelopment.' Walter particularly focuses on the role of addiction, narcotics, and blackness through his formulation of "Intoxicated blackness." Central to his thesis is that "[the] intoxicated black being in turn becomes toxin, where the addict takes on the role of the drug" (9). Further on, he argues, through his notion of intoxication, that blackness, due to its ontologically enveloped position, "is both intoxicated and… an intoxicating agent" (19). Thinking through this double-bind is key to my argument on the black trip, because blackness structurally occupies an object position of foreclosure—Ted showing this. Yet simultaneously this object position sustains a means of being read/engaged with in a way that can speak towards the material measure of violence that sustains coloniality, humanism, and the West. Thus the black trip is in line with Walter's arguments on intoxication and addiction, but refitted towards a psychedelic context.

examines the limits of its uses for all patients. In effect, the black trip is a destabilizing model of rereading the psychedelic archive, as it dares to sit with the violence rather than merely acknowledge it. It yearns to know how, within the history of science and psychedelics, it emerges the way it does and affects its discursive productions. To this end, the black trip sits with the residue of people like Ted, Jesse, and all the marginalized psychedelic subjects and does not recover them, but merely acknowledges the violence that will never be totally known. For these violences begin, once carefully deconstructed, to not just remap but undo the normative methods. The black trip then dares us, as researchers, to acknowledge our limitations yet continue to work towards and with the violated. The black trip does not offer a solution, but it offers a mode of thinking through the ineffable violations and continual reimagining that psychedelic science mobilizes. I may not have found Ted, nor will I truly ever, but I sit with him now. He is nothing, but we must not fear nothing, it is a scarily generative way to exist within humanism's violent epistemology.

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Chicas Malas, Mujeres Chingonas: Rebellious Daughters in Sandra Cisneros and Ana Castillo's Poetry

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Abstract

This research examines the bicultural identity of these Chicana poets and how their daughterhood intersects with their autonomy and personhood. In having the Mexican culture at home and the American one outside, Castillo and Cisneros straddle the border of their identity. Gender roles and fixed expectations for women hinder their need to leave the household—the culture at home teaching them what women will become. The poets disagree with the proposed future and are, subsequently, influenced by American society's liberties and opportunities for women. The study illustrates the ways in which these women attempt to leave the household, focusing on machismo, traditional values for women, and daughters emulating their fathers to achieve it. It is because of their bicultural identity, being both Mexican and American, that Castillo and Cisneros can surpass these hindrances and embrace their own aspirations and passions as writers.

Introduction

"I will overcome the tradition of silence," Anzaldúa wrote in her piece 'How to Tame a Wild Tongue' from Borderlands/La Frontera: the New Mestiza where she writes about breaking the linguistic borders by freely switching from English to Spanish in her writing (Anzaldúa 114). Her diaphanous political stance introduces biculturalism, the identity of an individual forming within two cultures. Anzaldúa's writing is pivotal to the 1980s Chicana Feminism that moved other women writers such as Ana Castillo and Sandra Cisneros to share their experiences through literature. In this research, and in Anzaldúa's writing, the personhood established within the works of Chicana literature is the identity of Mexican-American, specifically the effects of having the traditional conservative Mexican presence at home whilst living in America and interacting with U.S. values and social structures, and how this bicultural life relates to a daughter's identity.

Because of this, this research seeks to demonstrate the bicultural life related to the identity of the daughter raised in Chicana. Narratives commonly seen in Chicana literature center their stories on the lives of bicultural women searching for their own voices and autonomy. It is a journey of juggling both identities to overcome the fixed traditional roles expected of women in Mexican culture in an attempt to gain her own personality through the values of American society. In representation of this struggle, the works analyzed in this research are the poetry books of Sandra Cisneros My Wicked, Wicked Ways (1987) and Ana Castillo's My Father Was a Toltec (1995). Both Chicanas are seen as Madrinas of Mexican-American literature and have pioneered several stories that reflect their own experiences in their writing.

The focus on poetry is purposeful given that the poet's persona is closer to the authentic voice of the writer in comparison with their fictional voices, in which the writer's experiences may influence the characterization of the protagonist but not necessarily cast themself as the character itself. For instance, Esperanza from the House on Mango Street is not Sandra Cisneros. Even so, Cisneros's life in Chicago and the impoverished neighborhood she grew up in is represented in the setting of Mango street. Conversely, the persona of the poet in My Wicked, Wicked Ways is seen in Cisneros's poetry, her poems reflecting her family life, her own daughterhood identity, and how she struggles with the bicultural personhood. Thus establishing the poet's persona as more closely related and authentic in captivating the life of the poet.

As mentioned before, this research will focus on My Father Was a Toltec by Ana Castillo, a poetry book that centers on the daughter emulating her father as a method to reject the fixed roles of women. In embracing masculinity, she finds herself infringing on several taboos forced upon women and facing the anger and rejection from her family. This coincides with My Wicked, Wicked Ways by Cisneros, which capitalizes on the daughter's nascent freedom and her desire to be a writer rather than a mother or a wife. She commits multiple acts of defiance and faces the anger and abandonment of her family. Both these works reflect the borderlands of identity that is being a Mexican-American woman. It's a further illustration of what it entails to embrace one's autonomy against a patriarchal society. By utilizing a close reading analysis of their poetry, the lens will grant insight to the poet's internal struggle and means to overcome the machista attitudes that impedes a daughter's identity. In my research, the readings of My Wicked, Wicked Ways and My Father Was a Toltec are analyzed in a Daughterhood lens that captivates the experience of a woman breaking out of the cycle and escaping the narrative of womanhood in Mexican culture, and being able to do so because of their bicultural identity.

Previous Research

In support of this, Raymund Paredes notes that "in considering the nature of [daughters] in Mexican-American culture, the scholar or imaginative writer must engage a complex of cultural traditions often described as among the most patriarchal in the world" (Paredes 136). Historically, Mexican culture has perceived women less than men, often limiting their freedom financially, educationally, and sexually. Even Cherríe Moraga, playwright and daughter of an Anglo father with a Chicana mother, admits "If I had been born to a Chicano father, sometimes I think I would never have been able to write a line" (Moraga 112-113). Both Paredes and Moraga agree that the presence of the father, who represents the traditionally patriarchal culture of Mexico, oppresses and limits the daughter. The perceptions of women seen in Mexican culture are mostly written by men, and their figures are represented through the lens of a virgin, such as the infamous Virgin of Guadalupe, or as whores, seen in the unfavorable Malinche.

This limited viewpoint is suffocating to women in Mexican culture, and as stated in the article In Father's Footsteps: Bad Girls in Ana Castillo's and Sandra Cisneros's Poetry by Adriana Estill "limits women's possibilities to a choice between moral opposites" (Estill 47). Her article centers on daughters emulating their fathers and rejecting their mothers, embodying 'bad girls' by shifting the gender expectations and embracing their newfound independence through the adoption of the father's dominant features. These limits of womanhood that exist within Mexican culture are more explicit compared to life in the United States.

The machista values of traditional households are instituted by the father who ordains it to their daughters. Given the toxic patriarchal environment, the Mexican-American daughters search for other paths in which they can be more than mothers or wives, and virgins or whores. With their biculturalism, Chicana daughters, Castillo and Cisneros, are able to embrace the societal expectations of women in the United States and become writers. My Father Was a Toltec and My Wicked, Wicked Ways are connected given their reflection to their borderlands of identity and how chosen introspective scenes emphasize the lives of the poets and the means they use to escape the predetermined roles.

My Father was a Toltec

My Father Was a Toltec opens with the section titled ‘The Toltec.’ The Toltecs were the indigenous people of pre colonial Mesoamerica that ruled in Mexico before the Aztecs. However, Castillo refers to the Chicago gang that her father ran with; the section title alludes to the gang activity as an enunciation on the inadequacy of Castillo’s father as a paternal figure and husband. The poems ‘Saturdays’ and ‘Suede Coat’ follow each other in the section and capitalize on the infidelity the father commits against Castillo’s mother, supporting the belief of incompetent husbands in Mexican culture. The poems stated exhibit the behavior women are expected to adhere to despite their husband’s adultery; the women keep quiet, act as if they don’t know, and expect their daughters to follow in the same example while their husbands exploit them.

As is shown in ‘Saturdays,’ Castillo’s mom works from “5 to 5” and does the laundry on Saturdays, as the title implies (Castillo, line 2). She irons her husband’s clothes “bought on her credit,” adjusts his tie to the “tailor-made silk suit” and watches him leave to meet the mistress (Castillo, lines 14-16). Emphasis on who manages the finances and who dons the suit is purposeful in showcasing the mother’s hard work being taken advantage of by the husband. Estill interprets this as “the father’s exploitation of the mother’s labor in order to look good for another woman” (Estill 52). The last sentence of the poem illustrates the extent of gender roles in Mexican society: “That’s why he married her, a Mexican / woman, like his mother, not like / they were in Chicago, not like / the ones he was going out to meet” (Castillo, lines 19-22). In the daughter’s reasoning for the behavior, she places an intentional emphasis on the cultural background, she “believes that [the father] knew…his wife would subordinate herself to him” as read by Estill. Given the culture’s demands of women, the father is familiar with the attention from his mother and now his wife. In that mindset, the father reaps the advantages of the culture’s expectations for women and Castillo acknowledges what is to become of her future.

This pairs with the central theme of the ‘Suede Coat’ as it amplifies the adultery of the father and the subservience of not only the mother, but the woman in the family that Castillo identifies with:

> To whom did the suede coat with
>
> fur collar belong?
>
> The woman in my family
>
> have always been polite
>
> or too ashamed to ask.
>
> You never told, of course,
>
> what we of course knew. (Castillo lines 11-17)

Castillo’s use of ‘we’ in the last line can be interpreted as a form of identification to the women who are expected to be silent and inactive despite the blatant infidelities of their husbands. The daughter takes note of the treatment and sees that these “women are scripted as too passive, subordinate, and silent to demand answers” as analyzed by Estill. In relation to this, Castillo’s book Massacre of the Dreamers notes that this type of observation works as a lesson given by the mother that teaches daughters the opposite: “Witnessing our mothers’ endurance of husbands’ physical abuse, alcoholism and extramarital affairs, sometimes serves some women…as an example of what to not tolerate” (Castillo 131). Because Castillo has grown up biculturally, the relationship to the U.S. culture enables her to elude the oppressive Mexican culture, the attempts of maternal figures to mold daughters, and retain the dominant features of paternal figures.

To achieve the freedom Castillo seeks from predetermined roles of women, she resorts to emulating her father, as can be seen in her longer poem ‘Daddy With the Chesterfields in a Rolled Up Sleeve.’ The poem focuses on the father and his interactions with his interpersonal relationships to women, highlighting the ones with his mother and wife. Both women set the expectations of how women should behave in the lives of men, with Castillo observing the interactions. In comparison to the poems

viewed before, ‘Daddy with the Chesterfields’ reiterates the theme of women’s exploitation, but also introduces more details of the father, makes up his characterization, as well as the personal development of the daughter and how she chooses to escape the roles of women.

The poem begins with the father, his “tribe” of the Toltecs, his characteristics that are later repeated to describe Castillo: “He smokes cigarettes, / doesn’t ask permission, speaks English / with a crooked smile,” and the laziness of men while the wives “work the assembly line” (Castillo, lines 25-27, 36). In the same poem, Castillo describes herself as well, using petite language to represent how women are expected to act, such as including introspective scenes of herself as the “most delicate of offspring” and giving flowers to the Virgin (Castillo, line 56). Her grandmother, the mother of her father, dies and Castillo notices the women shifting in place: “Mami makes enchiladas for Daddy’s birthday, / never as good as the memory of his mother’s. / Mami takes her place now” (Castillo, lines 69-71). It’s the inevitable cycle of womanhood in Mexican culture, the next woman in line takes the place of the one before to take care of the men; once the suegra is gone, the wife takes her place, and once the mother is gone, the daughter takes the place.

Castillo’s relationship with her mother is tumultuous. Her mother, forced to work and care for the family, leaves her daughter with “a quarter for lunch, / crackers for breakfast on my pillow / that rats get before [the daughter] wake[s]” (Castillo, lines 87-89). Castillo even outwardly protests “Don’t leave me / with this mami” which emphasizes her longing for a maternal figure and the refusal to be like her mother (Castillo, lines 90-91). The mother’s enabling of the patriarchy is clearly shown in the next stanzas by scorning her daughter for “not forgiving [her husband] / when she caught [him] / with [his] girlfriend” (Castillo, lines 129-131). Castillo refuses to conform and begins to emulate her father’s masculinity, repeating the same characteristics to emphasize the severance of womanhood’s fixed roles:

> I speak English with a crooked smile,
>
> say “man,” smoke cigarettes…
>
> women tolerate my cigarette
>
> and cognac breath, unmade bed,
>
> and my inability to keep a budget (Castillo lines 114-115, 148-150)

The repetition of the father's features, such as his smoking, speaking English with a crooked smile, and his irresponsible demeanor, is emphasized within Castillo's adult characterization. In her emulation of her father, she escapes the predetermined role of women by refusing to conform to the 'womanly' duties and practices forced upon women. The last stanza of the poem cements Castillo's choice to surpass the expectations by adopting the patriarch's characteristics:

> Oh Daddy, with the Chesterfields
>
> rolled up in a sleeve,
>
> you got a woman for a son. (Castillo lines 156-158)

My Wicked, Wicked Ways

The same statement echoes the last line in Sandra Cisneros's epigraph to her section titled 'My Wicked, Wicked Ways' with Maxine Hong Kingston's quote "Isn't a bad girl almost like a boy?" (Cisneros 2). Freedom, autonomy, refusing to conform – all attributes designated as admirable male characteristics, yet treated as rebellious when a woman is involved. With Cisneros's poetry, the struggles of daughterhood are unequivocally seen and expressed, translucent with her manipulation of language and diaphanous experiences of being the only girl in a family of men. This struggle is explicitly shown in the poem 'His Story,' in which Cisneros's personal experience with being the only daughter in a family of men is represented.

'His Story' begins with a father's disappointment. His sorrow at having an only daughter "whom no one came for / and no one chased away," implying that women are meant to be with someone or they'll lose value the longer they're alone and single (Cisneros, lines 5-6). As the poem continues, Cisneros lists women in the family that haven't followed the strict roles of women and the consequences they paid for that sin, including loneliness, voodoo, and prostitution, among others. However, most importantly the father attempts to scare Cisneros into obeying him by retelling the story of "a girl with both my names / who was arrested for audacious crimes / that began by disobeying fathers" and another instance where a different Sandra Cisneros was "three

times cursed a widow" (Cisneros, lines 27-29, 32-33). It's the same story of women too different to be controlled and feared for it instead of celebrated or admired.

The last stanza not only embodies the father's disappointment of having a daughter, but also includes Cisneros's own perception of her situation:

> You see.
>
> An unlucky fate is mine
>
> to be born a woman in a family of men. (Cisneros lines 34-36)

The statement alludes to a woman's placement in the patriarchal world where less value is seen in being born a daughter instead of a son. Therein lies the double standard and gender roles of women needing to be married, of following the strict cultural values and expectations. In comparison to her brother's, Cisneros was the first to leave home without being married – both actions, leaving home and leaving without being married, break the social norm and her ideals are treated as being too white. The last sentence verifies once again the father disappointment at his daughter's actions, he would be prouder if she were a son:

> Six sons, my father groans,
>
> all home.
>
> And one female,
>
> gone. (Cisneros lines 37-40)

The pride of having six sons is not lost on Cisneros's father and is clearly shown in Cisneros's memoir House of My Own with the essay 'Only Daughter' in which she explains the makeup of her identity being represented by "I am the only daughter in a Mexican family of six sons" (Cisneros 183). In the same essay, Cisneros reveals her father's mode of thinking and introduces the audience to her introspective experience of what it means to be a woman in a Mexican family: "Being an only daughter for my father

meant my destiny would lead me to become someone's wife" (Cisneros 184). Her essay supports the poem and coincides with the pride the father feels, "I have seven sons.' As if he deserved a medal" Cisneros states. It was an erasure of her personhood, being in a family of six sons meant that the men would receive the attention, the women would be casted aside, yet Cisneros refused to be forgotten: "I'd tug my father's sleeve and whisper, 'Not seven sons. Six! And one daughter" (Cisneros 188).

This struggle of daughterhood is represented through Cisneros's poetry and coincides with Castillo's own experiences of womanhood in a Mexican household. Both these works offer the infidelity of fathers, the exploitation of mothers, the struggles of daughters attempting to disavow the cultural expectations. They pair together in this sense, but more so on their bicultural identities that resonate in the works. Circling back to Castillo's unfaithful father, Cisneros also contributes to the pattern of a husband's infidelity, the mother's passivity and enforcement of the daughter to follow in her footsteps as mother and wife to a patriarchal society. The pattern is clearly shown in 'My Wicked, Wicked ways,' the eponymous poem that pairs with Castillo's 'Saturdays' and 'Suede Coat;' all which offer the same overarching themes of a father's exploitation of a mother and the daughter's predestined life.

The eponymous poem offers an intricate description of the father, the clothes he's wearing, his appearance, while the mother receives no visual descriptors and is casted aside visually in the poem. Yet, Cisneros manipulates the language to reveal the mother's emotions:

> Here is my mother.
>
> She is not crying.
>
> She cannot look into the lens
>
> because the sun is bright. (Cisneros lines 12-15)

In utilizing a close reading lens, one can infer that the addition of 'not' in the second sentence alludes to an excuse for the mother's behavior. It's because of the sun that she cannot look into the camera, not because of the tears. The second part of the stanza offers a reason for her sadness:

The woman,

the one my father knows,

is not here.

She does not come till later. (Cisneros lines 16-19)

The existence of a mistress and an affair is clearly shown with the mention of another woman. It is in the following stanza that the reaction towards the adultery and the consequences the father faces are revealed: “My mother will get very mad…My father will say nothing. / After a while everyone / will forget it” (Cisneros, lines 20-25). In comparison to Castillo’s mother, Cisneros’s mother has a reaction, a burst of anger, and yet, despite the reaction both mothers remain passive in their anger and allow their husband to continue their adultery, granting the daughter a model of womanhood in Mexican culture.

Conclusion

The roles women play and the manner in which men treat women discourage Cisneros and Castillo to participate in the fixed roles. Instead, they choose to be “bad” and rebel against the narrative. Each poet materializes this rebellion in different ways: Cisneros leaves home and chases the freedom it brings, while Castillo focuses on adopting dominant characteristics and embracing her sexuality. Both poets reject the role for women in the patriarchal culture, and instead, choose to become writers. The works of Cisneros and Castillo capture the tumultuous relationship of a bicultural identity in daughterhood that creates discourse of tradition and values. By encompassing ‘rebellious girls,’ Cisneros and Castillo become bad, wicked, and defiant in their writing; overturning the society and its abstract rule by refusing to conform to the expectations for women.

The daughter's struggle is seen through Cisneros' poetry and coincides with Castillo's own experiences as a woman in a Mexican home. Both works feature the infidelity of fathers, the exploitation of mothers, and the struggles of daughters attempting to repudiate cultural expectations. They are matched in this sense, but even more so in their bicultural identities that resonate in the works.

As Estill states, the key to resisting the oppression for Chicanas is to gather “strengths of both Mexican and U.S culture” (Estill 47). Cisneros and Castillo both

utilize, embrace, and understand how their experiences shape and enable them to "initially reject the Mexican woman's prescribed role" and embrace the role of their choice. It is at this crossroad that Cisneros and Castillo emphasize with their writing the complexities of being a daughter in a traditional Mexican household and finding their identities through and because of their biculturalism. Both works emphasize the complex identity of daughters in Mexican cultures, having the U.S. societal values within reach while experiencing the patriarchal society at home. Cisneros and Castillo's introspective works offer visceral experiences that captivate the struggle of a bicultural identity. It is the strength of their biculturalism that allows them to transgress the limits of womanhood in Mexican culture and be more than what the limits say women can be.

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How TikTok Content Creators Provide Job Hunting Advice to Job Seeking Users

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Abstract

This research aims to understand how TikTok content creators provide job-hunting advice to job-seeking users. The purpose is to explore TikTok as a platform for job seekers to gain knowledge in job applications, and to identify the strategies and methods TikTok content creators use to offer job hunting tips. This research will conduct a content analysis of TikTok content creators with over 500k followers, examining the various approaches TikTok content creators use to give advice on resumes, interviews, and job searches. This research revealed that most TikTok influencers prefer sharing original content, with a focus on resume tips and career growth, while showing clear gender differences in advice, with female creators highlighting mental health and male creators focusing on tech industry guidance.

The purpose of this research is to investigate and analyze how TikTok content creators effectively deliver job hunting advice to job seekers. This study aims to understand the strategies, methods, and effectiveness of job-related content on TikTok, exploring its impact on users' job search strategies, career decisions, and overall engagement with career-related information on social media platforms.

Keywords: job hunting, job market, career growth, social media, Tik Tok, Tik Tok job hunting

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In the past, job seekers typically prepared detailed resumes and cover letters, submitting these documents either in person or through the mail to potential employers. This traditional, paper-based method was often a lengthy process, limiting the reach of applications and requiring applicants to physically visit businesses or rely on postal services, which further delayed the process. However, with the rise of the digital age, job applications have increasingly shifted toward digital platforms, leading to more efficient and widespread ways for individuals to connect with employers.

The late 20th century marked the beginning of this digital transformation with the introduction of online job boards like Monster.com and CareerBuilder.com. These platforms allowed job seekers to explore a wider array of opportunities, while employers could reach a larger pool of candidates (Boyd & Ellison, 2007). This evolution was further propelled by the emergence of professional networking platforms such as LinkedIn. LinkedIn revolutionized the job search process by integrating networking, job applications, and professional communication into a single, user-friendly platform (Skeels & Grudin, 2009). LinkedIn's ability to connect professionals worldwide, display skills and experiences, and facilitate direct communication between job seekers and employers has made it an essential tool in modern job searching.

As these digital platforms became standard practice, the role of social media in job seeking also expanded considerably. Originally intended for social interactions, social media platforms have evolved into critical tools for professional networking and career development. Among these, TikTok has emerged as a distinctive platform where job seekers can obtain valuable advice and insights from industry professionals in an engaging and accessible format (Abidin, 2020). Unlike traditional job search platforms, TikTok offers a more casual and approachable method, making career guidance accessible to a broader audience, particularly younger generations who may prefer this type of content.

On TikTok, content creators—many of whom are experienced professionals or career coaches—share advice on various aspects of the job search process, including resume writing, interview preparation, and networking techniques. These concise, engaging videos simplify complex topics into actionable steps, appealing particularly to users who may feel overwhelmed by more traditional job application processes (Van Hoye et al., 2009). TikTok's algorithm, which amplifies content based on user engagement, allows high-quality job-hunting advice to reach millions of users, potentially democratizing access to career guidance.

The impact of TikTok on the job-seeking process represents a notable shift in how individuals prepare for and approach the job market. Unlike earlier digital platforms, which primarily served as submission tools for applications, TikTok acts as an educational resource, offering tailored and dynamic content that can directly influence users' job search strategies. TikTok's role in shaping modern job-seeking practices underscores a broader trend: the integration of social media into professional development, challenging traditional methods of disseminating and consuming career advice.

This study aims to examine how TikTok content creators contribute to the job-hunting process by focusing on the strategies and techniques they employ to provide effective advice to job seekers. Through a content analysis of TikTok videos from creators with large followings, this research will explore how TikTok is used as a platform for career guidance and its influence on contemporary job-seeking practices.

Study Limitations

While there is a broad acknowledgment of social media's influence on job seeking, there is a notable gap in specific studies addressing TikTok's impact on the job search process. Existing literature often discusses the general influence of social media platforms without focusing specifically on TikTok as a medium for career advice and job-hunting strategies. This research aims to fill this gap by conducting a qualitative content analysis of TikTok videos created by influential content creators specializing in job application tips.

Literature Review

Original, Stitch, Duet Features on TikTok

TikTok's diverse video formats (Original, Duet, and Stitch) offer several ways for users to create and share content, each impacting viewer engagement and content interaction differently. The Original format, where users create videos from their own ideas without external content, is the most common format on TikTok. It allows for creative freedom and personal expression, contributing to the platform's dynamic content landscape (Anderson, 2020). This format fosters unique content creation and is fundamental to individual user branding on the platform (Anderson, 2020).

The Duet feature enables users to create videos alongside existing content, typically for reaction or collaborative purposes. Research highlights that Duets can enhance content visibility and engagement by leveraging the popularity of original videos while adding a new perspective or response (O'Toole, 2023). This feature often leads to viral content creation and increased interaction between users, demonstrating its effectiveness in fostering community engagement and collaborative content (MacKinnon et al., 2021).

The Stitch feature allows users to incorporate clips from other videos into their own, followed by additional original content. This format supports content remixing and extends conversations across different videos, thereby creating a more interconnected content ecosystem on TikTok (MacKinnon et al., 2021). Stitch enables users to build on existing content, encouraging innovative responses and discussions, which can enhance viewer engagement and content reach (Anderson, 2020).

TikTok's Original, Duet, and Stitch formats each offer distinct advantages for content creation and engagement. Understanding these features' impact helps in leveraging TikTok effectively for both personal expression and broader audience interaction.

Job Hunting Through Social Media

Social media has transformed the job search process, providing new ways for job seekers to connect with potential employers and access job opportunities. Platforms such as LinkedIn, Twitter, and Facebook are now essential tools for networking, job searching, and building a personal brand. Research highlights how social media influences job search strategies and outcomes by broadening the scope of professional connections and job leads (Van Hoye et al., 2009).

LinkedIn is a key player in the job search process. It functions as a professional networking site where users can highlight their skills, connect with industry peers, and explore job openings. Studies indicate that well-maintained LinkedIn profiles and active engagement on the platform improve job prospects and increase visibility to recruiters. The job search features and networking capabilities on LinkedIn enhance users' ability to find and apply for appropriate positions (Sullivan, 2016). This demonstrates the importance of an effective online presence for job seekers.

Twitter and Facebook also contribute to job hunting, though in different ways compared to LinkedIn. Twitter helps users stay informed about job opportunities and industry trends by following companies and engaging in professional conversations. Research shows that Twitter can provide valuable job leads and insights into job market trends (Koch et al., 2018). Facebook, with its various job search and professional development groups, offers networking opportunities and job leads, although its effectiveness can vary depending on group activity and focus (McDonald & Hite, 2019).

Interviews, Cover Letters, Resumes for Job Seekers

To succeed in the competitive job market, job seekers need to excel in several key areas of the application process. However, the job search process involves several challenges, and job seekers need guidance to successfully stand out from all the job applicants. One major hurdle is crafting a compelling resume and cover letter, which is important for making a positive first impression. Job seekers frequently encounter difficulties in tailoring these documents to specific job requirements and effectively highlighting their qualifications (He & Kang, 2021). Effective resume and cover letter writing is essential as these documents are typically the initial point of contact with potential employers and play a critical role in securing interviews. Mastery of these skills is essential for effectively managing the competitive job market and achieving favorable job search outcomes (Morrison, 2014).

Job seekers often have trouble preparing for interviews, which is an important part of the job application process. Interviews require candidates to show how well they fit the role and can contribute to the organization. Many job seekers are not ready for the types of questions they might be asked or how to talk about their experiences and skills effectively (Schmidt & Hunter, 1998). To make a strong impression and advance in the hiring process, it is important to practice answering common questions and understand what the company needs.

Resume and cover letter are important for standing out in a competitive job market and increasing the chances of securing an interview. Research shows that tailored resumes and cover letters, which highlight relevant skills and experiences specific to each job application, significantly improve a candidate's likelihood of being noticed by recruiters (He & Kang, 2021).

Effective resume and cover letter writing involves not only listing qualifications but also demonstrating how one's skills align with the job requirements and organizational goals. By ensuring that these application materials are tailored and polished, job seekers enhance their chances of making a positive impression and advancing through the hiring process.

Career Growth for Employers

Career growth is an important aspect of professional development that encompasses various strategies and motivations for advancing one’s career. Understanding why employees pursue career growth and how they achieve it is essential for both individuals and organizations. This review examines key factors influencing career growth, including personal ambitions, organizational support, and the impact of career development programs.

Employees often seek career growth to fulfill personal ambitions and enhance job satisfaction. Research indicates that career advancement opportunities can lead to increased motivation and engagement among employees (Joo & Park, 2010). Individuals are motivated by the prospect of improving their skills, achieving higher status, and obtaining better financial rewards. Career growth not only satisfies personal career aspirations but also contributes to overall job satisfaction and retention (Judge & Kammeyer-Mueller, 2012).

Companies that provide clear career paths, professional development opportunities, and mentoring programs are more likely to see their employees advance within the organization (Segers & Inceoglu, 2012). Career development programs are essential for helping employees achieve their career goals. (Segers & Inceoglu, 2012). Effective career development initiatives can help employees navigate their career paths more successfully and achieve their professional objectives. By aligning personal goals with organizational needs, these programs support both individual and organizational success. When organizations invest in their employees' growth through training and development, they help employees acquire the skills needed for promotions and increased responsibilities (Bai & Liu, 2018). Such support fosters a positive work environment and helps retain talented individuals.

Identifying Red Flags

When exploring job opportunities, it is important for job seekers to be aware of potential warning signs, known as "red flags," that could indicate problems with a job or workplace.

These red flags can appear during the application or interview process and often signal issues like a negative work environment or poor management practices. To protect their career development and ensure job satisfaction, job seekers need to be proactive in researching and evaluating potential employers.

In the job market, a "red flag" refers to warning signs that signal potential issues or challenges associated with a job opportunity or work environment. These indicators, which can emerge during the application or interview process, often hint at underlying problems such as an unsupportive organizational culture, poor management practices, or a lack of transparency.

To avoid these problematic situations, job seekers must be diligent in their research and evaluation of potential employers. Effective strategies include scrutinizing company reviews, seeking insights from current or former employees, and asking pointed questions during interviews. Identifying and avoiding these red flags is crucial for ensuring job satisfaction and aligning career opportunities with personal and professional goals, safeguarding one's career development and well-being (Kwantes, 2009).

Men in Tech Industry

Many men are drawn to careers in the tech industry due to the high salaries and job stability it offers. Studies show that men often choose tech jobs because of the financial rewards and strong career growth opportunities (Cross & Linehan, 2006). The opportunity to earn a good income and enjoy job security makes the tech industry appealing to men (Smith & Clark, 2020).

Another reason men are attracted to tech jobs is the excitement of working on innovative projects. The tech field is known for its rapid advancements and offers the opportunity to be involved in innovative work. Men are motivated by the chance to contribute to important technological developments and make a significant impact (Gallivan, 2004).

Societal expectations also play a role in why men pursue tech careers. Cultural norms often associate technology and engineering with masculinity, influencing men’s career choices from an early age (Pyrkosz-Pacyna et al., 2022). These societal beliefs align with personal interests, reinforcing the desire for a tech career as it fits with traditional gender roles and professional goals (Van Tuijl & Van Der Molen, 2015).

Gender Differences in Work Promotion

Gender disparities in the job industry have been well-documented. This study is also interested in whether men and women content creators are associated with different issues along stereotypical gender lines regarding technology careers and mental health issues.

Significant differences have long been found in the promotion of certain career paths by men and women. Traditionally, men have been overrepresented in STEM (Science,

Technology, Engineering, and Mathematics) fields, particularly technology, while women have leaned toward roles in healthcare, education, and mental health sectors (Charles & Bradley, 2009). These differences reflect both historical trends and societal expectations around gender roles, where men are often encouraged to pursue careers associated with technical expertise, innovation, and leadership, and women are steered toward careers that emphasize care, communication, and interpersonal skills. The persistence of these trends in the job industry continues to shape the ways in which men and women seek and promote career opportunities.

Women are more likely to promote careers related to mental health and wellness, reflecting their dominance in professions such as counseling, psychology, and social work (Karakcheyeva, 2024). These roles often align with traditional female-associated skills such as nurturing and caregiving. Men, on the other hand, are more likely to promote and dominate in the technology sector, where technical skills, problem-solving, and innovation are emphasized. This gender gap is particularly visible in leadership positions in tech companies, where men continue to hold the majority of executive roles, further reinforcing gendered pathways in career promotion (Cech, 2013).

Females, who represent a substantial portion of the workforce in mental health professions, have historically advocated for greater awareness and importance of mental health services. This gender disparity in promoting mental health careers can be attributed to the traditionally female-dominated nature of these fields and societal expectations around emotional labor (Salvatore & Marecek, 2018). Research by Crompton and Lyonette (2011) indicates that women's increased participation in these roles also stems from gender socialization, which encourages empathy, communication, and caregiving – skills essential in mental health services. These roles often offer women more flexibility and align with the value of interpersonal skills over technical ones, further embedding the association of women with mental health careers (Crompton & Lyonette, 2011).

In contrast, men continue to dominate and promote careers in the tech industry, a sector that has long been marked by gender inequality. Educational pathways and cultural expectations often lead men to enter STEM fields at higher rates, which in turn results in greater representation in tech-related careers. Cech (2013) highlights that gender stereotypes and socialization play a crucial role in steering men towards these fields, particularly by fostering an environment that rewards technical prowess and discourages female participation. The underrepresentation of women in tech jobs is also perpetuated by industry culture and hiring practices, which can reinforce male-dominated networks and further marginalize women in these roles. This ongoing gender disparity highlights the importance of addressing systemic issues in education, hiring, and promotion practices to foster a more inclusive workforce (Cech, 2013).

Based on this literature, this study predicts:

H1: Female content creators will address mental health issues more than male content creators.

H2: Male content creators will address the tech industry more than female content creators.

From these topics on job searching identified in the literature this study asks the following research question:

RQ1: How frequently do TikTok content creators give career advice on
a) Resume, b) Cover Letters, c) Interviews, d) Identifying Red Flags, e) Career Growth, and f) Job Hunting?

Methods

This study employed a quantitative content analysis to examine the types of career-related content produced by popular TikTok content creators. A coding assistant participated in the coding process. To ensure objectivity, the coder was not informed about the specific research objectives. Instead, the coder categorized the videos based solely on their own interpretations.

Seven TikTok content creators (four female content creators, three male) were selected for analysis, each with a minimum of 500,000 followers. These creators were chosen because their content primarily focused on providing career advice. The analysis was conducted over a one-year period, from July 1, 2023, to July 1, 2024. To manage the large volume of content produced by these creators, a skip rate sampling technique was implemented. The skip rate was calculated by dividing the total number of videos by the sample size, with the sample size being determined by the time frame.

For this study, it was estimated that each creator would upload content on average during the 22 working days of each month, excluding weekends. Over a year (12 months), this resulted in an estimated 154 videos per creator. By dividing 154 by 12 months, a skip rate of 13 was established. This means that every 13th video from each content creator was selected for analysis, until 22 videos per creator were recorded, resulting in 154 videos across all creators.

Before officially documenting 154 TikTok videos, two coders – one of the authors and one independent coder – were trained for approximately 2 weeks in sessions lasting between 2 and 5 hours a day, and the coding categories were revised using videos not in the final sample. After approximately 20 hours of training, agreement was achieved on 20 percent of the videos (Krippendorf's alpha: Red Flag 1.0, Duet 1.0, Stitch, 1.10, Cover letter tips 1.0, Job hunting tips 1.0, Tech industry 1.0, Original, 1.0, Resume tips .845, Interview tips .899, Career growth 1.0, Mental Health 1.0.

FORMAT	
Original	72.7%
Duet	14.3%
Stitch	13%
TOPIC	
Resume	16.9%
Cover Letter	10.4%
Interview	18.8%
Red Flag	9.1%
Career Growth	19.5%
Job Hunting	15.6%
Technology	3.2%
Mental Health	5.2%
CREATOR GENDER	
Female	57.1%
Male	42.9%

Table 1. Percentage of TikTok Videos by Format, Topic, and Creator Gender

The two coders also categorized each video based on the format of the video including whether it was Original Content, meaning if the videos were created entirely by the content creator, without incorporating existing TikTok videos; Duet videos, which involve two videos playing simultaneously side by side, often used for reactions or sharing the creator's opinions; or Stitch videos, which, combine a segment from another creator's video followed by the creator's own content, allowing for a sequential narrative. It is important for content creators to provide Original Content to address key points to convey in their unique thoughts.

Results & Discussion

This research aimed to analyze the content created by seven TikTok influencers who focus on career-related advice, revealing trends in both the type and format of the videos. Among the seven content creators, four were female and three were male. A total of 154 videos were examined, which garnered approximately 646,748 likes and 16,145,837 million views. The average video length was 84 seconds. Table 1 showcases that the majority of the videos were in an original format (72.7%), followed by duet (14.3%) and stitch (13.0%) formats, indicating that most content creators prefer creating their own original material rather than engaging with others' content.

In terms of content, resume tips (16.9%) and career growth advice (19.5%) were the topics most discussed, reflecting job seekers' significant interest in enhancing their job application materials and progressing in their careers. Interview tips also featured prominently, with 18.8% of the videos dedicated to this area, further emphasizing the importance of interview preparation. Other notable topics included cover letter tips (10.4%), job hunting strategies (15.6%), and identifying red flags in job searches (9.1%), highlighting a wide range of career advice tailored to different stages of the job search process. Additionally, 1.3% of the videos were placed in the "other" category, both of which were advertisements. There was also a category for the tech industry (3.2%) and 5.2% addressing mental health in the workplace.

The content analysis of the 154 TikTok videos revealed clear gender differences in the topics covered by the creators. All 5.2% of videos related to mental health were created by female content creators, while 3.2% of videos focused on landing jobs in the tech

industry were produced exclusively by male creators. This indicates that female creators may emphasize topics like workplace well-being and mental health, while male creators tend to focus on industry-specific advice, particularly in technology. The analysis further revealed that female content creators specifically addressed mental health topics related to anxiety, depression, and burnout. Of the 5.2% of videos dedicated to mental health, 3.9% focused on anxiety, 0.6% on depression, and 2.6% on burnout. These topics highlight common challenges that professionals, particularly women, may face in the workplace. The emphasis on burnout and anxiety suggests a growing awareness of the pressures and mental strain that can accompany career development and professional environments.

This analysis shows the variety of career advice on TikTok and highlights the different focuses male and female creators have, with women addressing mental health and men offering tech industry guidance. In addition, this research reveals that most creators prefer sharing original content, with fewer videos using duet or stitch formats. As a resume tips and career growth were the most mentioned categories.

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Artificial Microbiome Selection to Improve Engineered Nitrogen-Fixing Bacteria Fitness and Benefit Bioenergy Crops

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Abstract

Determining a more resource-efficient way to provide plants with the nitrogen they require for proper growth would minimize the damaging overuse of nitrogen-based fertilizers and serve as an excellent trait for biofuel crops that are intended to grow in less ideal soil and with minimal human input. One potential way to increase nitrogen use efficiency (NUE) includes artificially selecting the root microbiome of the plant via stress over multiple generations to induce resistance via symbiotic bacterial interactions. The root microbiome is known to provide numerous benefits to its host, including microbes that provide essential nitrogen to all plants, but the differential communication that takes place in these diverse communities from the host and between microbiome members is largely unknown. A variety of preliminary experiments were conducted to inform a host-mediated artificial root microbiome selection procedure that could induce bioenergy candidate crops with nitrogen stress resilience. To further deduce plant-microbe and microbe-microbe interactions, these experiments also tested the propagation and impact of a nitrogen-fixing strain engineered to overproduce nitrogen within a synthetic microbiome community.

Keywords: Microbiome selection, plant-microbe interactions, microbe-microbe interactions, rhizosphere microbiome, nitrogen uptake, nitrogen-use efficiency

Introduction

The Root Microbiome

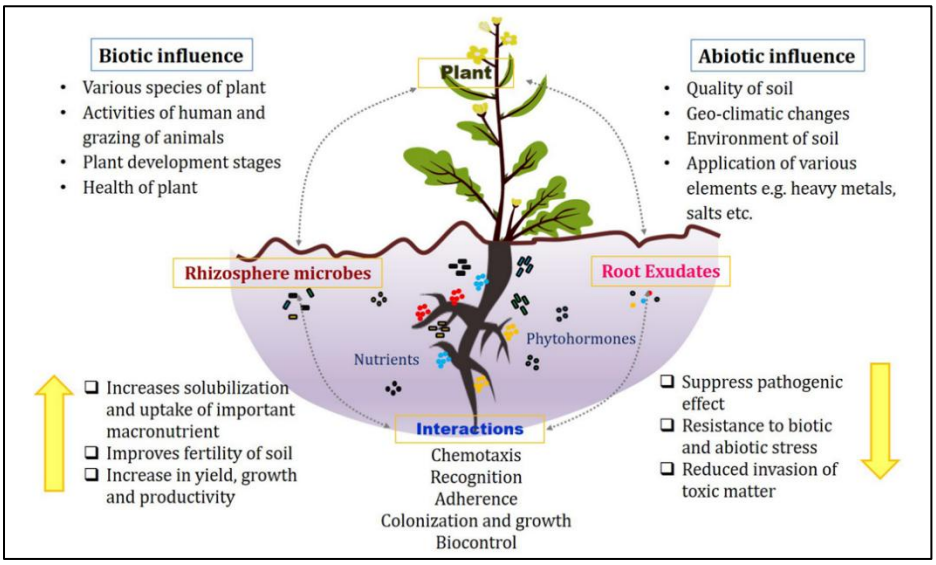


Figure 1: Interactions in the root microbiome between the plant host, microbes, and root exudates under biotic and abiotic influences (Vishwakarma et al., 2020).

The natural world is filled with a multitude of symbiotic relationships spanning all kingdoms and environmental conditions. Recent technological advances in metagenomic sequencing and growing interest have facilitated studying the effects of these commitments to both host and microbe, as well as begin uncovering how these relationships are established and maintained. The plant root microbiome is an especially fascinating community of free-living organisms encompassing bacteria, fungi, and archaea due to its impressive diversity in quantity and function. These microbes have provided plants with essential nutrients and have served as the first line of defense for biotic and abiotic stresses for millions of years in exchange for a cut of the host's carbon sources. Certain members of this community have well-studied, specialized functions, most notably the mechanisms used by leguminous plants to associate with nitrogen-fixing rhizobia bacteria (Franche et al., 2009). Our understanding of community-wide structure, while more complicated and expensive to study, has made some notable progress. One study in particular found a keystone microbe from a community of 185 that was singlehandedly responsible for maintaining proper root development, using its auxin degradation ability to act as a mediator between

arabidopis thaliana and its microbiome (Finkel et al., 2020). Even so, the majority of individual functions and how they take priority within a large community have not been well-studied due to their complexity. These studies also often focus on bacterial members, rather than other domains found in the root microbiome, due to how much easier these are to culture for experimental purposes as well as how plentiful they are in the community.

Recent efforts have sought to elucidate how a host is able to communicate with and manage such a multifaceted symbiotic system. For one, it's very likely that plants hold greater control than their interacting microbes on overall function, as multiple studies have found symbiotic hosts are capable of discriminating whether microbes are providing the intended benefit and can take action to penalize a symbiont when this doesn't happen. While this control has been more difficult to study in diverse root microbiome communities, studies have shown that pea plants can actively regulate which nodules are supplied carbon based on their nitrogen-fixing output (Moran, 2024b). Regarding communication with the comprehensive root microbiome, it's well-known that the host utilizes root exudates and other chemical signaling molecules to attract and regulate the microbes of choice. One established example is how flavonoids released by the roots of pea plants and other legumes are essential in recruiting essential N-fixing bacteria (Franche et al., 2009). It is also known that recruited microbes may aid in ensuring these interactions. Following the legume example, in response to flavonoid release, rhizobia microbes release nod factors that signal the development of small lumps in the plant root to house rhizobia and specialize in anaerobic nitrogen fixation. Beyond well-studied plant-microbe interactions, it's been discovered that the plant immune system plays an integral role in microbiome assembly by serving as an initial screen for bacterial association. The host typically uses Microbe-Assisted Molecular Patterns (MAMPs), or conserved structures in the microbe surface, to detect the presence of pathogens, and researchers have found these same immune responses also occur for beneficial bacteria, except these commensal bacteria are typically the only ones that can suppress or evade these immune responses to establish themselves in the root (Teixeira et al., 2019).

One consistent finding from studying host-microbe interactions is how often the benefit of an individual microbe to an inoculated host can be diminished or lost when in the presence of a community. Why this occurs likely depends on a variety of external factors, but may be due to competition with other microbes for the same energy provided by the host or outright growth inhibition by other community members. At the same time, it's been found that microbe-microbe interactions can support a community for longer than individually grown cultures. One study found

that a collection of microbes in liquid media was able to consistently stabilize to a similar community at the family level of the taxonomic hierarchy, and these communities produced 25% more biomaterial than any growth achieved in monoculture while also promoting a less drastic change in media acidity, suggesting that microbial communities utilize nutrients more efficiently than individual members (Goldford et al., 2018).These seemingly contradicting views emphasize the importance of studying microbe-microbe interactions to understand what antagonistic, synergistic, or neutral mechanisms must take place in order for the root microbiome to balance multiple functions. This also highlights the importance of context, in this case the presence of a specific stress, as it is possible that stress-specific beneficial microbes are not completely lost but hidden behind additional microbes whose functions have been prioritized by the host, instead. While this concept of hidden or background microbes hasn't been directly studied in the plant root microbiome, it is supported by recent studies from the gut microbiome field. One presentation I recently attended focused on gut microbiome recovery in patients after antibiotic use and discovered that while antibiotics do eliminate gut microbe abundance, certain microbes continued propagating in very small frequencies and successfully reestablished themselves once conditions were more favorable. Moreover, these findings reflect microbiome recovery strategies after ecological disruption, notably how multiple strains have demonstrated they can enter a dormancy stage, or a decrease in metabolic activity that can be reversed under the right environmental conditions (Shade, 2023). Still, dormancy mechanisms in the root microbiome are poorly studied despite their presumed importance in contributing to plant stress resilience.

Regarding the organization of the root microbiome, we know that there must be some compartmentalization taking place in order to efficiently sustain the amount of microbes that colonize plant roots. One general agreed-upon distinction lies in the differing composition between the more diverse rhizosphere that comprises the soil immediately surrounding the roots and the less abundant, likely more selective endosphere comprised of microbes that colonize inside root tissue (Trivedi et al., 2020). Some researchers create even more distinction in the rhizosphere by grouping those that colonize the root surface into the rhizoplane to better highlight the increasing host filtering that occurs from the initial reservoir of microbes in the soil (Bai et al., 2022). In addition to this initial sorting, stochastic or gradient differences in mineral availability, oxygen availability, and other soil properties create further microbiome partitioning, as a study on the root microbiome of three bioenergy crops found that differences in abiotic conditions were correlated with differing community composition along a depth gradient that likely lead to further niche development (Benucci et al., 2023). Researchers

discovered that while microbial diversity decreased with depth, bacterial networks strengthened or were maintained with increasing depth, highlighting the importance of host communication in maintaining stable microbe-microbe interactions despite varying microenvironments. Moreover, one notable pattern inferred from this investigation's models is how, in the absence of stress, processes such as ecological drift, referring to differences in community composition due to chance alone, or passive dispersal, describing the random movement of microbes, had a larger impact on microbiome composition than host selection. It would be interesting to perform this study in a stressed environment, as host selection would likely be stronger in order to recruit the necessary microbes to promote a specific resilience.

This recruitment is primarily performed by root exudates, a vast assortment of compounds secreted by the root system that function as the primary currency by which microbes use to establish themselves in the root. These host secretions are often coupled with nutrient utilization by microbes and have been found to drive rhizosphere assembly (Beattie, 2018). This study found a distinction within this compartment between bacteria that responded positively to plant exudates by increasing abundance near roots and bacteria that responded negatively by decreasing or showing no difference in abundance near roots. Microbial preferences to energy supply likely create the foundation for chemical compartmentalization throughout the rhizosphere, one that is possibly further enforced by microbe-microbe interactions. Microbial members can be equipped with a large variety of mechanisms to compete with or inhibit other members, notably antagonistic secretion systems and antibiotic production (Geller & Levy, 2023). Conversely, members also employ interactions that allow them to grow alongside one another, with the most notable being metabolic cross-feeding, where the biochemical byproducts of one strain serve as energy sources for other bacteria (Goldford et al., 2018). These assorted mechanisms likely provide the push and pull factors that spatially organize and stabilize the root microbiome.

The environment plays an important role in the root microbiome, as multiple factors such as climate and soil properties may determine what reservoir of soil bacteria are available for association, as well as what specific functionalities would be most beneficial for a particular plant host, for example additional aid in dryer or hotter environments. Diversity often plays an essential role in providing microbial communities with these resiliencies. For example, one review paper notes how alleviating salt stress depends on species diversity, as the beneficial mechanisms rely on synergies among different microbial species (Bai et al., 2022). Diversity also plays a role in providing stress resilience to the plant via functional

redundancy, or multiple strains that can take on the same role in the microbiome (Shade, 2023). Shade specifically notes how genes involved in nitrogen fixation are distributed throughout a broad range of microbial taxa, and this demonstrates how much functional redundancy exists for this very essential function, likely contributing to microbiome stability in the event of environmental disturbances. The environment itself may act as a contributor to microbiome stability through what is deemed soil legacy, referring to the variety of preexisting activity from previous microbial associations that can often promote resilience for subsequent plant growth (Jing et al., 2022). Positive legacies, ranging from chemical compounds to microbial activity, have been widely observed in promoting agricultural yield through sustainable practices such as crop rotating. Moreover, these legacies have even led to generational disease resistance, as highlighted in a study where *arabidopsis thaliana* was planted in a field and inoculated with a pathogen (Teixeira et al., 2019). Not only was there a notable increase in three specific bacteria, the supposed beneficial associations that brought about this resistance, but this led to continued conferred resistance to the subsequent generation of plants in that field.

Another important contributor to root microbiome composition is plant host. Typically, no two plant species will associate with an identical microbe community. While environment certainly has an impact on composition, as one study found that 48.3% of rhizosphere variation was explained by site, this dominance decreases as host filtering plays a bigger role in composition, with 52.8% of the variation in the root endosphere being explained by crop site (Xiong et al., 2021). Moreover, despite environmental differences between the two sites in this experiment, higher taxonomic classification remained relatively consistent within each crop, and each observed a distinct set of dominant taxa. Dominant taxa are a consistent observation in microbiomes often referred to as the core microbiome, and it is often determined in plants as the microbial members present in 80% of samples in any given species. Core microbiome members are assumed to play essential roles in microbiome function, as many are already known to provide hosts with benefits such as growth promotion and nutrient acquisition (Trivedi et al., 2020). This review also discusses how much of the core microbiome overlaps with the heritable microbiome, which does not mean these microbes are inherited, as the large majority of the plant microbiome is horizontally transmitted, but instead describes the microbes whose association has been linked to the host genome. Plant genetics has a significant impact on microbiome composition even within the same species, as genotypes, or genomic variations of the same plant host often due to adaptations gained from their native environment, have also been found to create differences in microbial composition (Edwards et al., 2023). Edwards' study on the differences in microbiome composition among three natural accessions, or

genotypes, of *panicum virgatum* found that host genotype was a significant effect on the variation in community composition, and observed that differences in microbial enrichment patterns were due to affinity for strains local to a genotype's native environment.

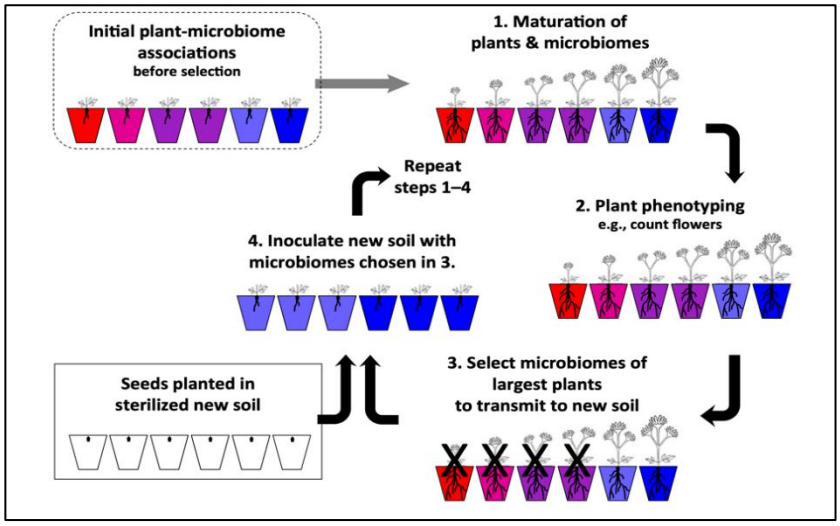
A prominent feature of microbial communities is their dynamic approach to handling disturbance. Trivedi et al., 2020 discusses how plants recruit a stress microbiome by reusing the same pathways to respond to both abiotic and biotic stresses. Notably, specificity in microbial recruitment under these conditions appears to be more focused on microbe function rather than an exact species to allow for a more flexible approach to changes in the host's environment. Studies on specific associations provide an even clearer depiction of what is possible in symbiotic systems. Researchers have been able to simulate the evolution of an established symbiotic relationship had the insect host *plautia stali* lost its essential nutrient-providing gut microbe *pantoea* by replacing the current symbiont with *E. coli* (Moran, 2024a). With time, mutant *E. coli* was able to establish in the insect gut for near normal insect development, and genetic analysis found that a single inactivation of a gene in the e. coli genome made this bacterium more metabolically compatible for symbiotic association with the insect. This discovery suggests that once a relationship mechanism has been established, it becomes easier for the host to recover a beneficial interaction with a different microbe if, say, the current strain has degraded over time or has developed parasitic habits. This adaptability may be due to how much faster bacteria evolve significant genomic changes compared to other organisms. In addition to their smaller genomes and shorter reproductive cycles that allow for higher mutation rates, therefore a larger chance for beneficial changes to occur, bacteria can use mechanisms for horizontal gene transfer to exchange genetic information with other strains. These combined factors create higher opportunities for advantageous genomic changes to occur and propagate quickly. While symbiotic studies this thorough be difficult to investigate in root microbiomes at the moment, this adaptable potential explains

why the root microbiome has been used as an environmental stress buffer for plants for the past millions of years.

Artificial Microbiome Selection

Artificial microbiome selection is a protocol used to induce a specific stress resistance in a host species through its existing associations with a microbial community (Mueller & Sachs, 2015). This practice parallels how breeders take advantage of the natural genetic variation in a community by selecting those who display a desired phenotype to mate over multiple generations until an enhanced phenotype is achieved. Microbiome selection instead takes advantage of the diversity and adaptability of a microbe community by stressing a host's environment over multiple generations until a specific resistance is amplified. By transferring the microbiome of an organism that is consistently exposed to a certain stress over multiple generations, the result is a community strengthened by bacterial interactions essential to this increased resistance. Multiple selection lines, or groups of plants in identical conditions with the same replicate size, are utilized to increase the random odds for additional beneficial interactions to be established. This procedure differs from traditional selection in that the latter may come at some noticeable cost to the host over time, including increased vulnerability to genetic disorders or viability issues such as decreases in fertility, overall health, and life expectancy.

Figure 2: Host-mediated plant root microbiome selection strategy. From Mueller & Sachs, 2015.



Root microbiome selection offers compelling benefits toward the development of renewable energy sources. Biofuel grasses have great potential

as an alternative to current unsustainable fuels due to their extensive root architecture, large biomass yield, and versatile environmental range. These crops can grow in poor field conditions so as not to compete with agricultural fields and don't require much human input to cultivate. The discovery and use of stress-tolerant bacterial communities would enhance the production of bioenergy crops through more efficient growth in environments struggling with nutrient imbalances, organic pollutants, heavy metals, or salt stress without the additional need of bioremediation treatments. One successful iteration of artificial microbiome selection in plants has been the improvement of both sodium and aluminum salt stress tolerance in *brachypodium distachyon*, a model bioenergy grass (Mueller et al., 2021). A collection of soil samples from three different field sites were consolidated and used to obtain the microbiome community that would be used for the initial generation of genetically identical plants. After transferring the microbiomes of the largest plants from each cohort for nine generations, the resulting microbiomes were able to sustain their *brachypodium* hosts significantly better than control plants without a microbial inoculant, with up to a 205% increase in seed production that serves as a typical indicator of plant fitness.

In addition to providing a crucial protocol for the successful development of stress-resistant microbiomes for bioenergy crops, Mueller's experiment also provides key insights for future iterations of this process. One highlight includes how many generations would be needed to obtain the beneficial bacterial interactions. While the salt stress microbiome selection experiment underwent eight generational microbiome transfers, Figure 2 in Mueller's paper shows that the benefits to the host measured via aboveground biomass production gradually increased until the 3rd generation under Sodium stress and 5th generation under Aluminum stress, after which biomass production decreased and failed to recover earlier levels. This was likely due to too sharp of an increase in salt treatment, preventing the additional stress-tolerant bacterial interactions from establishing in a timely manner. These results may also reflect the idea of diminishing returns epistasis, or the evolutionary concept that beneficial changes are established in a logarithmic pattern where initial selection leads to exponential gains that eventually slow down and become stagnant as acquired changes have less of an impact on the trait of interest (Wünsche et al., 2017). This study's findings suggests that constant stress can eventually lead to a plateau in phenotypic benefit, meaning additional beneficial interactions may have been acquired but were lost because their impact wasn't phenotypically beneficial at the time. Despite these limitations, the results of Mueller's artificial selection experiment were successful, and it's likely that a future iteration under the right environmental conditions will not require more

than nine generations of plant growth so long as the stress-resistance indicator is increasing at a sufficient rate.

Artificial microbiome selection is a promising procedure that allows researchers to develop stress-tolerant organisms without needing to be completely aware of what mechanisms created them. However, with increasing interest regarding how plant-microbe and microbe-microbe interactions take place, one way to begin inspecting is the use of synthetic communities as the starter microbiome inoculum. Rather than obtaining the microbiome from soil samples, researchers can artificially create a controlled microbial treatment by growing an existing collection of microbes in liquid culture that are later consolidated in the same buffer for inoculation. This enables complete control of what is included in a microbiome, expanding the possibilities for what microbiome selection experiments could be designed. This approach also simplifies and expands microbiome analyses with, for instance, the development of rapid screens to detect specific bacterial propagation or more manageable sample preparation for metagenomic sequencing. For these reasons, and the availability of a bacterial database isolated from various *panicum virgatum* field sites by the Juenger lab, preliminary experiments involved the use of core microbes from this bioenergy grass.

Nitrogen Selection

Nitrogen is a crucial component to all living things, but the majority of the world's source is biologically inaccessible in its stable atmospheric N_2 form and therefore must be converted, or fixed, into more active inorganic compounds such as ammonia and then nitrate to be readily used. The world relied on diazotrophic bacteria in the soil for accessible nitrogen sources until the Haber-Bosch process developed in the early 20^{th} century revolutionized agricultural output by making it possible to synthetically mass-produce ammonia (Mus et al., 2022). While this invention dramatically increased food production and has become essential in supporting current human population levels, the overuse of synthetic fertilizers has had detrimental effects on the environment. Overapplication of nitrogen in agricultural fields leads to nutrient imbalances that disrupt mineral cycling processes and reduce soil quality over time. This imbalance drives microbial life to convert excess nitrogen to nitrous oxide, a greenhouse gas more potent than carbon dioxide that significantly contributes to climate change. Overuse also creates nitrogen runoff to aquatic ecosystems who are incapable of dealing with a mass influx of nutrients, leading to eutrophication, or oxygen depletion that can severely reduce biodiversity. Determining more efficient methods to provide crops

with the nitrogen required for proper growth and fitness would serve as a monumental alternative to the current use of synthetic fertilizers. Artificial microbiome selection serves as a promising strategy to increase nitrogen use efficiency (NUE) in plants via symbiotic interactions that are gradually tailored to an individual species and strengthened using nitrogen deficiency with each microbiome transfer. While this approach would undoubtedly benefit food crops, microbial selection could also enhance the biomass yield of bioenergy-hopeful grasses that researchers hope to grow in low-quality soil with very minimal fertilizer or other human intervention.

Once internal seed stores run out, plants depend entirely on microbial associations to obtain this essential nutrient for universal biomolecules such as nucleic acids, amino acids, or energy compounds, as well as vital plant function such as the synthesis of chlorophyll necessary for photosynthesis and plant growth (Franche et al., 2009). There are two primary methods plants have developed to interact with diazotrophic bacteria. The most well-researched system is the compartmentalization of this conversion to small root organs called nodules that support N-fixing bacteria that only grow in anaerobic, or oxygen-less, conditions. Root nodulation evolved independently across a variety of plant lineages, with the most notable being the legume family and their association with rhizobium bacteria. Grasses, on the other hand, use a likely earlier-established association method involving aerobic bacteria in the soil who’ve developed a large assortment of mechanisms effective at maintaining minimal oxygen levels for nitrogen fixation while in oxygenated conditions. These interactions can be intimate, such as cases found in maize and rice where endophytic diazotrophic bacteria permeate inside their host’s root tissue, but are usually looser, for instance those created under proximity to the host leading to their establishment in the rhizosphere.

Many labs have attempted to engineer diazotrophic strains in an effort to better support plant host growth and function. Mus et al. (2022) describe the varying strategies utilized by the Ané lab to engineer azotobacter vinelandii to overproduce nitrogen, and their strains were implemented in our preliminary microbiome experiments. Azotobacter is an aerobic microbe that takes advantage of its high respiration rate to deplete its nearby environment of oxygen and ensure the efficient activity of nitrogenase, the enzyme responsible for converting atmospheric nitrogen to ammonia. While multiple insertion and deletion combinations concentrated to the primary NifL-NifA nitrogenase regulatory system were attempted, one of the more successful and well-studied engineered strains was AvFM2, shortened to FM2 for this paper, and thus became our engineered azotobacter strain of choice for preliminary microbiome experiments. This specific

strain had the C-terminal domain of the NifL antiactivator deleted, as well as the insertion of a kanamycin resistance gene to ensure the selective propagation of the engineered strain in the presence of kanamycin.

However, the long-term viability of the engineered strains has been called into question due to the possibility for deletions to be regained via homologous recombination, including genetic exchange that could occur within the strain or with nearby bacteria. Moreover, some engineered strains have been found to be prone to cheaters, or individual azotobacter cells that stop fixing nitrogen but still take advantage of the ammonia excreted by other members. Considering how diazotrophy is already an energy-intensive process, it's reasonable that modifying a strain to overproduce nitrogen may create an unstable bacterium. One way to possibly minimize these issues may be to include the engineered strain in a microbial community. Under nitrogen-deficiency, we infer that a microbiome may be able to provide this strain with the ideal conditions for proper function in exchange for being essential in alleviating stress for the plant host. A variety of strategies could make this possible, with the most straightforward being cross feeding networks that could develop and supply FM2 with ample metabolites to sustain an overproduction of nitrogen (Geller & Levy, 2023). The variety of methods bacteria have developed to share genetic information with other strains, known as horizontal gene transfer (HGT) and highlighted as a means of community rescue to disturbances by Shade in a 2023 review, combined with their rapid evolutionary ability under the right circumstances, may also prove valuable. The engineered microbe could, for example, acquire an additional metabolic pathway from another microbe to support its energy-intensive needs, or a random mutation could emerge that grants FM2 with a metabolic advantage.

Preliminary experiments conducted over the course of three semesters will aid in determining how to best implement an artificial microbiome selection experiment utilizing synthetic communities to promote NUE biofuel crops. For the majority of conducted experiments, we focused on studying the perennial bioenergy candidate *panicum virgatum* variety *alamo*, more commonly known as switchgrass. We also investigated the model grass *panicum hallii* variety *filipes*, also known as Hall's panicgrass, due to being a smaller and more manageable relative of the biofuel of interest. Another aspect of these preliminary studies is the integration of the beneficial although unstable engineered bacterium into a microbe

community to determine what plant-microbe and microbe-microbe interactions may benefit the strain's propagation.

Results

Nitrogen Stress

For the nitrogen stress experiment, a linear regression statistical analysis was used to determine the effect of the different nitrogen input treatments on the harvested data of maximum shoot length and dried aboveground biomass. We used the following input amounts determined by a study from Poiré et al., 2014 to indicate varying levels of Nitrogen stress: 0uM N indicates no N input, 600uM N indicates typical N input, and 6000uM N indicates over-abundant input. Pairwise comparisons of the estimated marginal means were used to determine the significant differences between the N treatments within the different plant systems used in this experiment, allowing us to essentially obtain the same output as an analysis of variation (ANOVA) model.

The datasets were first evaluated to ensure they met analysis assumptions, after which the significance results were visualized in a table using the kable function in the knitr R package. Highlighted rows indicate which treatment-plant interactions were significant based on the typical significance p-value of less than 0.05.

Results from the maximum leaf length dataset in Table 1 indicate that only the nitrogen treatments from *panicum hallii* variety *filipes* and *panicum virgatum* variety *alamo* displayed any significant differences in aboveground length. *Panicum hallii* shoot length was significantly different with every treatment contrast, while *panicum virgatum* shoot lengths were only significant when comparing no N input with an over-abundant N input and a typical N input with an over-abundant N input.

contrast	plant	estimate	SE	df	t.ratio	p.value
0 - 600	brachy	-0.7750000	0.9594284	247	-0.8077726	0.6985670
0 - 6000	brachy	-0.2000000	0.9594284	247	-0.2084574	0.9763314
600 - 6000	brachy	0.5750000	0.9594284	247	0.5993152	0.8206024
0 - 600	fil	-1.7769048	0.5578689	247	-3.1851658	0.0046368
0 - 6000	fil	-4.2197619	0.5578689	247	-7.5640751	0.0000000
600 - 6000	fil	-2.4428571	0.5617838	247	-4.3483940	0.0000595
0 - 600	rice	-0.8125000	1.0726736	247	-0.7574532	0.7294018
0 - 6000	rice	-2.5075000	1.1147551	247	-2.2493730	0.0651155
600 - 6000	rice	-1.6950000	1.0062570	247	-1.6844603	0.2131777
0 - 600	virg	-0.7441645	0.6347225	247	-1.1724249	0.4707747
0 - 6000	virg	-2.5141164	0.6025306	247	-4.1725950	0.0001233
600 - 6000	virg	-1.7699519	0.6204982	247	-2.8524690	0.0130188

Table 1: Results from the linear model analysis on the significance of maximum leaf length as an indicator of nitrogen treatment. Rows highlighted in green indicate significant contrasts.

Results from the log2-transformed aboveground biomass dataset in Table 2 indicate that only the nitrogen treatments from *panicum hallii* variety *filipes* and *panicum virgatum* variety *alamo* displayed any significant differences in aboveground length. *Panicum hallii* shoot biomass was significantly different with every treatment contrast, while *panicum virgatum* shoot biomass was only significant when comparing no N input with an over-abundant N input.

contrast	plant	estimate	SE	df	t.ratio	p.value
0 - 600	brachy	-0.1223882	0.2779649	247	-0.4403009	0.8987116
0 - 6000	brachy	0.0122476	0.2779649	247	0.0440616	0.9989302
600 - 6000	brachy	0.1346358	0.2779649	247	0.4843626	0.8787862
0 - 600	fil	-0.3865487	0.1616254	247	-2.3916343	0.0459563
0 - 6000	fil	-1.0246171	0.1616254	247	-6.3394576	0.0000000
600 - 6000	fil	-0.6380684	0.1627596	247	-3.9203120	0.0003364
0 - 600	rice	0.1446553	0.3107742	247	0.4654676	0.8875075
0 - 6000	rice	-0.1598578	0.3229660	247	-0.4949680	0.8737789
600 - 6000	rice	-0.3045132	0.2915321	247	-1.0445272	0.5495285
0 - 600	virg	-0.3925574	0.1838913	247	-2.1347249	0.0849969
0 - 6000	virg	-0.7797609	0.1745647	247	-4.4668867	0.0000359
600 - 6000	virg	-0.3872035	0.1797703	247	-2.1538793	0.0813693

Table 2: Results from the linear model analysis on the significance of aboveground biomass as an indicator of nitrogen treatment. Rows highlighted in green indicate significant contrasts.

To visualize the distributions of maximum shoot length and aboveground biomass within the nitrogen treatments among the plant systems involved in this experiment, beeswarm plots were created using the ggbeeswarm extension package to the conventional ggplot2 library. Point transparency was included to more easily view overlapping data. Unlike boxplot visualizations, beeswarm plots provide a more detailed view of dataset density such as clustering and outlier groups.

For the maximum leaf length dataset (Figure 3), you can clearly see that *brachypodium distachyon* and *oryza sativa* did not respond distinctively to the nitrogen treatments, as both plant systems clustered within the same range and dealt with plenty of outlier groups. *Panicum hallii* variety *filipes* and *panicum virgatum* variety *alamo* responded much more favorably, with the *virgatum* 0uM N treatment displaying an outlier cluster on the higher end of the distribution that probably prevented all the treatments for this plant from being significant.

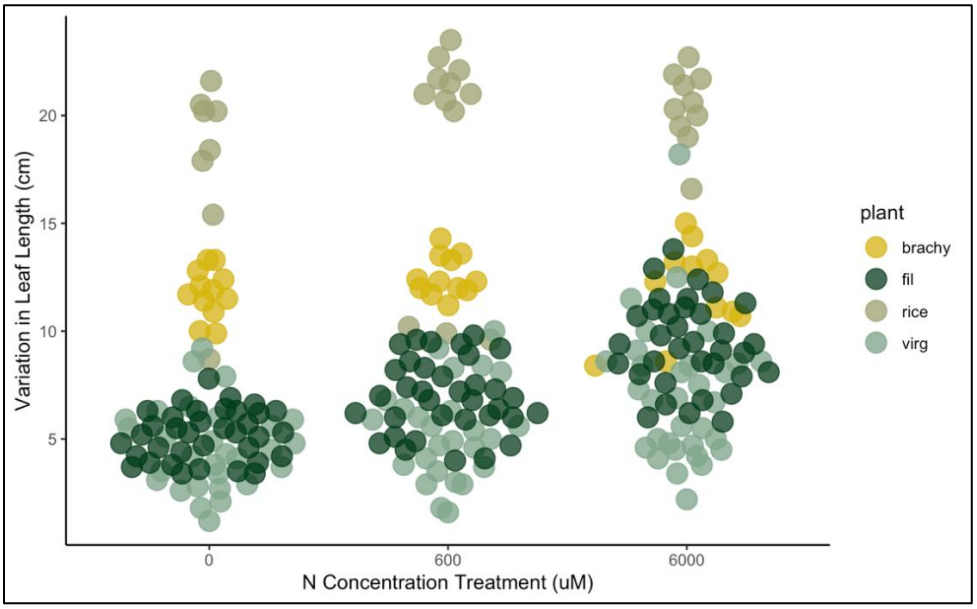


Figure 3: Beeswarm plot visualizing the distribution of maximum leaf length from different model plant systems in different nitrogen inputs.

For the aboveground biomass dataset (Figure 4), you can clearly see that *brachypodium distachyon* and *oryza sativa* did not respond favorably to the nitrogen treatments, as both plant systems clustered around similar but rather dispersed datapoint ranges per treatment, and also dealt with outlier groups. *Panicum hallii* variety *filipes* and *panicum virgatum* variety *alamo* responded more distinctively, with the virgatum dataset exhibiting a larger amount of dispersal and outliers that probably prevented all the treatments for this plant from being significant. If you focus on where the three sets of data center, you can observe a prevalent upwards trend.

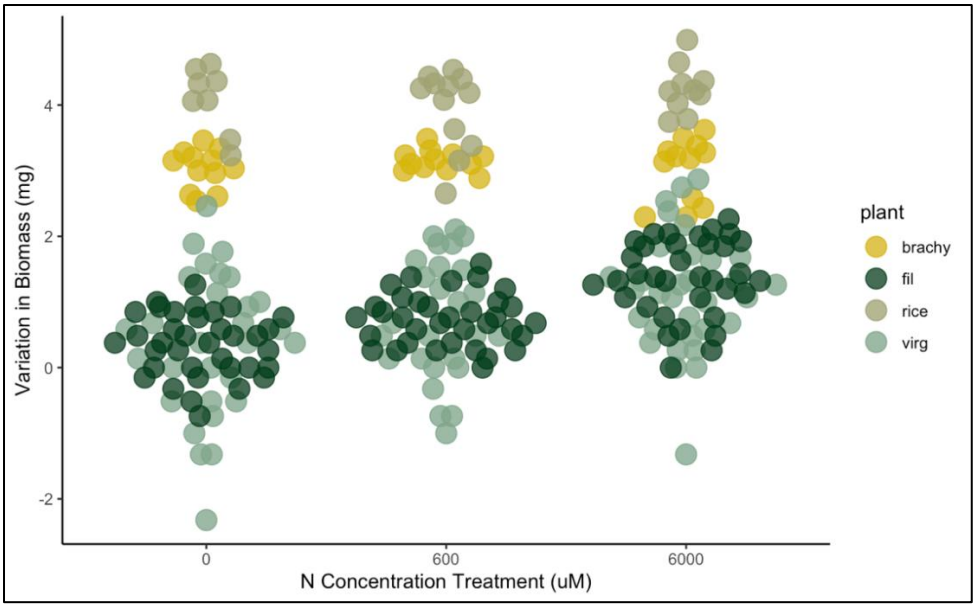


Figure 4: Beeswarm plot visualizing the distribution of above-ground biomass from different model plant systems in different nitrogen inputs.

Synthetic Communities

For the syncom experiment, an analysis of variation (ANOVA) statistical model was used to determine the effect of different synthetic community treatments on the harvested data of *panicum virgatum* maximum aboveground length, maximum belowground length, germination rate, two chlorophyll measurements, and aboveground biomass. Tukey's HSD test was then used to determine what specific treatments experienced significant differences from one another.

The datasets were first evaluated to ensure they met analysis assumptions, after which the significance results were visualized in a table using the kable function in the knitr R package. Highlighted rows indicate which treatment contrasts were significant based on the typical significance p-value of less than 0.05. Of the six ANOVA analyses that were run, only two exhibited significant contrasts.

Results from the square root-transformed maximum aboveground length post-hoc analysis displayed in Table 3 indicate that the only significant comparisons involved the FM2 engineered microbe-only treatment with the synthetic community treatment that included engineered microbe, as well as the FM2 treatment with the control PBS buffer-only treatment.

contrast	estimate	SE	df	t.ratio	p.value
FM2 - (FM2 + Syncom)	0.4169766	0.1341953	154	3.1072364	0.0119397
FM2 - PBS	0.3666666	0.1333845	154	2.7489448	0.0335116
FM2 - Syncom	0.3373132	0.1421883	154	2.3722986	0.0867954
(FM2 + Syncom) - PBS	-0.0503101	0.1341953	154	-0.3749017	0.9819710
(FM2 + Syncom) - Syncom	-0.0796635	0.1429493	154	-0.5572848	0.9444375
PBS - Syncom	-0.0293534	0.1421883	154	-0.2064402	0.9968759

Table 3: Results from the ANOVA analysis on the significance of maximum aboveground length among different synthetic community treatments. Rows highlighted in lilac indicate significant comparisons.

Results from the maximum belowground length post-hoc analysis displayed in Table 4 indicate that the only significant comparisons involved the FM2 engineered microbe-only treatment with the synthetic community treatment that did not include the engineered microbe, as well as the FM2 treatment with the control PBS buffer-only treatment.

contrast	estimate	SE	df	t.ratio	p.value
FM2 - (FM2 + Syncom)	1.0256765	0.5634628	152	1.8203091	0.2678091
FM2 - PBS	1.5905696	0.5707269	152	2.7869188	0.0302498
FM2 - Syncom	1.7830560	0.5920870	152	3.0114766	0.0159433
(FM2 + Syncom) - PBS	0.5648931	0.5740683	152	0.9840172	0.7587670
(FM2 + Syncom) - Syncom	0.7573795	0.5953085	152	1.2722472	0.5819816
PBS - Syncom	0.1924864	0.6021885	152	0.3196448	0.9886617

Table 4: Results from the ANOVA analysis on the significance of maximum belowground length among different synthetic community treatments. Rows highlighted in lilac indicate significant comparisons.

To visualize the distributions of maximum shoot length and maximum root length within the different synthetic community treatments in the *panicum virgatum* plant system, beeswarm plots of the significant datasets were created using the ggbeeswarm extension package to the conventional ggplot2 library.

For the square root-transformed maximum leaf length dataset (Figure 5), all treatments display a large range in shoot length as well as outliers or outlier groups. However, all treatments are shown to have a density cluster around the middle of the spread of points that seem to be more or less distinct when compared to the FM2 treatment cluster. It is clear from this visualization that the engineered microbe treatment plants were significantly larger than the rest of the treatments. This includes the synthetic community-only treatment that was technically not statistically significant (p = 0.087), as this was more likely due to outliers, such as the datapoint at the top of this treatment range, combined with the observation of two similar-sized clusters instead of one larger cluster as seen in the statistically significant treatments.

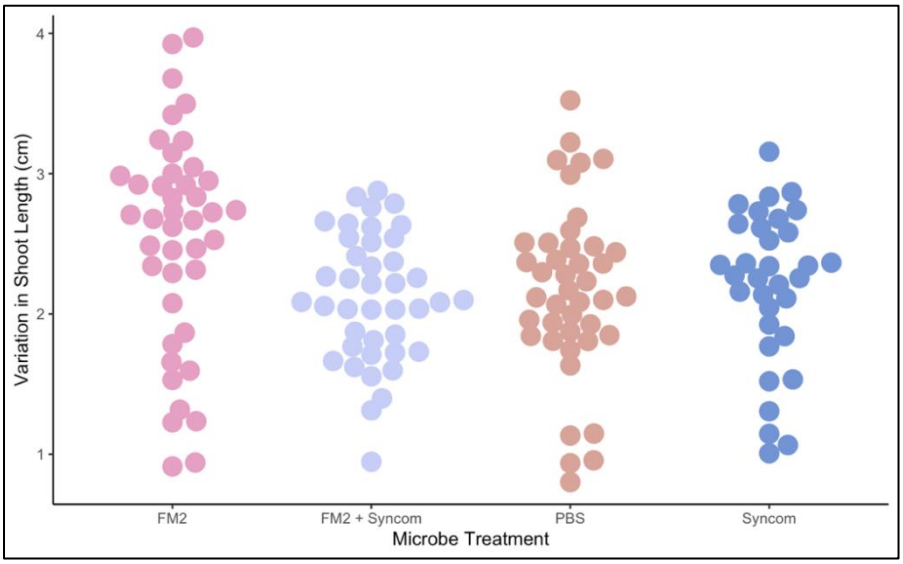


Figure 5: Beeswarm plot visualizing the square root-transformed distribution of maximum aboveground length of panicum virgatum under nitrogen stress and varying bacterial inoculation treatments.

For the maximum root length dataset (Figure 6), all treatments display a large range in shoot length as well as outliers or outlier groups. The FM2 engineered microbe treatment exhibits a surprisingly uniform spread, while the PBS control and synthetic community-only treatments show a dispersed but more obvious cluster. This likely contributed to the ANOVA model's ability to determine these treatments to be statistically significant from the engineered microbe treatment. The same would've been said for the synthetic community treatment that includes the FM2 microbe if it weren't for the three outliers at the top of this treatment range that undoubtedly prevented a statistically significant contrast with the FM2 treatment ($p = 0.27$).

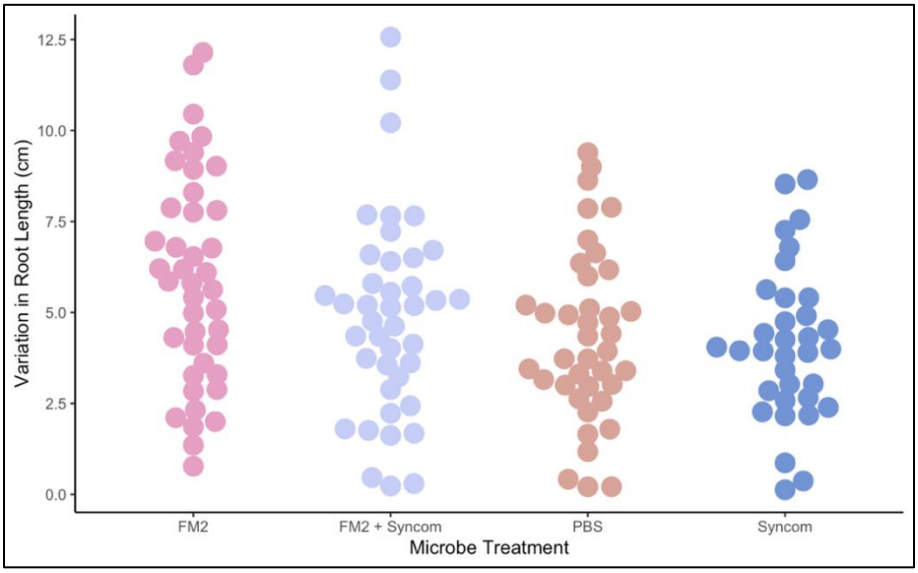


Figure 6: Beeswarm plot visualizing the distribution of maximum belowground length of panicum virgatum under nitrogen stress and varying bacterial inoculation treatments.

Preliminary Nitrogen Selection

The results of the preliminary nitrogen selection experiment unfortunately were not properly analyzed in time for this paper's submission. However, future analyses could include more ANOVA models for the aboveground biomass, belowground biomass, and engineered microbe colony count data collected during the experimental harvest. The most essential insights collected from this study would be found in the filtered microbiome samples, as metagenomic sequencing

could be used to determine the resulting bacterial composition and abundances of each selection line after 30 days of symbiotic activity. I do not know the methods by which this type of data is prepared, analyzed, or visualized, but I would love to learn how this is done if these samples are indeed to be sent for sequencing.

From observations made while harvesting this experiment, it was obvious that the *panicum hallii* variety *filipes* plants belonging to the 600uM nitrogen input positive control treatment grew most favorably, while the plants in the 0uM nitrogen input negative control treatment grew least favorably. From handling alone, it could be discerned that the selection lines that received the synthetic community treatment, with and without the addition of the engineered FM2 microbe, grew more-or-less on par from one another but better than the negative no-input control. The top-performing plants from the engineered microbe-only treatment line did not perform better than the plants from the two selection lines with a synthetic community treatment, though this may be due to the much larger repeat size of the syncom selection lines rather than a significant difference.

In addition, an initial engineered microbe screen was performed with the obtained filtered microbiome samples. While it was determined that a dilution after the 10^{-4}, or fourth, series was unnecessary, as it did not yield cell cultures, this initial screen did not provide clear enough results to justify using the data to determine whether the engineered microbe propagated. The primary issue is that while the synthetic communities used for this experiment were created with the intention that they could be easily screened for the engineered microbe, more than the distinctly yellow cell colonies were able to grow in this screen, and not in the expected treatments. From this, it is difficult to determine solely from culture growth whether cross contamination is the culprit, as the FM2 engineered microbe is already known to take longer to develop its typical yellow phenotype as you increase the kanamycin dose in a plate, and this morphology may take even longer to be observed, if at all, after interacting with the synthetic community for an extended period. This observation does not rule out the possibility that other stresses or even evolutionary changes that emerged from interacting with a community of microbes resulted in visual variations. An improved screen or PCR confirmation or both would be needed to determine what is truly growing on the plates, and so data from this initial engineered microbe screen was not used for further analyses.

Discussion

Optimal Selection Strategies

One crucial consideration when designing an NUE experiment is ensuring that microbiome selection occurs in a directional matter. There is no straightforward method to continually increase nitrogen deficiency akin to the salt stress treatment from the last artificial selection study, but a couple improvements from previous selection investigations may be promising. One straightforward change from the salt resistance experiment includes watering with the 0uM nitrogen Hoagland solution rather than sterile water to only promote nitrogen stress specificity in the microbiome. Similar to the experiment by Mueller et al., 2021, a more complicated addition may be to normalize seed weight along a gradient such that the heaviest seeds from the inbred stock are used in the starter generation followed by progressively lighter seeds in order to simulate a decrease in initial nitrogen input from seed nutrient stores. On the other hand, previous studies aiming to induce nitrogen efficient crops via microbiome selection were able to show improved aboveground biomass without the diligent preparation modeled by the salt stress experimental procedure. Specifically, Garcia et al. increased aboveground biomass for *brassica rapa* by 36% compared to the microbiome-less control over nine generations of microbiome transfer, and this was observed under one small dose of nitrogen-based fertilizer for each 10-day generation. While these findings involve a different plant and don't compare to the benefits produced with Mueller's protocols, it is still impressive that significant selection was observed over much less stressful conditions and with a much smaller generational length. This prompts reconsideration into the balance between how long one generation of growth should take at minimum and how many generations are needed for adequate selection.

The experiments described in this paper provide a couple of insights into how to best go about performing a Nitrogen Use Efficiency (NUE) microbiome selection experiment. For one, according to the nitrogen stress experiment, it would likely be better to use a plant model with a smaller seed, as the smaller-seeded plants of *panicum virgatum* variety *alamo* and *panicum hallii* variety *filipes* were the only ones that showed differential responses between the nitrogen treatments. This is likely due to how all seeds supply an initial nutrient source that provides seedlings with adequate support before being able to establish themselves in an environment. If you assume that seed size is proportional to initial

N input, then a smaller seed will depend more on its environment and thus have a stronger response to Nitrogen deficiency.

Panicum hallii may be the best plant to use for an NUE selection experiment. This was the only plant from the nitrogen microbox experiment that displayed significant differences between all treatments on both measured indicators. In addition, this plant is the closest relative to the biofuel crop of interest, easier to grow and study, and can self-pollinate to create identical plants to reduce genetic differences as a confounding factor in microbiome responses. Hallii also features preexisting genomic resources that could provide a successful selection experiment alongside robust subsequent experiments utilizing the Juenger lab's existing mutant population or genomic analyses or both. With recent advances in differential gene expression analyses, It would be interesting to determine the role that genomics, from both the host and symbiont, plays in determining what changes created a more nitrogen-efficient root microbiome. Furthermore, *panicum hallii* is a perennial like its larger cousin, and there is interest in studying how microbiome selection would behave in this system, mainly because it's assumed that a plant that can be efficient enough to live longer than a season may have more established strategies for establishing and maintaining their microbiome.

The primary downside to using *panicum hallii* to perform a nitrogen efficiency selection experiment is how long this plant takes to germinate after transfer, extending how long each round of selection would take. One way to minimize this may be to perform *hallii* germination in another vessel the same week that rocket pots preparation occurs and then transferring the seedlings to the rocket pots. This vessel could be petri dishes where seeds germinate with hydrated autoclaved profile soil or on top of Hoagland solution solidified with agar. Both methods are known to provide appropriate conditions for sprouting in a sterile environment, and could benefit selection studies by minimizing the number of pots that have to be prepared to account for germination issues while also allowing further experimental normalization by being able to transfer seedlings that are a similar size. Moreover, this option would minimize the threat of contamination, as the bulk of incidents involving the presence of mold occurred during this vulnerable seedling stage. It may be possible to perform a nitrogen use selection experiment on a different, perhaps faster growing plant such as the previously used *brachypodium distachyon*, but experimental results indicate this crop may take longer to see noticeable differences. Another downside to using *panicum hallii* is the extensive and more fragile root architecture that makes harvesting the root microbiome a more delicate process. Despite the stresses that the preliminary selection experiment went through, the harvested plants still generated an ample

aggregate of roots, going so far down the rocket pot that it reached the polyfill filling at the base in one sample. This could be alleviated by performing the selection experiment in larger rocket pots.

The Ideal Selection Trait

A big question surrounding a host-mediated microbiome selection experiment that induces more efficient nitrogen use is determining what trait would be easiest to implement as the ideal selection indicator. The previous selection experiment used plant height as a rather accurate proxy for aboveground biomass to determine what microbiomes would be transferred to the next generation and successfully evoke the microbial interactions that promote salt tolerance. Plants have well-known and rather fast-acting stress responses to an overabundance of salt ions, mainly limiting certain types of transport in the membrane or compartmentalizing these ions into the cell vacuoles, which have consequences on what nutrients the plant can access and therefore negatively affecting how many nutrients the plant can allocate to aboveground biomass. Plant responses to Nitrogen stress, on the other hand, aren't as fast acting. Because it is such a crucial nutrient to growth and development, plants have developed a multitude of often more gradual responses to deal with this stress, including enhanced uptake mechanisms, internal nitrogen recycling, and of course mechanisms that serve to enhance interactions with N-fixing microbes. Plants may exhaust these methods before showing physical signs of stress, very unlike the immediate osmotic shock experienced with salt stress. This may explain why typical indicators of Nitrogen stress, such as the chlorophyll measurements attempted with the syncom microbox experiment, did not show observable nor statistical significance.

These responses are further confounded by the fact that selection cycles using *panicum virgatum* or *panicum hallii* are occurring with relatively young plants, as the younger the plant, the more it depends on initial nutrient inputs. It is suggested to limit a selection cycle to less than 30 days to minimize how long a microbiome selection experiment would take. This means selection will occur roughly halfway through maturity, as the cycle from germination to flowering takes minimum about 6-8 weeks for these plants. This differs from the shorter cycle of the previous *brachypodium* experiment where microbiomes were harvested after approximately 3 weeks, and flowering was reported to occur slightly after 30 days or as early as 20 (Mueller et al., 2021). It's not very clear how this would affect successful microbiome transmission between generations. Perhaps a longer selection generation means fewer total generations need to be performed, or a younger plant may mean more heightened microbial activity than one closer to

maturation, similar to how you typically want fresh plant tissue to get better genomic readings or more amplified immune response assay results. Based on current data and results from past NUE microbiome selection experiments (Mus et al., 2022), it may be more ideal to use aboveground length as the nitrogen use indicator and instead focus on obtaining more generations by growing *panicum hallii* up until there is enough differentiation to perform selection. This idea also supports observations from the preliminary selection experiment, as there were certain pots from the collection who clearly made the right interactions earlier than the rest and were the ones to propagate most successfully. Tracking aboveground length from generation to generation will be beneficial in determining if NUE is indeed increasing and when to stop the microbiome transfer.

The idea of trade-offs would continually permeate discussions surrounding beneficial interactions between plant and microbe, specifically that there are carbon costs to these associations for the host. This would help explain the results of the synthetic community microbox experiment, where there was a significant increase in shoot length for plants who were given the engineered microbe inoculum, but this increase was lost when the treatment included FM2 in the community. It is probable that the carbon costs associated with interacting with the entire community cancelled out the benefits of including a microbe that overproduces nitrogen for use by the plant. It is interesting that the host seemingly decided to act differentially between aboveground and belowground growth, as this carbon cost is evidently not present in root growth. This has implications into what the ideal selection indicator could be, as it's safe to assume that a measure that is less affected by these trade-offs would be the preference. It may be that a phenotype that focuses on what happens in the roots would be a better indicator of Nitrogen Use Efficiency (NUE), but a previous study using *brachypodium distachyon* observed that over an extended growth period, nitrogen inputs have no significant effect on root biomass while having a significant increase in shoot biomass with either a 500uM or 5000uM Nitrogen input (Barhoumi, 2017). Even if plants for this study were grown for 60 days rather than the 30 days these are typically grown for selection experiments, these findings suggest that root growth more or less stays the same with varying N additions. Despite any differential growth that may occur earlier in the host's life cycle, it seems that maximum shoot length could act as a potential proxy for nitrogen use efficiency and serve as the best selection indicator for a host-mediated microbiome selection experiment, as supported from findings with the Nitrogen stress experiment. Additionally, the two plants with the largest roots from the synthetic community treatment including the FM2 microbe in Figure 6 overlap with the three largest plants in this treatment in terms of shoot length. This data supports the continued use of aboveground height

as the selection indicator rather than attempt more rhizosphere-specific methods that would be more expensive or time-consuming. To ensure that the most successful associations are passed to the next generation, a good update to the current selection protocol may be to perform mixed microbiome harvesting on the three largest plants in terms of shoot length rather than the largest two, or harvest the largest 3-5 and using the ones with the largest root systems for microbiome transfer.

I am a bit weary of the idea that microbiome associations have such a significant effect on plant growth due to current symbiosis research indicating that hosts have more control over who they associate with than the symbiont has on the plant, to the point that there have been cases where the host can shut off whatever benefit it provides to a cheating microbe (Moran, 2024b). The idea of a heavy carbon cost for association also doesn't support the general motif observed in symbiotic relationships where if there isn't a need for the association, one won't be created, such as when N-fixing root nodules don't develop when the legume senses sufficient nitrogen in its environment. However, the bulk of these findings focus on relationships that only involve one well-established symbiont or symbiotic organ rather than a free-living community. The methodology used in plant-microbe experiments may also play a role in the extent to which bacterial associations have an impact on plant growth compared to field conditions. A direct microbial inoculation, for example, may limit the plant's ability to fight off a cheater compared to a less invasive interaction. In addition, these association experiments usually use younger seedlings that may have less developed mechanisms to manage microbial interactions, making it easier for microbes to take advantage of the host's energy source. One alternative hypothesis to why a beneficial increase in aboveground length was lost when the engineered microbe was inoculated within a syncom treatment would simply be due to competition with the community. Diversity is usually a beneficial characteristic, but in situations such as what this experiment observed where the nitrogen stress isn't as initially responsive as other abiotic stresses, the beneficial microbes may be prone to lose priority over other interactions the host would benefit from and become background microbes. This may make it more difficult for the host to communicate with the essential microbes it needs to minimize the stress once it has escalated. Due to this, the plant will continue to use its built-in mechanisms for dealing with nitrogen stress rather than dedicate energy to recruit bacterial aid. This may come at the cost of minimized aboveground length, a known symptom of nitrogen stress that perhaps isn't as evident in root length due to the additional energy provided for this tissue to locate a nitrogen source or associate with beneficial symbionts. An experiment that determines the culprit of shorter plant length when a synthetic community is

present would be helpful in deciding if shoot length truly is the better selection trait for a NUE microbiome selection study.

Creating Synthetic Communities

The most obscure aspect of the experiments building towards an NUE selection experiment would undoubtedly be the use of synthetic communities. A couple of factors may complicate the successful use of syncoms in a selection experiment, but not enough resources have been allocated to determine if these are significant confounding variables to determine how to minimize them. For one, unlike the previous salt stress selection experiment where a diverse microbiome was captured from a collection of field root samples, this new iteration uses a previously collected database of microbes collected from three different fields of *panicum virgatum*. Only those who are considered core microbes, or those found in abundance in a specific plant microbiome throughout an array of different environments, were used in preliminary experiments (Edwards et al., 2023). The switchgrass core microbiome consists of around 92 microbes according to Edwards, but to make experiments more manageable, only about 20-30 of them were used to create the synthetic community treatments. Diversity is often a great advantage for communities, especially when it comes to disturbance resilience mechanisms such as functional redundancy (Shade, 2023). With nitrogen being an essential nutrient, core microbes more than likely have mechanisms in place to compensate in the event of a nitrogen-limited environment. However, a less variable microbiome may limit the benefits it can provide and ultimately serve as a disadvantage at amplifying the most helpful interactions. It may be beneficial to at minimum include most of the core microbiome in future syncom selection experiments.

The fact that we are not well informed on the underlying mechanisms behind plant-microbe and microbe-microbe interactions complicates the use of synthetic communities in microbiome selection efforts. Efforts and estimates were made to determine what should and shouldn’t be included in the synthetic community treatments to most benefit the engineered N-fixing microbe, but none of these efforts were validated due to time constraints. For example, antagonistic interactions between the FM2 microbe and prospective members of the synthetic community were tested via the Burkholder agar diffusion assay (Burkholder et al., 1966). Three microbes were found to not allow the engineered microbe to grow in their proximity, but because we prioritized including as large of a number of members in the syncom experiment as readily available, these were still included in the treatment for a total of 26 syncom members. For the preliminary selection

experiment, we modified the syncom treatment around the ecological theory that when more than one member of a community performs the same function or uses the same energy source as another, those will compete for the same niche (Segura Munoz et al., 2022). We also modified the syncom to more efficiently screen the resulting microbiome for the propagation of the FM2 microbe through a Kanamycin screen in Burks media. For these reasons, 4 microbes that were known to fix nitrogen and 8 microbes that grew on half-dose kanamycin plates were not included in the selection experiment, for a total of 23 microbes in the synthetic community treatments. Despite the benefits of these changes, the idea that nitrogen-fixing bacteria cannot coexist was not verified neither was it directly supported by the previous Burkholder assay. Moreover, we are not aware of the complete functions of the core microbes and so aren't sure about the consequences of leaving multiple strains out.

Manipulating syncoms is further complicated by our limited understanding of how plant microbiomes are arranged in space. For example, it doesn't necessarily matter that two species aren't able to coexist in a plate when one typically resides in the root tissue endosphere while another finds its niche in the rhizosphere. Extrinsic factors such as spatial microbe locality and abundance likely play a significant role in their function throughout the root microbiome, but these unfortunately haven't been well studied due to the complex and often costly methodologies needed to study them, such as fluorescent labeling and metagenomic analyses. Another disadvantage to syncoms compared to the root microbiome samples used in the salt stress selection experiment may be that these originate from soil that contain, in addition to the all-essential bacterial members, a likely important of organic particles in the soil from a history of preexisting networks (Jing et al., 2022). These likely were not filtered out for the starter inoculum of the salt stress selection experiment, making it possible for these to have aided in making plant-microbe and microbe-microbe interactions more stable. This is very different from the microbes used to create synthetic communities who were isolated and have gone through multiple growth cycles via plating or liquid culture, meaning whatever beneficial compounds they contained were likely lost or degraded due to lack of use. It may be beneficial to determine how significant this disruption is by reincluding the organic particles from root samples and measuring syncom stability in a preliminary experiment.

For a future selection experiment utilizing synthetic communities, I would aim to include as many microbes as possible, or at minimum the core microbiome, rather than risk unintended consequences from not including a known member. Rather than utilizing a kanamycin screen to detect the presence of an engineered

microbe, a better method may be to develop a qPCR protocol from the available database of microbes to develop primers that would only amplify FM2. This would prevent the removal of possibly essential core microbes from the synthetic community treatments. This would also minimize the resources spent on creating a morphology-focused screen for a microbe that is known to change morphology with stress and whose identity would have to be confirmed with PCR either way.

Limitations

A consistent issue throughout the implementation of selection experiments was often growth chamber conditions. The stress experiment dealt with the least amount of growth issues, with ideal moisture and light conditions that led to less noise in the experiment. This is made obvious by comparing the more clustered beeswarm visualizations from this experiment with the larger dispersal of the syncom experiment data, as it dealt with larger, more frequent gaps and less centralized treatment distributions. The synthetic community experiment was likely affected by the decreased light conditions due to a change in the growth chamber that was used for this experiment. This experiment may have also been possibly affected by the use of PBS buffer to create the synthetic community treatments, as it's possible that this solution added ions to the microbox environment that may have contributed negatively to plant development. I've also been told by my supervisors that the reason PBS wasn't used as the buffer for microbiome transfer and filtering is because this solution can be harsher than alternatives on microbial activity. This may have had a more prolonged effect in the microbox experiments, as the added changes have no way of being minimized over time like a pot experiment though watering. Thus, PBS may have also interrupted plant-microbe and microbe-microbe interactions that possibly further contributed to the noisy syncom experiment data.

A stressed environment was observed by how much thinner the leaves of plants in the synthetic community experiment were growing compared to the fuller leaves of the nitrogen experiment plants. The control treatments of each experiment using *panicum virgatum* variety *alamo* didn't differ in the comparable maximum aboveground length dataset, as the median length of the nitrogen stress experiment was 4.3cm (Q1 = 3.1, Q3 = 5.9) while the median length in the syncom experiment was 4.6cm (Q1 = 3.4, Q3 = 6.1), and a t-test showed that these two datasets weren't statistically significant ($p = 0.38$). However, thinner leaves may indicate that the plants stretched themselves out to find a better light source than what was currently being provided, which was likely due to not enough light

radiating from the growth chamber, as the chamber that housed the syncom experiment had lower lighting capabilities.

Other facets of the syncom experiment may have contributed to noisy experimental results. For one, cooler temperatures less than 22°C may have lead to stressed *panicum* virgatum germination and growth conditions, which I unfortunately did not keep track of during the experimental timeline. Another factor may be the protocol used for data collection, as harvesting the plants out of the microboxes was a delicate process that may have led to less accurate belowground length data due to root breakage. Furthermore, the methods used to measure the resulting data may have had an impact on the accuracy of the analysis. This experiment was conducted with the aid of three freshman students who, for the sake of making sure data would be analyzed in time for their presentation, independently collected data by randomly being assigned a treatment to measure. While each student was properly trained on how to use the ImageJ software needed for data collection, this decision may have led to an increase in variability due to differences in how each student individually chose to take measurements and interpret the images.

The preliminary selection experiment was subjected to a variety of conditions that contributed to less-than-ideal plant growth and development. For one, germination conditions were not ideal, as the chamber was set with growth conditions for the model *arabidopsis thaliana* rather than the *panicum hallii* variety *filipes* plant used for this experiment that prefers warmer growing conditions. The seedlings dealt with 20°C during the day cycle and 16°C during the night cycle, where seeds were able to germinate but weren't shooting upwards, with some displaying a red phenotype that I now know is often an indicator of cold stress. After two weeks, the culprit for poor growth conditions was discovered and a lab protocol on ideal *panicum hallii* germination was used to increase growth chamber temperatures to 24°C during the day and 22°C during the night.

Despite the changes, much of the damage had already been done. While growth conditions definitely improved, the majority of the plants clearly grew smaller than the *panicum hallii* growth that had been observed in previous microbox experiments in a similar time range. Moreover, the transparent caps originally meant to shield the seedlings from outside contaminants and increase priority effects for the syncom inoculum were kept on for three weeks rather than five days with the previous experiment. This created an overly humid environment that worsened the cold growth conditions for an already vulnerable seedling plant, as the experiment dealt with contamination issues in the form of a cotton-like mold.

From an experiment consisting of 120 pots, just as many plants were lost to contamination (n=15) as were lost to a lack of germination (n=15). This replication loss may be attributed to the clear caps, as these were typically removed after the seedlings were close to reaching the cap top, only the *brachypodium distachyon* plant used for the previous iteration of selection experiments took 4-5 days from seed transfer to accomplish this height. This was far faster than the *panicum hallii* used in this selection experiment that typically takes 5-10 days from seed transfer to pots to germinate and minimum another week to sprout up and develop leaves. The decision to keep caps on was made worse by the fact that growth chamber temperatures were not maintained constant between day and night cycles, as was the case with the previous selection experiment. This worsened the condensation on the incompletely sealed cap-rocket pot system that likely created a home for possible contaminants to propagate and reach the seedling. Furthermore, overwatering may have also taken place given that I followed watering recommendations for a largely uncapped experiment, meaning I may have allowed seedlings to go through an unnaturally damp hydration regimen that didn't allow the soil to dry.

Future Microbiome Experiments

There are a handful of interesting experiments that would provide greater insight into how plant-microbe and microbe-microbe interactions maintain a root microbiome, such as testing different synthetic communities or how plant microbiomes stabilize over multiple generations. The issue is how time- and resource-consuming it is to prepare a semi-sterile root microbiome experiment, and how oversights can lead to unideal conditions for the entire experiment that you may not observe are an issue until it's too late. Because of this, it may be ideal to develop a simpler protocol that utilizes less space to test root microbiomes.

One way to do this may be to conduct plate experiments. This system has been successfully used to study the effect of synthetic communities on *arabidopsis thaliana* before, meaning one would simply have to determine the best inoculation method for a larger seed (Finkel et al., 2020). For example, seeds could be placed in solidified Hoagland solution using agar with the synthetic communities inoculated into the cooling media, similar to how Burkholder assays are conducted. This medium would allow bacteria to grow for a couple of days or up to 2 weeks while being able to interact with its preferred niche of the plant root. Plates would be wrapped in micropore tape to keep a sterile environment while allowing for gas exchange and to prevent the media from drying out too quickly. The risk of contamination will be larger with every microbial addition and with every additional

day plants remain inside the plates, but this may be solved by filtering the working syncom solutions before inoculation and transferring seedlings to syncom plates a couple days after germinating to minimize the amount of time seedlings spend in the same plate. Longer petri dish plates could be used to minimize the risk of the leaf reaching the top of the plate and causing the primary root to leave the media surface, or we could transfer seeds such that the root is embedded in the media. Microbiome transfer would involve cutting out the root with surrounding agar and shaking the material in falcon tubes to be filtered for the next generation. Analysis-wise, this method would make it possible to collect both root and shoot data for an experiment, if desired. One could even cover where in the plate the root starts with aluminum to mimic the dark belowground conditions and encourage more realistic root development. Sterile dividers could be used to plate more seedlings per plate while limiting interactions between the plants in case a size ideal for individual seedling growth isn't available. This setup would also simplify data collection, as you could readily use a scanner to visualize and even automate the collection of a large amount of data. In case there are issues, an alternative to petri dish plates may be to instead pour the identical media about half-way into long cylindrical tubes. Their tops could be covered by semi-transparent micropore tape, or liquid culture cape, or a clearer alternative. This may be more space-efficient than plates with the right vials, but data harvesting methods might be tricker to develop considering the root microbiome would be less accessible with this setup. It may take some initial effort to develop, but if successful, these protocols would be completely sterile alternatives that provide more streamlined setup and harvest methods. This would also expand the possibilities for further plant root microbiome discovery. For example, by utilizing semi-transparent agar media rather than soil, you may now more easily visualize what niches are created in the root microbiome with the right tools and software.

One experiment I would love to conduct if resources were available or a faster alternative were developed would be to study the root microbiome via microbe knockouts, similar to typical molecular biology experiments where a specific gene is inactivated to determine its function. I would make multiple synthetic communities where each has a member missing or a random number of members missing, inoculate genetically identical plants with these treatments, and induce a specific stress, such as nitrogen deficiency. After a couple of weeks, this would allow me to determine what microbes are essential in delivering the nitrogen stress relief, if the removal of a single microbe has impacts on microbiome function, etc. After determining the beneficial microbes, you could conduct further experiments to uncover how these interact with each other and the host plant to uncover which microbes are essential, which are amplifiers or stabilizers, what

redundancy is prevalent, and other interesting finds. If time permits, it would be interesting to develop isolation or tracking methods for these bacteria to discern how they perform in an NUE microbiome selection experiment throughout the different microbiome generations. If money permits, RNA sequencing of the resulting microbiomes after each generation could be performed to track the activity of the bacterial communities to determine what genetic expression changes or random mutations occurred to strengthen the nitrogen stress-relieving interactions. Already being aware of the beneficial microbes would make analysis much easier, and could answer an additional question of whether the significant changes originated from the known beneficial microbes or from adaptations to the microbial community.

Materials and Methods

Summer 2023

While multiple experiments were attempted, the most useful involved testing various nitrogen concentrations on different plant systems using Microbox cups. The goals of this experiment included determining which plant host would be best for N-fixing experiments, how well plants could grow in this experimental cup system, what nitrogen concentration would be ideal for selection experiments, and what phenotypic measurements would work best as indicators of nitrogen stress.

To set up this experiment, a specific weight of autoclavable profile soil was added to microboxes. These cups are neat systems due to providing very sterile environments for seedlings to grow, as they are sealed to only provide gas exchange via the filters on the top. These cups are also much easier to handle considering the seeds you add to this system don't even have to be watered. The profile soil was hydrated with a pre-determined amount of modifiable Hoagland solution, a fertilizer typically used in hydroponics (Poire et al. 2014). Three levels of nitrogen input were used: 0uM N, the most stressed N input, 600uM N, the typical input in a Hoagland solution, and 6000uM N, an over-abundance of N input. The cups were sealed, covered in aluminum foil, and autoclaved for sterilization. Once cooled, 4 different types of sterilized seeds were added to the hydrated soil: *Oryza sativa*, also known as rice, *brachypodium distachyon*, the grass used in the previous microbiome selection experiment, *panicum virgatum*, also known as switchgrass and the lab's biofuel crop of interest, as well as *panicum hallii*, a smaller cousin of switchgrass often used as a proxy for the lab's biofuel work. Multiple seeds were added to the cups, and each seed-treatment combination was designated 3 cup replicates. The microboxes were sealed and placed in a growth

chamber for about 30 days, a comparable amount of time we would allow seedlings to grow for a microbiome selection cycle.

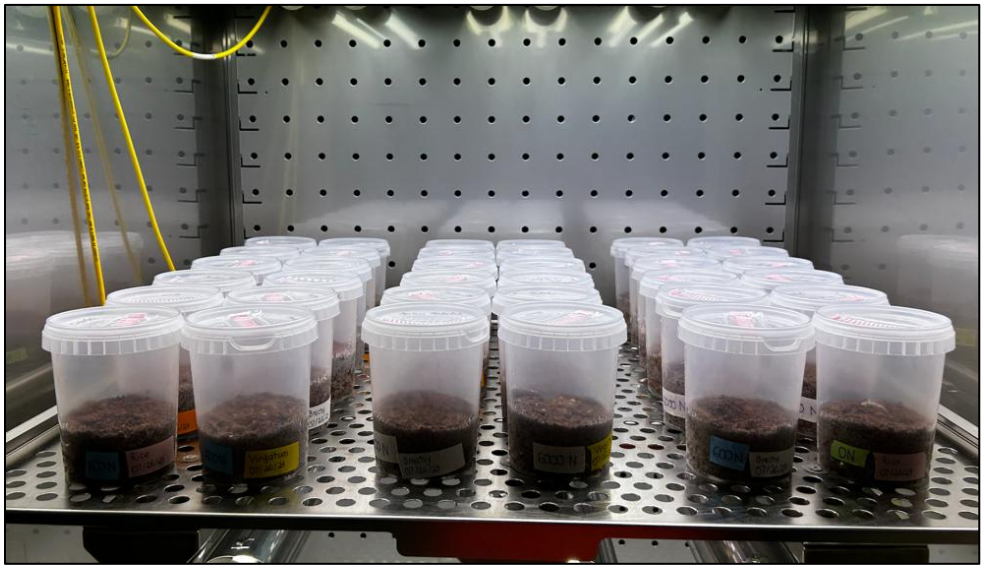


Figure 7: Nitrogen treatment experiment utilizing a microbox setup inside the growth chamber.

After 30 days, harvesting of this experiment took place. Data was collected on two forms of aboveground phenotype: Aboveground biomass, a very typical indicator of plant health, and maximum shoot length, mainly because I noticed there were obvious differences in how tall a plant got depending on the nitrogen treatment. To harvest this data, scissors were used to snip off the largest-growing green shoots in a respective cup. The largest tiller of these were immediately measured with a ruler, recorded, and added to a labelled manilla envelope. These manilla envelopes would be left in an incubator for three days to dry the collected shoot. A week later, the remaining envelope contents would be weighed and recorded to get biomass measurements.

Fall 2023

For this semester, I mentored three students from the X-Plants Freshman Research Initiative (FRI) program and continued developing projects for the nitrogen selection experiment. The focus with my team was to learn more about

the engineered nitrogen-fixing strain, develop protocols for creating a synthetic community around this strain, and try more methods to easily and sensitively distinguish nitrogen stress.

The most exciting project was one where we inoculated nitrogen-stressed seedlings with various synthetic communities to see how much of a benefit the engineered microbe can have on *panicum virgatum* growth depending on whether it is in a synthetic community. For this experiment, we used the same Microbox setup as the last experiment, except this time only one nitrogen treatment, 0uM N, was used and only *panicum virgatum* seeds, 7 per cup, were needed for this experiment. A total of 4 microbe treatments were used each with 8 cup replicates: The PBS inoculum as our control, the FM2 engineered microbe only, a synthetic community composed of about 20 individuals, and that same synthetic community with the addition of the engineered microbe.

Once the sterilized *panicum virgatum* seeds were added to the cups, these were placed in the fridge for a couple of days for a vernalization treatment that served to standardize seedling development. While this was occurring, liquid cultures of each intended microbe we intended to include in a synthetic community determined from previous screens were made. After three days, the cultures were foggy and ready to be used to make syncom treatments. Before creating a synthetic community, you must first normalize the varying bacterial densities to make sure one microbe doesn't completely overpower others purely because more of it was originally added. Optical Density (OD) is a typical fogginess measurement used to measure bacterial density and can be obtained in a high-throughput fashion using a 96-well plate and a spectrophotometer.

The OD values were used to calculate how much of each liquid culture would be needed to create stock syncom solutions for the three bacterial inoculants with an OD of 0.01 in PBS buffer. These solutions were then further diluted 1:10 to create the three working syncom treatments with an OD of 0.001. The working syncom treatments were then slowly inoculated at 5mL intervals throughout the cup soil surface, sealed, and transferred to a growth chamber.

After 30 days of growing in the growth chamber, harvesting of this experiment took place. An even larger amount of data was collected for this experiment to take advantage of the larger repeat size and new phenotyping protocols. This included: Maximum shoot length, maximum root length, germination rate, 2 different chlorophyll measurements, biomass, and root sampling for possible sequencing. To harvest this data, I began by carefully removing the entirety of the microbox contents onto a sheet of aluminum foil. I

carefully removed the seedlings, trying my best not to rip the fragile root system, removed as much soil as possible from the roots, and organized these onto a sterile transparent sheet. Up to 4 replicates of a treatment could be placed onto this sterile sheet, which were then transferred onto a scanner to image the seedlings. These images would later be used to trace and measure the maximum root and shoot lengths using ImageJ software, as well as document how many seedlings in a cup ended up germinating. After scanning, the three largest seedlings were weighed and cut up into glass 5mL vials. An acetone solution would later be added to these vials for two days, whose resulting supernatant would be analyzed via a spectrophotometer to calculate the two chlorophyll values. Once the vials were complete, the rest of the seedling shoots were snipped and collected in pre-labelled manila envelopes to collect biomass data. The seedling shoot tissue used for the chlorophyll assay would be collected after the end of that protocol and dried to collect the rest of the experiment's biomass data. The imaged roots were collected into 2mL tubes and stored in the -80 freezer for possible metagenomic sequencing efforts to determine resulting synthetic community compositions.

Spring 2024

The use of microboxes for selection experiments was questioned due to continued concerns over how well plants can grow inside these cups. It was decided to reroute the experiment using previously established methods from Ulrich's salt stress scheme to determine if this might improve experimental outcomes. Thus, the bulk of this semester was aimed towards developing and setting up a preliminary open-air selection experiment using a synthetic microbe community that could successfully be screened for the presence of the engineered N-fixing microbe.

Figure 8: Synthetic community selection experiment utilizing an open-air rocket pot setup in the growth chamber.

Dr. Ulrich Mueller, the developer of the methods for host-mediated microbiome selection for biofuel grasses, was essential in setting up this nitrogen stress experiment. He provided a detailed tutorial of how he set up the rocket pots, a smaller and longer alternative to regular pots to allow a greater amount of plants to fit in a growth chamber at once while also providing enough room for roots to grow.

My experiment included a total of 120 pots with the following treatments: 12 pots with non-stressed Hoagland solution (positive control), 12 pots with stressed Hoagland solution (negative control), 16 pots with only the engineered FM2 microbe treatment, 40 pots with a synthetic community inoculum, and 40 pots with a synthetic community inoculum that includes the engineered FM2 microbe. For each pot, I added 6 grams of compacted polyfill filling to allow for pot drainage. After that, I added profile soil up to about 1cm and compacted this to 1.5cm with a pre-made wooden dowel.

These pots must be hydrated and autoclaved to provide a semi-sterile environment in order for priority effects to promote more efficient establishment of the synthetic communities. Pots were hydrated with Hoagland solution according to which nitrogen treatment would be added to a certain line of pots: stressed, 0uM N input, or not stressed, 600uM N input. The tops and drainage holes on the bottom of each pot were covered with aluminum and assembled in large autoclavable bins.

These bins were covered in aluminum and autoclaved with the 30-minute liquid setting twice with a minimum 10 hour gap between cycles where the pots remain inside the autoclave overnight. These bins were removed from the autoclave and allowed to cool for minimum a day, maximum a week, before seeds could be added onto the pots.

For this experiment, we used *panicum hallii* seeds instead of the switchgrass seeds used in previous semesters due to poor *panicum virgatum* germination rates. We did not want to risk an avoidable loss of repetition power for any treatment line in an already time-consuming setup protocol, and the Juenger lab already had a well-established protocol for successful *hallii* germination. To prepare seeds for transfer, the needed amount was poured into a small manilla envelope and left at minimum 72 hours in an incubator to heat treat them. Once this was done, a 2mL tube was filled about a third with seeds and 1mL of half-diluted bleach solution. These tubes were placed in an inverter for 2hrs. The tubes were then transferred to a sterlized flow hood where the bleach solution in each tube was removed and rinsed out with milli-q water multiple times. The sterilized seeds were poured onto a sterile petri plate for easier transfer, and one *panicum hallii* seed was added to the center of each pot with bead-sterilized forceps. After filling a rack with planted pots, each pot was covered with a transparent ethanol-sterilized plastic lid to protect the seedling from cross contamination. The rack was removed out of the flow hood and into a transparent plastic bin with lid that was sterilized with ethanol beforehand. These bins served as transport devices that minimized possible pot contamination when moving the bins. After placing all the necessary racks with pots into the respective growth chamber, the chamber was locked to prevent people outside of the primary handlers from compromising the experiment.

Current lab practice regarding the use of synthetic communities in experiments prefer inoculating this treatment after seedlings had begun germinating, under the guise that microbes could more easily find their niche in the root complex if a root had already been established, rather than have the microbes roaming around in a setting unlike a typical soil. Due to this, synthetic communities were added to their respective experimental treatment about 10 days after seed transfer. Two days before inoculation, individual community microbes were independently grown in liquid culture. On treatment day, a high-throughput spectrophotometer was used to calculate the optical density, or OD, of each microbe that is a measure of culture fogginess as a proxy for bacterial growth. This is used to calculate how much liquid culture to add in consortium with the rest of the microbes to create a more even synthetic community. Microbes were added to

the treatment working solutions with a PBS buffer background and were gently mixed well by swirling the solutions with circling motions before being used. To inoculate a pot with the synthetic community, a rack and the respective treatments were placed in the flow hood. A sterile 25mL serological pipette with adjustable pipetting gun was used to inoculate each rocket pot by gently dispensing 5mL of the respective synthetic community treatment in circular motions around the seedling, making sure to not dispense too close to the pot surface to minimize cross contamination, being sure to put the cap back on the pot after inoculating, and repeating with the same serological pipette until you run out of the treatment. You would continue this process until every pot has their intended syncom treatment and all the racks can be locked back into the growth chamber. The transparent caps would be removed from the rocket pots once the seedlings were close to reaching the cap tops to limit cross contamination and allow priority effects to better establish what was inoculated to interact with the plant.

Watering depended on what stage of the experiment the pots were in. The rocket pots were easier to maintain when the caps were on, as the trapped moisture meant the seeds could go on for up to 3 or 4 days before the soil around the seed began to dry and were watered accordingly. Watering was increased to every two or three days after the caps were removed. Five-liter plastic carboys were bleach-sterilized and subsequently autoclaved weekly for sterile storage of the respective Hoagland working solution that would serve as the watering treatment. An ethanol-sterilized graduated dispenser was attached to the carboy to easily water the majority 0uM nitrogen treated pots, while serological pipettes were used to quickly water the remaining 600uM nitrogen treated pots. To determine how much to water, a rack of pots and a small, easily sterilizable kitchen scale were brought into the flow hood. Three pots from the left, middle, and right of the scale were taken out of the rack and weighed. The average of the three was subtracted from 275 grams, the average weight of a fully hydrated rocket pot, to determine how many mL to water that specific rack. To operate the dispenser, the knob was adjusted to the calculated mL amount, the spout was sprayed with ethanol and left to dry for about 3min, and the entire device was rinsed 2-3 times before use by pressing on top of the instrument and allowing it to gradually return to its stationary form. To hydrate each pot, one hand gently pressed down on the top of the device while the other hand moved in consistent circular motions though the pot surface for even watering.

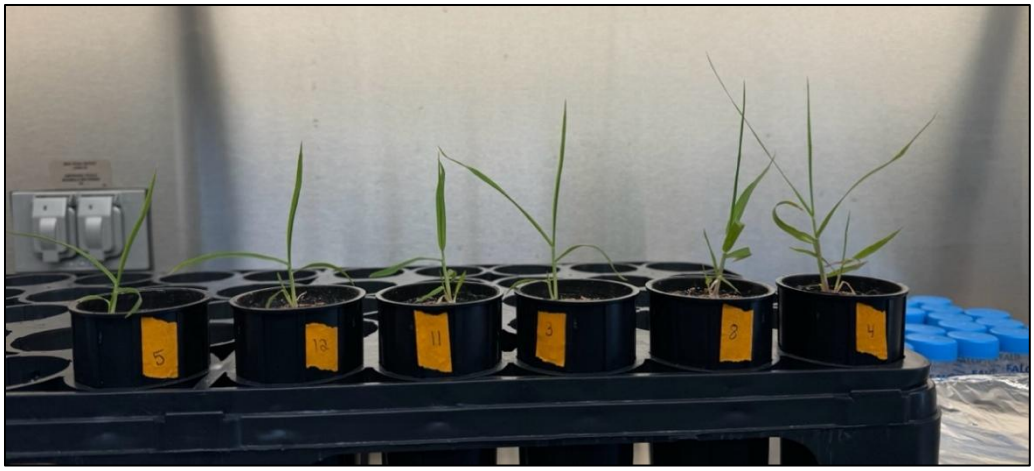


Figure 9: Selection line of the 600uM nitrogen input positive control treatment of the selection treatment. Plants were ranked in ascending order.

After 30 days, the selection cycle was complete and data harvesting took place. All members of the same treatment were collected into one rack, placed in the flow hood, and ascendingly ordered. The two largest plants would be harvested for the next microbiome generation while the rest of the selection line would be thrown away. An autoclaved aluminum bin was added to the flow hood and used to collect the soil while data harvesting occurred. This harvesting included collecting the aboveground biomass to dry as well as collecting the belowground biomass to weigh and filter the resulting microbiome. To collect the plant material, a rocket pot was held above the aluminum bin and squeezed at the sides to loosen the soil. The plant was held at the base with bead-sterilized forceps while the pot was placed on its side to remove the soil until the majority of the root could be removed from the pot. Bead-sterilized scissors were used to snip the aboveground biomass that was collected in a pre-labelled manilla envelope and the belowground biomass that was collected in pre-labelled 50mL falcon tubes containing 40mL half-concentration Hoagland solution. The next largest plant in the selection line received the same procedure and the belowground biomass was collected into the same 50mL falcon tube. Once each treatment set went through the same process, the falcon tubes with root content would be inverted 50 times and allowed to settle for 1min before being filtered for microbiome transfer. To filter these samples, a sterile and disposable 20mL syringe was used to aspirate the suspended buffer. A 2uM sterile and disposable filter was attached to this syringe to dispense the filtered microbiome onto another pre-labeled falcon tube. This was done until each belowground sample collected about 35-40mL of filtered microbiome. Back in the lab, the aboveground samples were placed in an incubator to dry, the root samples

left over from filtering were weighed to get a rough estimate of belowground biomass, and the filtered microbiome samples were used to create glycerol stocks of each collected sample.

In addition, the filtered microbiome samples were used in a dilution series screen that detects the presence and amount of the FM2 engineered microbe. Burks media plates with a half-concentration input of Kanamycin antibiotic were prepared for this protocol. A sterile 96-well plate was used to create the dilution series that allows us to more easily determine which dilution is needed to clearly visualize individual colonies. To prepare the plate, a multichannel pipette was used to make sure each well contained 90uL of half-Hoagland solution. To start, ten well-mixed microliters of each filtered microbiome sample were pipetted onto the first row of a sterile 96-well plate with cap. To make the rest of the dilution series, a multichannel pipette was used to mix and aspirate 10uL of the last performed dilution and dispense the solute onto the next row of wells. This was repeated 7 times until a total of 8 series dilutions were made, making sure a new set of pipette tips was used for each dilution. A multichannel pipette was used to aspirate 2uL of the resulting dilution sets and gently dispense them as dots onto a pre-labelled Burks plate, with each plate being able to comfortably fit two microbiome sample dilution series. The plates were left in the flow hood until the dispensed samples had completely dried to minimize the chance that the samples could contaminate one another. After a couple days, pictures of these plates were taken to quantify colonies that would be used to calculate how well the engineered microbe was allowed to propagate in the different samples.

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The biggest thank you the McNair directors, Dr. Eric Dieter and Dr. Nabeeha Chaudhary, for their unfaltering trust and support these past two years. It was truly an honor to participate in such a reassuring program alongside the most amazing and motivated cohort of researchers I know. I sincerely don't know where I would be if it weren't for this program. Thank you!!

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Stellar Properties as a Function of Position in the Praesepe Open Cluster

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ABSTRACT

Stellar rotation has been a prominent topic in astronomy since at least the late 1970's, when Skumanich discovered that a low mass star's rate of rotation decays proportional to the inverse square root of the star's age. A better understanding of the relationships among stellar properties of stars in star clusters will contribute to our understanding of the formation and dynamics of stars and star clusters, stellar life cycles, and modeling star clusters. Here we investigate the 670-Myr-old Praesepe open cluster, utilizing the most complete sample of stellar rotation data for the cluster as published by Rampalli et al. (2023). We explore correlations among rotation period, mass, and spectral type as functions of position within Praesepe. Slicing Praesepe into 50 radial shells centered at the center of mass, we find that there is a low average rotation period of 5.65 days per rotation in the radial shell at 17±1 parsecs from the center of mass. In addition, there is a noticeably low number of stars between 0.6 and 0.8 $M_{\odot}$ in the 0-to-10-day rotation period range. More investigation into Praesepe, including statistical analysis, as well as comparisons with other clusters, will help determine the relevance and meaning of these observations.

[6] Compare this average period to the 25.4 days per rotation at 16 degrees solar latitude documented by NASA (Williams 2024).

[7] There is a star listed as 640.53 pc from Earth, however this star is also listed as 0 $M_{\odot}$ and is therefore excluded from later analysis.

1. INTRODUCTION

1.1. Stellar Rotation

Stars are dynamic entities — evidenced, for example, by their rotation. Stellar rotation has been a prominent topic in astronomy since at least the late 1970's, when Skumanich discovered that a low mass star's rate of rotation decays proportional to the inverse square root of the star's age (Skumanich 1972). Stars acquire their rotation from immense molecular gas clouds that — given enough average density — collapse under gravitational influence to form stars (Williams et al. 2000; Corsaro et al. 2017). Moreover, stars exhibit differential rotation — their latitudes rotate at different rates, with a faster rotation at the stellar equator. We understand stellar differential rotation to be a primary driver of the internal dynamos that make the magnetic fields of a star. Therefore, stellar rotation is the ultimate cause of the star spots that we can utilize to determine the rotation rates of the star.

1.2. Star Spots

Star spots are relatively cool areas on a star's surface. These points represent strong solar magnetic field lines arcing through the surface of the star at that point (see Figure 1). These areas are directly visible on the surface of the Sun and are called sunspots. Since star spots are carried along with stellar rotation, they allow visual determination of stellar rotation periods (Hanslmeier 2024; Rampalli et al. 2021). For instance, utilizing the sunspots in solar images from the Solar and Heliospheric Observatory (SOHO) (2024), we calculated an average of 23±1 days per rotation of the Sun.[1]

1.3. Star Clusters

Clusters of stars provide the baseline of their mutual environment from which to investigate stellar properties. We are studying an open star cluster, which is a loose collection of gravitationally bound young stars. The Praesepe cluster, the focus of this work, is a roughly flattened-oval-shaped 670-Myrs-old cluster comprising 1030 known stars with masses up to 1.6 $M_{\odot}$ (Rampalli et al. 2021, 2023). Praesepe's stars vary in distance from 89.3 to 251.1 parsecs (pc) from Earth[2] (Rampalli et al. 2023) (see Figure 2).

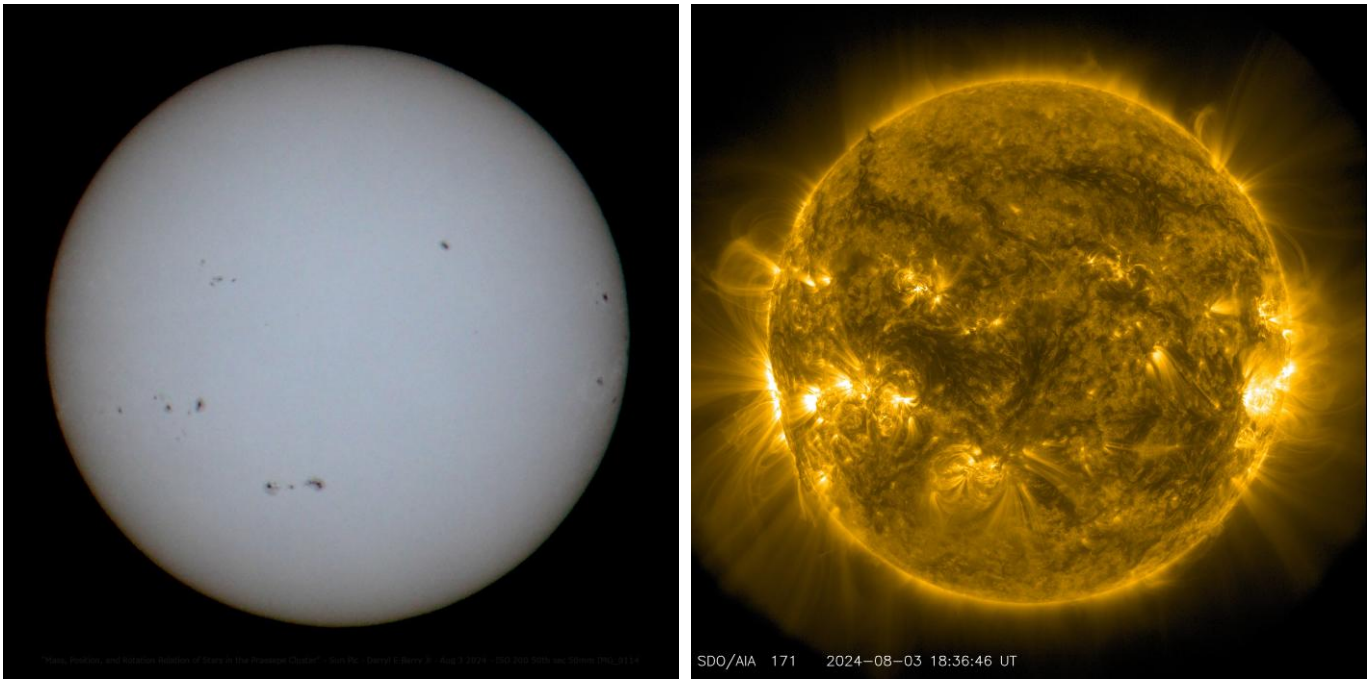

Figure 1. These solar images show how star spots correspond to areas of increased solar activity. Left: Taken August 3, 2024, at 12:35 pm by the first author. Right: Taken at 12:36 pm on the same day by the Atmospheric Imaging Assembly & Helioseismic and Magnetic Imager (AIA/HMI) instruments aboard the Solar and Heliospheric Observatory (SOHO) (2024).

Figure 2. An image of the Praesepe open cluster, taken on March 6, 2022 (Lauterbach 2022).

1.4. Relevance & Purposes

Purposes for understanding relationships between various stellar properties of the members of star clusters include: understanding general trends in star formation and distribution (Healy et al. 2021), understanding the cosmic environment of stars (Healy & McCullough 2020), considering whether dynamics within the cluster's structure affect and disturb the alignment of spins between the various stars in the cluster (Corsaro et al. 2017), informing the creation of models for predicting cluster evolution (Healy et al. 2021), and better understanding stellar rotation over time (Rampalli et al. 2021).

2. THE LITERATURE

2.1. Spin Alignments

Continuing from Skumanich (1972), astrophysicists have been working to better understand stellar rotation. Rampalli et al. (2021) found that the period of rotation[8] of stars remains stable over several years of observation, which means that single measurements are representative of a star's rotation period. Corsaro et al. (2017) found that for clusters showing strong alignment between its stars' axes of rotation, rotational kinetic energy must make up at least 50% of the energy from its early formation period. Healy et al. (2021) found the spin alignments of stars in Praesepe to be isotropic or only

[8] Sometimes abbreviated as P_{rot}.

moderately aligned. Prior to this, studying cluster NGC 2516, Healy & McCullough (2020) similarly found discrepant to moderately uniform spin alignments. They conclude that turbulence would dominate a region where there is an isotropic distribution of rotation axes, whereas rotation would dominate regions containing star clusters with axes in alignment (Healy & McCullough 2020).

2.2. Stellar Rotation

Rampalli et al. (2021) presents a digestible investigation of stellar rotation, focused on data acquired by the Kepler (recommissioned *K2*) and *Gaia* satellites. Rampalli et al. (2021) focused on observational data and comparative results — including between contemporary and previous observations. As stated, they found stability in stellar rotation periods over time. In the process, they pioneered rotation data on 220 Praesepe stars, expanding the stellar catalog (Rampalli et al. 2021).

The work by Hanslmeier and Braj˘sa discusses several methods of determining the rotation rates of stars (Hanslmeier 2024). They highlight that, unlike the Sun, which we can observe in detail, distant stars appear as point sources despite their enormous sizes. Therefore, gathering rotation rates of distant stars necessitates indirect methods (Hanslmeier 2024). Methods described included: spectral line examination — searching for the telltale broadening of stellar spectral lines due to stellar rotation, and light curve analysis — utilizing the fact that star spots periodically alter the luminosity of a star to track its period of rotation (Hanslmeier 2024). It is of note that Rampalli et al. (2021) utilized the light curve method to determine rotation periods in Praesepe.

Arnold Hanslmeier and Roman Braj˘sa Hanslmeier, through their book *Stellar Rotation*, present a comprehensive assessment of stellar rotation (Hanslmeier 2024). We focused on the chapter dealing with stellar rotation and the H-R diagram — a way of classifying stars via their masses, sizes, and temperatures. Though our early ideas on analysis were vague, it seems that simply placing stars in an H-R diagram covers much of our initial intent for analyzing Praesepe.

2.3. Star Clusters

Hanslmeier (2024) presents a very broad take on stars, discussing their fundamental physics, formation, rotation, and remnants of stars including black holes. Furthermore, Hanslmeier (2024) confirms that the origins of stellar rotation are the immense gas clouds that eventually collapse under gravitational influence to form stars. This notion is confirmed by Williams et al. (2000) and Healy & McCullough (2020). However, Williams et al. (2000) state that not all large molecular clouds clump to form stars, dependent upon the structure and average density of the cloud in question.

2.4. Mass & Spectral Type

The masses of the Praesepe stars were estimated from stellar absolute magnitudes (Rampalli et al. 2021) — where more massive stars are intrinsically brighter. Rampalli and her team utilized apparent magnitude data and *Gaia* distances to

calculate the absolute magnitude of Praesepe stars. Having an absolute magnitude for the stars, they then interpolated the effective masses of Praesepe's stars (Rampalli et al. 2021) — Rampalli notes that the tenth decimal place is the greatest accuracy to which we can be practically sure (Rampalli 2024).

Stars are also categorized into spectral types. Categories include — from the brightest and most massive stars to the dimmest and least massive stars — O, B, A, F, G, K, and M type stars. Numbers attached to the spectral type sub-categorize each letter type from 0 — for the hottest and brightest stars in that letter category, to 9 — for the coolest and dimmest stars in that letter category. Praesepe comprises F, G, K, and M stars ranging from M5.5 to F4 (Rampalli et al. 2023).

3. THE DATA

Over three years and three campaigns utilizing *K2*, Rampalli et al. added 220 new Praesepe rotators to the catalog and confirmed 793 previous measurements (Rampalli et al. 2021). Our analysis focused on the data in Table 4, which includes mass data, star designations, periods of rotation, effective temperatures, position, and magnitude (Rampalli 2024). Furthermore, Rampalli et al. (2021) reference several catalogs and other sources of information that may provide future research with either data to process or methods by which to creatively maneuver current and new data.

Rampalli prominently referenced the data of S. T. Douglas. In his "*K2* Rotation Periods for Low-mass Hyads and a Quantitative Comparison of the Distribution of Slow Rotators in the Hyades and Praesepe," we found further references to data caches (Douglas et al. 2019). Other data sources we accessed include Cotarˇ et al. (2019) — from which we acquired the *Gaia* magnitude of the Sun, and Kuiper (1938) — from which we acquired a mass-luminosity relation. With this information we experimented with deriving stellar masses.

4. ANALYSIS & RESULTS

4.1. Familiarization with the Data

We expected that the more massive stars of Praesepe are generally towards the center of the cluster, and that these stars also have the lowest period of rotation and therefore spin faster. We plotted the stellar masses using axes of declination (δ), right ascension (α), and distance (pc), and recreated plots to confirm that they matched those in Rampalli et al. (2021). Upon visual assessment of mass distribution in the plots, the masses of the stars seemed randomly placed throughout the cluster. However, rotation seemed generally faster towards the center of the cluster.

In Figure 3 we show period of rotation versus $M_\odot$ — with spectral types encoded in the color of the marker — like various magnitude versus period of rotation plots in Rampalli et al. (2021), for instance their Figures 6 and 7. We find a noticeably low number of stars in the 0.6 to 0.8 $M_\odot$ range having rotation periods under 10 days per rotation. Furthermore, there is a large range of rotation periods for M-type stars, particularly with the 0.4 $M_\odot$ stars ranging from 0.42 days per

rotation to 40.13 days per rotation (Rampalli et al. 2023).

4.2. Slice Analysis

We converted the given coordinates from equatorial coordinates to a Praesepe centered spherical coordinate system for analysis. The initial procedure was to manually program a conversion first to Cartesian coordinates and then to spherical coordinates utilizing equations 1 through 4, with ϕ as the azimuthal angle or α, θ as the polar angle or δ, and ρ as the radial distance from the origin (in this case being distance from Earth).

$$radians = degrees \times \frac{\pi}{180} \quad (1)$$

$$x = \rho \cos\phi \sin\theta \quad (2)$$

$$y = \rho \sin\phi \sin\theta \quad (3)$$

$$z = \rho \cos\theta \quad (4)$$

Recreating earlier plots confirmed the accuracy of the conversion. We then shifted the coordinate system so that the origin is within Praesepe rather than at Earth and masked out the seventeen data points lacking distance data. Masking brought our number of data points down from 1030 to 1013 — matching Rampalli et al. (2021) analysis number of stars. The origin of the new coordinate system was at the center of each axis, but not quite at the center of mass.

We then attempted to slightly shift the origin of the coordinate system to coincide with the center of mass of the cluster (Michel van Biezen 2013b,a),

$$CM^j = \frac{\sum_{i=1}^{1013} [M_i^j \tilde{x}_i^j]}{\sum_{i=1}^{1013} M_i^j} \quad (5)$$

where M is the mass of the star and CM is the cluster's center of mass. The superscript *j* denotes axis x, y, or z, and individual stars are denoted by the subscript *i* located at position ˜*x* on the *j* axis. We repeated this process for each axis, which provided the center of mass for the cluster as a three-vector. However, the attempted shift to the center of mass caused an unknown error. Moreover, converting to spherical coordinates centered at Praesepe resulted in errors. Nevertheless, we could slice Praesepe into various slices along each axis and do some initial analysis.

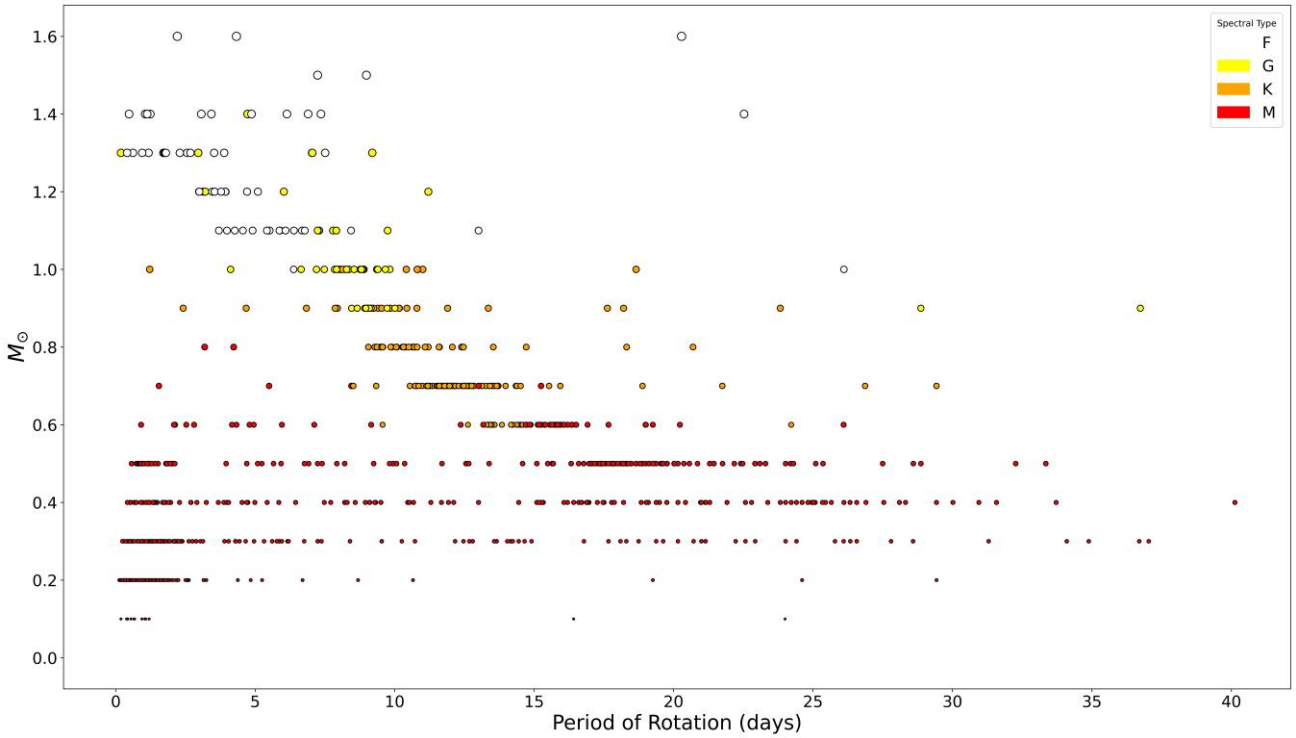

Figure 3. This figure is a plot of Praesepe showing $M_\odot$ on the y-axis and P_{rot} on the x-axis. We remove extraneous data points — it includes only stars with distances from Earth greater than 0 pc, for 1013 total rotators — and includes spectral types encoded in the color of the marker.

Analysis suggested an asymmetrical distribution of stellar properties but was ultimately inconclusive. For instance, we encountered an error in properly accounting for all the stars in the cluster — more slices of Praesepe resulted in fewer stars and less mass being accounted for. Moreover, overlapping the three axial slices proved untenable. For example, the slice with minimum rotation period on one axis rarely overlapped with a minimum on the other two axes. Nevertheless, in one two-dimensional plot we did get an overlap suggestive of an asymmetrical distribution of mass and rotation. We concluded that conversion and accounting errors were due to manually coding the coordinate conversion and manually slicing Praesepe, which automatic processes embedded in coding libraries could alleviate.

We restarted analysis by applying the astropy SkyCoord library to convert to Cartesian coordinates. In this assessment we did not mask out the 0 distance stars, analyzing all 1030 known stars. We found that the default conversion resulted in a unit-less coordinate system — with correct relative distances among stars evidenced by faithful representations of Praesepe per comparison to previous plots. In the SkyCoord coordinate system, the breadth of Praesepe along the x, y, and z axes was 0.2344, 0.2095, and 0.2155 Units, respectively.

We had difficulty arranging a slicing code while the center of Praesepe was in the center of each axis. Therefore, we reset the axes so that the origin of each axis was in the corner of the plot, with each axis starting at 0 and its breadth as the maximum value of the axis. We then sliced Praesepe into every denomination of slices from 0 to 50 slices, placed the data into tables, and saved the tables in a CSV file for analysis in Excel.

While the output was better than before, we found that there were still some discrepancies. Slight variability remained in accounting for all stars among the different denominations of slices of Praesepe. Total cluster mass varied between 523 and 525 $M_{\odot}$, and the total number of stars varied between 1027 and 1030 stars. In addition, the axial overlap issue was still present — areas of interest in one axial slice did not always overlap with positions in the other two axial slices. Nevertheless, the analysis still suggested asymmetrical distribution of mass and rotation, and the process helped the progression from slice analysis to cube analysis of Praesepe.

4.3. Cube Analysis

Cubing Praesepe allowed for the direct three-dimensional placement of analyzed properties, without requiring the subsequent overlapping of planar slices along different axes. Moreover, the total number of stars and mass remained consistent at 1026 stars and about 522.5 $M_{\odot}$, regardless of how many cubes we broke Praesepe into.[9]

For instance, cubing Praesepe into 512 cubes (8 slices along each axis), the cube with the most total mass was cube 294, with x, y, and z upper and lower end coordinates 0.1172 to 0.1466, 0.1047 to 0.1310, and 0.1346 to 0.1617, respectively. Cube 294 comprises the 5th slice along the x-axis, the 5th slice along the y-axis, and the 6th slice along the z-axis. The total number of stars in Cube 294 is three hundred and ninety-nine, totaling 235.7 $M_{\odot}$ and having a mean period of rotation of 8.8. The average $M_{\odot}$ in Cube 294 is 0.59 $M_{\odot}$ (see Table 1).

The cube with the lowest mean period of rotation is cube 374, encompassing the 6th, 7th, and 6th slices along the x, y, and z axis, respectively. There are two stars in this cube, sharing 0.4 $M_{\odot}$ and having a mean period of rotation of 0.27 days. The coordinates for cube 374 along the x, y, and z axes are 0.1465 to 0.1759, 0.1571 to 0.1834, and 0.1346 to 0.1617, respectively. Recall that the full breadth of the x, y, and z axes were 0.2344, 0.2095, and 0.2155 units, respectively. And finally, the cube with the highest average $M_{\odot}$ is cube 191. This cube contains one star at 1.4 solar masses and a mean period of rotation of 6.14. The coordinates of the cube are 0.1465 to 0.1759, 0.1309 to 0.1572, and 0.1885 to 0.2155.

We overlaid these properties onto plots as colored-in fill areas to get a visual assessment of their placements within the cluster (see

[9] Cube analysis primarily utilized Version 9 of the cubed code.

Figure 4). We found that the highest concentration of mass is toward the center of the cluster, which is unsurprising. However, a consistent pattern seems to be that concentrations of lowest period of rotation (and highest *average* mass) are not in the center of the cluster, nor the outskirts, but in the central region relative to the boundary and center of mass of the cluster. We then examined Praesepe sliced into even more cubes to analyze at a higher resolution.

Cubing by 50 slices per axis yields 125,000 cubes. The largest number of stars in a single cube were 19 in cube 68,935 (see Table 2). Looking for the cube of largest mass, we found that to be in cube 58,975 with a single star at 1.6 $M_\odot$ and period of rotation of 20.29 days[10]. The cube with the highest total $M_\odot$ is cube 68,935, containing nineteen stars, having a total of 16 $M_\odot$ and a mean period of rotation of 9.60 days. The cube with the lowest period of rotation is cube 68,793 having one star of 0.2 $M_\odot$ and period of rotation of 0.18 days.

Most illuminating was looking at the cubes with the 42 lowest average period of rotation in the 125,000-cube data. We stopped at 42 because beyond that the cubes increasingly had more than one star. We found that these lowest period of rotation regions seem to make a rough circuit largely between the center and edge of the cluster. In other words, none of the lowest period of rotation cubes are in the central region, though a few low period of rotation regions stretch out to the edge of the cluster (see Figure 5).

It seemed significant that none of the fastest rotation cubes were in the center of the cluster — contrary to expectations. However, Dr. Andrew Loveridge suggested considering all stars rather than singling out stars, looking for an overall pattern for a more rigorous assessment. Therefore, we addressed this broader scope in the radial analysis, also placing the axes in parsecs to determine precise rather than relative placements within the cluster.

4.4. Radial Analysis

Radial shells allow for a direct assessment of stellar properties as a function of radial distance from the center of mass of Praesepe. We masked out stars with distance from Earth of 0, which brought our number of stars down to 1013. However, we noticed during our analysis that there is a star with 0 mass, which complicated some calculations via division by 0. Therefore, after masking this star out we analyzed 1012 Praesepe stars in our radial analysis. Moreover, utilizing Equation 5 early in the process, we set the origin to the center of mass of Praesepe from the start.

For this analysis we added distance into the SkyCoord coordinate transformation, putting a distance scale in parsecs into each axis. Setting the axes in parsecs allowed for precise distance determination rather than relative placement along the axis. Our early number of radial slices were in the thousands, which reduced the analysis virtually to examining individual stars.

[10] As stated, the stars in Praesepe range up to 1.6 $M_\odot$, however, there are only three stars having 1.6 $M_\odot$ in the cluster.

Therefore, no pattern could be seen. We decreased the number of slices, and included spectral type, to see

512 Total Cubes	# Stars	x: 0 to 0.2344	y: 0 to 0.2095	z: 0 to 0.2155	$M_\odot$	P_{rot}	Cube
Lowest Mean P_{rot}	2	0.1465 to 0.1759	0.1571 to 0.1834	0.1346 to 0.1617	0.4	0.27	374
Highest Mass	399	0.1172 to 0.1466	0.1047 to 0.1310	0.1346 to 0.1617	235.7	8.8	294
Highest Avg Mass	1	0.1465 to 0.1759	0.1309 to 0.1572	0.1885 to 0.2155	1.4	6.14	191

Table 1. A table presenting the number of stars, range of coordinates, total $M_\odot$, mean P_{rot}, and cube number of the cubes of Praesepe with the lowest P_{rot}, highest $M_\odot$, and highest average $M_\odot$ from the 512 cube (8 by 8 by 8) unit-less analysis.

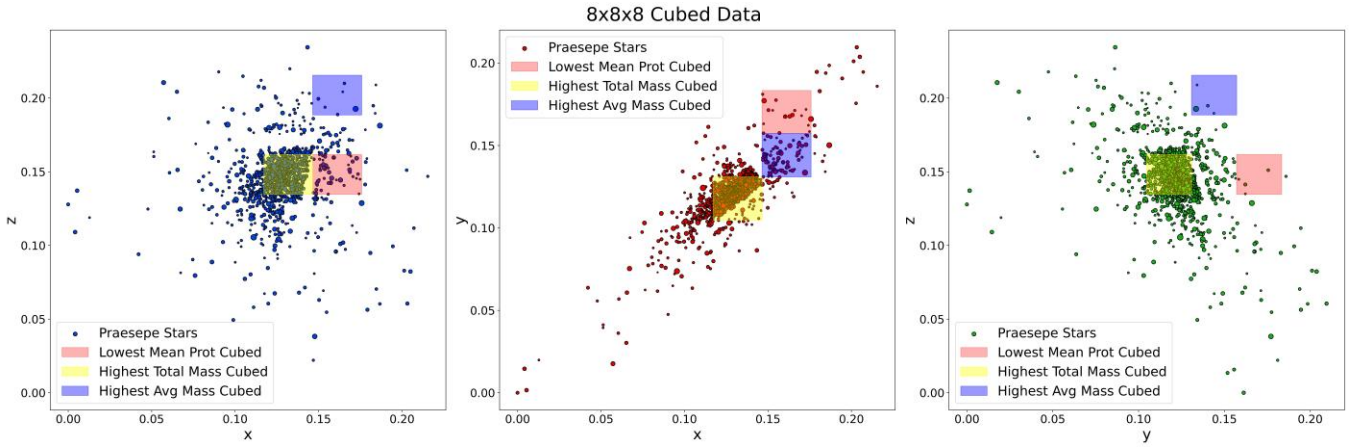

Figure 4. A plot of the 8 x 8 x 8 unit-less cube analysis, with locations of lowest P_{rot} highlighted in red, highest total $M_\odot$ highlighted in yellow, and highest average $M_\odot$ highlighted in blue.

125k Total Cubes	# Stars	x Coords	y Coords	z Coords	$M_\odot$	P_{rot}	Cube
Lowest Mean P_{rot}	1	0.1265 to 0.1313	0.1047 to 0.1090	0.1809 to 0.1854	0.2	0.18	68,793
Highest Mass	19	0.1265 to 0.1313	0.1173 to 0.1216	0.1465 to 0.1509	16	9.60	68,935
Highest Avg Mass	1	0.1078 to 0.1126	0.1215 to 0.1258	0.1034 to 0.1078	1.6	20.29	58,975

Table 2. A table presenting the number of stars, coordinates, total solar mass, mean P_{rot}, and cube number of the cubes of Praesepe with the lowest P_{rot}, highest mass, and highest average mass from the 125,000 cube (50 by 50 by 50) analysis.

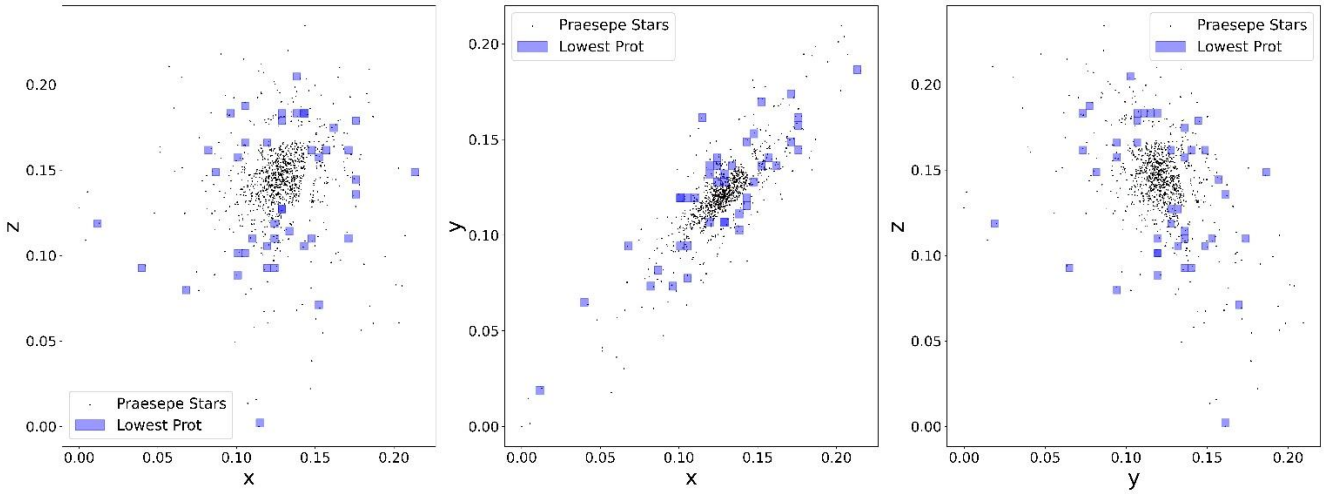

Figure 5. A plot of the 50 x 50 x 50 cube showing the 42 cubes with the lowest average P_{rot}. Most cubes contained one star. Notice the distribution mostly in a rough ring about the cluster between its center and edge.

if that could thereby illuminate a pattern.

To compare more directly to the 50-cubed data in which we first noticed that the fastest rotating stars are distributed about a ring around the cluster, we analyzed a 25-radial-slicing and a 50-radial-slicing of Praesepe. Both the change to a lower number of radial slices and accounting for spectral type proved fruitful. In both the 50 radial sliced plot and the 25 radial sliced plot we noticed a dip in the average period of rotation at about 17 pc from the center of mass of Praesepe. In the 50slice analysis at 17±1 pc from the center of mass, period of rotation dipped to 5.65 days per rotation (see Figure 6 and Table 3).

In the 50-slice analysis the radial shells are 2 parsecs thick. The radial shell in question is shell 9 (counting outward from the center of the cluster). Shell 9 houses 56 stars totaling 27.7 $M_{\odot}$. The shell contains 2 F-type stars, 3 G-type-stars, 6 K-type stars, and 45 M-type stars. Moreover, immediately beyond the relatively linear decrease in period of rotation from the center of mass to 17±1 pc the period of rotation distribution as a function of distance becomes apparently random (see Figure 6).

5. DISCUSSION

It seems that there are some characteristics of Praesepe where there is a virtually linear decrease in period of rotation until a minimum at 17±1 pc from the center of mass. The average period of rotation at that range, 5.65 days per rotation, is a rate of rotation at which there are few rotators in the 0.6 to 0.8 $M_{\odot}$ range (see Figure 3). Therefore, while the

center of the cluster is the highest overall concentration of mass as expected, the focus of lowest period of rotation seems to be 17 parsecs from the center of mass.

Given the broad range of period of rotation for M-type stars, we suspected the cause of the dip may be a prominence of M-type stars in the radial region. However, radial slice 9 does not have the highest count of M-type stars. Five shells in total have more M-type stars than shell 9, including shell 3 with 132 M-type stars, shell 2 with 122, shell 4 with 116, shell 5 with 80, and shell 6 with 55. However, shell 9 does have the 2nd highest ratio of M-type stars to other types of stars.

To wit, compared to Shell 9's 45 M-type stars there are 11 of the other three types of stars. Therefore, the ratio of M-type to other-type in Shell 9 is 4 to 1. Only shell 5 has a higher ratio — 4.44 M-type stars to any other type of star. Yet, these other shells still lag shells with lower M-type to other type ratios as far as lowest period of rotation.

Reconsidering Figure 3 along with the minimum average 5.65 day period of rotation at 17±1 pc from the center of mass, we can see that the 5-to-6 period of rotation range is the central region of absence of 0.6 to 0.8 $M_{\odot}$ stars of low period of rotation. It is an open question whether there is a correlation.

A hypothesis for the ring of low period of rotation is that it relates to a process in the cloud collapse that spawned Praesepe, or perhaps a dynamic inherent in star clusters if the pattern reveals itself in other clusters. To this end, we are gathering rotation and other data for stars in several other star clusters to cross analyze. Furthermore, statistical analysis on the likelihood of the pattern of average period of rotation from the center of mass to 20 parsecs — of Praesepe and other clusters — may help determine if the pattern is due to randomness.

Lastly, consolidating the coding analyses may allow for a direct comparison of the cube analysis versus the radial slice analysis, to see how the distances to the lowest cube period of rotation areas correlate with the 17±1 period of rotation marker in the radial slice analysis.

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Thank you to Dr. Viranga Perera for being a great mentor and joining me in this endeavor. Thank you to Dr. Steven Finkelstein for his AST 376R course, which provided the foundation of many of the coding tools we applied to this analysis and report. Thank you to Dr. Andrew Loveridge for his valuable input. Thank you to Michael Gadja — my lab partner in Dr. Greg Sitz's awesome modern laboratory — for introducing me to Python coding. Thank you to the edX's HarvardX CS50 Python Course, which was (and is) integral to my budding coding skill. We also received coding assistance from ChatGPT, Stackoverflow, and Cognitive Class.

7. BIOGRAPHY

With aspirations to be a college or university professor and astrophysics researcher, Darryl E Berry Jr was born in New Orleans, Louisiana. However, he has lived in

Kentucky, Georgia, and Texas, and has traveled internationally. He earned two *Summa Cum Laude* associate degrees from Palo Alto College in San Antonio, TX, before transferring to The University of Texas at Austin. He is earning therefrom both a B.S. in Physics and a B.S. in Astronomy and has earned University Honors in multiple semesters. His ongoing research includes properties of stars as functions of position within star clusters. He enjoys reading, writing, teaching, and movies — and the occasional video game.

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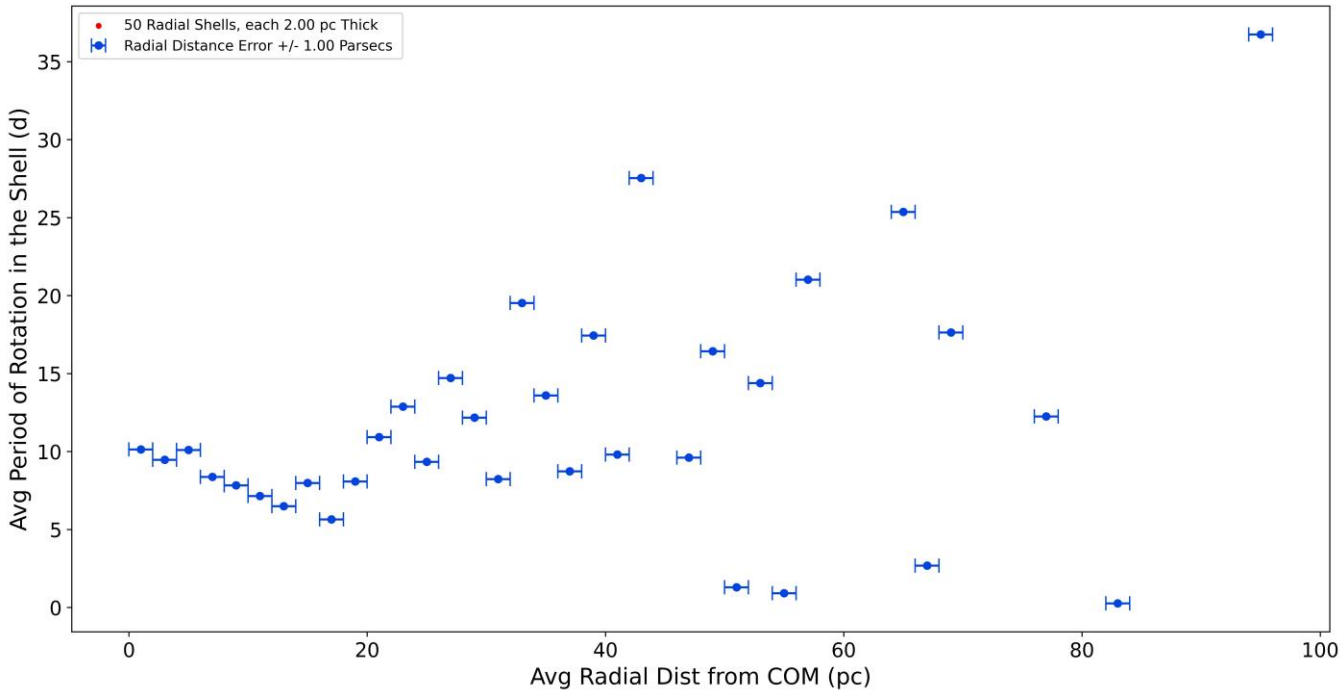

Figure 6. A plot of Praesepe with 50 radial slices outward from the center of mass of the cluster. Notice the dip in P_{rot} at 17 ± 1 parsecs from the center of mass of the cluster.

50 Radial Slices	Shell #	Inner Bound (pc)	Outer Bound (pc)	Avg Dist (pc)	$M_{\odot}$	P_{rot} (d)	F	G	K	M

Radial Shell Data	9	16	18	17±1	27.7	5.65	2	3	6	45

Table 3. Data on radial shell 9 of the 50-slice analysis, the region of Praesepe where there is a dip in average P_{rot} to 5.65 days per rotation. F, G, K, and M refer to stellar spectral type. Inner Bound and Outer Bound refer to the lower and upper radial bounds of the shell in parsecs, which are averaged to produce the average distance from the center of mass of Praesepe.

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BIO PAGE

Chasity D. Webb

Chasity Webb, a native of Dallas, Texas, is a passionate advocate, researcher, and community leader dedicated to enhancing the lives of parents and primary caregivers of special needs children, particularly those with Autism. With an academic background in Criminal Justice from Cedar Valley College, a Bachelor's degree in Advertising, and coursework in Speech Language and Hearing at the University of Texas at Austin, Chasity is currently pursuing a Master's degree in speech pathology at Texas Christian University's Davies School of Communication Sciences and Disorders. Driven by a desire to support and uplift families facing the unique challenges of caring for nonverbal autistic children, Chasity's research is focused on illuminating the struggles experienced by primary caregivers and developing much-needed resources to alleviate their mental, physical, and emotional burdens. In her leisure time, Chasity indulges in her love for romance literature, enjoys a diverse range of TV shows and movies, and cherishes moments spent with her family, including her beloved dog, Groot.

Wendi Deng

Wendi received his BS in Biochemistry from the University of Texas at Austin. Currently, his research at Dr. Bryan Davies' lab focuses on exploring the mechanism by which microcins are taken up by outer membrane receptors of Gram-negative bacteria. This work can provide insights into new methods for delivering proteins into Gram-negative bacteria, paving the way for the development of novel antibiotics. For his Ph.D. research, he is interested in exploring the molecular pathways of diseases to identify novel biomarkers. In his free time, he likes to play games, watch dramas, and draw.

Norma Gay

Driven by a deep passion for understanding and addressing complex social issues, Norma has dedicated her academic journey to studying Social Work, Sociology, and Urban Studies, along with earning a certificate in Public Policy and a minor in Government. Through her work with diverse populations, including individuals

experiencing homelessness, low-income youth of color, refugees, and asylees, she has gained valuable insight into the resilience of marginalized communities and the systemic challenges they face. Norma finds joy and fulfillment in building connections and advocating for those often overlooked. She upholds values of love, peace, community, and human dignity in both her personal and professional life. When she is not engaged in research or service, she enjoys grounding herself in nature, practicing yoga, or taking restorative naps.

Kinda Abou-Hamdan

At the University of Texas at Austin, Kinda Abou-Hamdan pursued a dual degree in Public Health (B.S.) and Plan II (B.A.), with a minor in Anthropology and a Bridging Disciplines Program Certificate in Patients, Practitioners, and Cultures of Care. She was selected as a Dean's Distinguished Graduate for the Class of 2025. Throughout her college career, she has helped coordinate an annual health symposium, along with other outreach events, for the local non-profit People's Community Clinic. This hands-on engagement with community health prepared her to pursue research in a clinical setting, where she could further explore the complex factors influencing health and healthcare. By researching healthcare utilization, health-promoting behaviors, beliefs, and motivations among Asian Americans, she hopes to inform health promotion and healthy aging initiatives. The field of public health will continue to guide her work both academically and professionally, shaping the impact she hopes to make on the world.

Oluwasemilore M. Ojerinde

Oluwasemilore M. Ojerinde was born in Abuja, Nigeria, and moved to the U.S.A. at seven years old to pursue a better life and education with her family. Her hometown Dallas, Texas, holds a special place in her heart as it was the foundation location where she built herself to experience the opportunities she has now in education and work. Oluwasemilore is a multifaceted undergraduate student who loves to learn about her environment through mindfulness, stimulate and unravel her curiosities and is fascinated with all psychological studies about the human mind and behavior. Her current research interests include traditional and non-traditional therapy treatments that best cater to racial and ethnic groups to develop better culturally sensitive approaches to therapy. She is about to graduate in Spring 2024 with her B.A. in Psychology and a minor in Sociology, both from the College of Liberal Arts. Her future endeavors include going to graduate school to pursue a Ph.D. in Psychology, opening her own clinic/research lab, and hopefully becoming a tenured professor at a higher education university. In her free time, she loves to watch television, bake, explore new places,

serve in her church ministry, and converse with new people. Her life's mission is to continuously foster her determination and passion to better the psychological well-being of individuals, especially young adults and ethnic-minority demographics in and out of the professional workplace. Oluwasemilore believes that with faith, purpose, vision, and perseverance: everyone can achieve their full potential.

Michael Cordova

Michael is a Black Studies researcher that studied at UT Austin. Their work focuses on the intersection between blackness (as a cultural production) and psychedelics. Michael examines the ways that the archive and film serve to structure, struggle against, and perhaps even rupture normative constructions of black existences through trippy aesthetics.

Saraí Cantú Dávila

From a family of passionate storytellers, Saraí Cantú Dávila hopes her work will illustrate life and meaning of culture, and inspire compassion through her narratives. Born and raised in Rio Grande Valley of Texas, Cantú is a writer, researcher, and avid reader. She received a B.A. in English and Mexican-American Latino Studies (College of Liberal Arts), a minor in Philosophy, and a certificate in Creative Writing from the University of Texas at Austin. Attending graduate school promptly after graduation, Cantú's research will focus on Latino culture in the U.S., studying language, identity, and folklore. She has committed her life to tell the stories of people marginalized and ignored, making room for their narratives to be seen. In her free time, Cantú enjoys visiting family in the RGV and playing with her dog Apollo. She also loves writing stories and reading books of fiction and poetry.

Rose Park

Rose Park is a storyteller and innovator studying Radio-Television-Film and Human Dimensions of Organizations at The University of Texas at Austin. Passionate about the intersection of media, technology, and culture, she actively researches and analyzes trends through social media and news outlets. Recognized with national and international journalism awards for her compelling coverage of current events, Rose is driven to push creative boundaries. With her diverse expertise in storytelling, research,

and marketing, she is eager to introduce innovative perspectives to the industry and make a lasting impact as she embarks on her media career.

Andrea Pantoja

Andrea is a Biology student with a minor in Social Entrepreneurship at the University of Texas at Austin. She loves research, mentoring, and being curious, even if it sometimes results in distractions for herself and others. While Andrea is indecisive about what PhD program to pursue, she is enthusiastic about anything involving interactions, sustainability, and using multidisciplinary methods to uncover what's really happening within a system. She has thoroughly enjoyed the journey that research has provided for her, and hopes to promote diversity and a welcoming research environment wherever it is that she ends up. Currently, you can find her awake at very late hours in coffee shops, out with her night owl friends, and overall trying her best to balance everything she signs up for. Andrea has no real hobbies at the moment but hopes to start that up a bit more as well as apply to PhD programs during her gap year.

Darryl Berry

With aspirations to be a college or university professor and astrophysics researcher, Darryl E Berry Jr was born in New Orleans, Louisiana. However, he has lived in Kentucky, Georgia, and Texas, and has traveled internationally. He earned two Summa Cum Laude associate degrees from Palo Alto College in San Antonio, TX, before transferring to The University of Texas at Austin. He is earning therefrom both a B.S. in Physics and a B.S. in Astronomy and has earned University Honors in multiple semesters. Darryl is pursuing a Ph.D. in Physics with an Astrophysics and Cosmology focus at Southern Methodist University in Dallas, Texas. Darryl enjoys reading, writing, and movies — and the occasional video game.