STANFORD
Undergraduate Research Journal

VOLUME 13 | SPRING 2014
Stanford, CA 94305

JOURNAL STAFF

Executive Board

Editors-in-Chief Laurie Rumker
James Nie

Financial Officer Anthony Cordova

Production Board

Lead **Emily Alsentzer**

Kate Nelson
Vivian Wang
Elijah Williams

Photography Kate Nelson

Outreach Board

Lead **Catherine Dong**

Courtney Noh
Emily Kelly
Helen Xiong
Kapil Kanagal
William Du

Editorial Board

Natural Sciences

Section Editor **Devan Diwanji**

Associate Editors Eric Liaw
Naomi Shah
Sophia Edelstein
Steven Lee
Taide Ding

Humanities

Section Editors **Allison Dods**
Alec Arceneaux

Associate Editors Melodyanne Chang
Shu Chen Ong
Neel Thakkar
Lauren Wedekind

Social Sciences

Section Editor **Jodie Ha**

Associate Editors Alex Zivkovic
Alexandros Cristodoulou
Jian Yang Lum
Kimberly Tan
Roman Larson
Sonali Singh

Engineering

Section Editor **Evelyn Chang**

Associate Editors Albert Chu
Cathy Wong
Cheenar Banerjee
Nathan Kondamuri
Shivani Baisiwala

The mission of the Stanford Undergraduate Research Journal is to encourage, recognize, and reward intellectual activity beyond the classroom, while providing a forum for the exchange of research and ideas. SURJ encourages students to become interested in research by displaying examples of what is studied and by offering the means of communicating knowledge between these disciplines to achieve a holistic effect.

A LETTER FROM THE EDITORS

Dear Reader,

It is with great excitement that we present the thirteenth volume of the Stanford Undergraduate Research Journal (SURJ). By providing an interdisciplinary forum for the exchange and dissemination of undergraduate research, we hope to foster the investigative spirit among undergraduates. The articles in this issue reflect the intellectual vitality of undergraduate researchers at Stanford and other academic institutions worldwide. We encourage you to explore the cholera crisis in Haiti with Jenny Chen ('17), delve into the notion of a citizen's council with Rohan Sampath ('16), discover the melodies of organic chemistry with Sherman Leung ('16), and challenge your interpretation of an iconic musical with David Kay ('16).

We would like to sincerely thank our staff, whose tireless efforts made this volume possible. In particular, we would like to acknowledge our Social Science Section Editor Jodie Ha ('14), Natural Science Section Editor Devan Diwanji ('15), Engineering Section Editor Evelyn Chang ('15), and Humanities Section Editors Allison Dods ('16) and Alec Arceneaux ('16). This volume of SURJ is a testament to your dedication and leadership. We would also like to thank Emily Alsentzer ('16), our Production Officer, whose direction and diligence were crucial in the redesign and production of the journal. We would also like to recognize Catherine Dong ('16), our Outreach Officer, for increasing SURJ's presence on campus, and Anthony Cordova ('15), our Financial Officer, for keeping the lights on.

We would like to express our gratitude for the support of our authors, the ASSU Publications Board, and the Office of the Vice Provost of Undergraduate Education. We would also like to thank the many students who submitted papers to the journal. Our editorial staff was impressed by the diligence and creativity reflected in each of the papers we received.

On behalf of the entire 2013-2014 SURJ staff, we would like to thank you for reading this journal and we hope you enjoy the wonderful work of these young authors.

Sincerely,

James Nie and Laurie Rumker
Editors-in-Chief

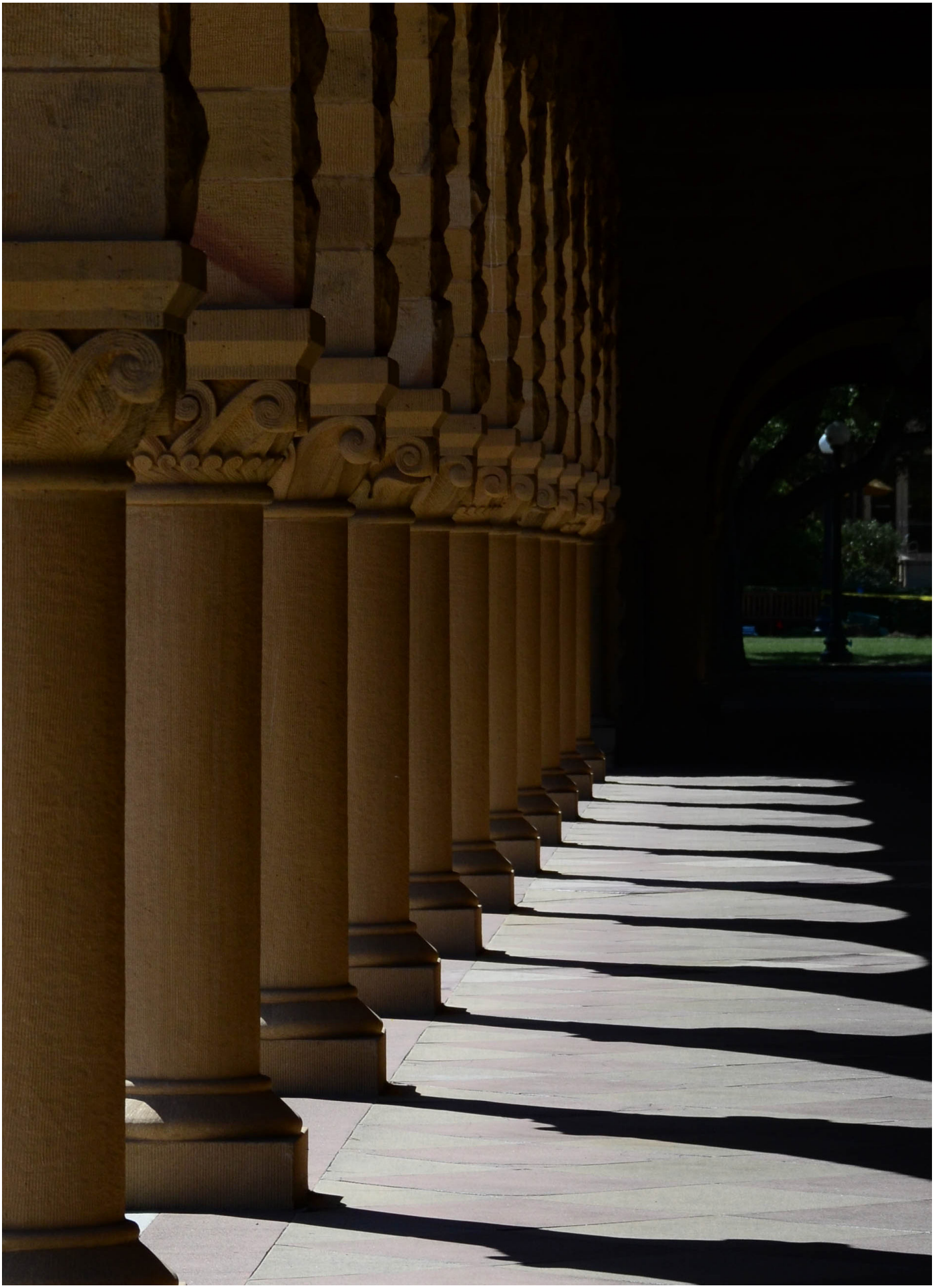
TABLE OF CONTENTS

SOCIAL SCIENCES

ENGINEERING

HUMANITIES

NATURAL SCIENCES



social sciences

Employing a Vaccine-Centered Maximalist Policy to Mitigate the Cholera Crisis in Haiti

Jenny Y. Chen

Stanford University

Despite the fact that cholera is both highly preventable and treatable, the 2010 outbreak of the disease in Haiti continues to claim victims to this day. The nation's heavy dependence on foreign aid and the lack of coordination among NGOs for delivering relief has protracted the disease's presence on the island. The absence of a public agency for overseeing efficient water use and distribution has also led to the squandering of precious natural water resources. Moreover, the limited amount of centralized governmental oversight for the health sector has weakened Haiti's response to the cholera. In response to the disease outbreak, minimalist policy makers aiming to reduce costs have focused more exclusively on treating the disease's symptoms and distributing water treatment kits. However, to effectively resolve the cholera crisis in Haiti and prevent future outbreaks, policymakers ought to employ a maximalist approach that combats the disease on multiple fronts including implementing a cholera vaccine program and improving water, sanitation, and health care systems. Increasing the distribution of an oral cholera vaccine would build up herd immunity in this nation where few members have been vaccinated previously. Epidemiological simulations predict that vaccinating just 10% of the Haitian population could avert 63,000 cases and 900 deaths. Therefore, the long-term benefits of investing in such vaccination measures in addition to increasing the distribution of cholera treatment kits, repairing water systems, and improving public safety education would be well worth the initial costs. Taking these steps would ultimately reduce cholera cases and fatalities and prevent future epidemics of water-borne diseases in Haiti.

WHEN CHOLERA STRIKES A RESOURCE-POOR COUNTRY

On January 12, 2010 a magnitude 7.0 earthquake ravaged Haiti, causing over 300,000 deaths and thousands more injuries [1]. However, even as the nation seemed to be on the verge of recovery from the quake and its aftershocks, the initial death toll was further compounded just nine months later when a second disaster struck – an outbreak of cholera. By February 2013, more than 8,000 people had died from the cholera epidemic and more than 640,000 others had fallen ill [2]. Arguably the poorest nation in the Western Hemisphere, Haiti had already been struggling with its economy and government budget, and when the earthquake devastated much of Haiti's already scarce water and sanitation infrastructure, the country was set back even further.

Haiti, unfortunately, is only one of several countries today which still struggle to provide their citizens with adequate water resources. To address this major issue, the member states of the United Nations Millennium Summit in 2000 established eight Millennium Development Goals (MDGs), the first of which included the provision of basic human needs such as safe drinking water. The U.N. aimed to provide access to water to 88% of the world's population by 2015 [3]. Though this goal was one of the first MDGs to be met when the 88% mark was reached in 2012, today around a billion people still lack access to safe drinking water, and between 2.4 and 2.9 billion lack adequate sanitation systems [4]. These continued difficulties in providing basic human needs point to systemic institutional, economic, and policy issues, particularly in Haiti. For example, in 2012, Haitians reported spending an average of 20% of their income on water alone [5]. Critics may blame exorbitant water prices, but at the same time, water that is too cheap is also damaging as it does not generate enough revenue to maintain water quality. This situation can be remedied through government water subsidies and more stringent water distribution management. Nevertheless, according to Deborah Sontag, investigative journalist for The New York Times, "in 2008, only 12% of the [Haitian] population had access to piped, treated water, and only 17% to 'improved sanitation,' which includes the simplest of pit latrines" [6]. Indeed, Haiti is one of the few countries where access to safe water and sanitation systems has actually declined through the past decades. In 1985, around 60% of Haitians were able to obtain safe water, but most likely due to increases in population density, this number is now less than 30% in urban areas and even lower in rural villages [7]. This widespread lack of access to safe water enables the spread and persistence of various water-borne illnesses, including typhoid and other diarrheal diseases. Haiti has the highest child mortality rates in the Western Hemisphere – one in eight children die before the age of five. Of these deaths, 37%

are due to diarrheal diseases, which could be better prevented and treated with the renovation of Haiti's health facilities as well as its water and sanitation systems [8].

Researchers were able to identify a broken sewage pipe in a U.N. camp of Nepalese peacekeepers which was leaking into the Artibonite River as the original source of the cholera outbreak [9]. When these peacekeepers arrived in Haiti to help restore order after the earthquake, they brought with them the El Tor strain of Vibrio cholera, the most virulent form of the cholera bacterium. Since cholera had not struck Haiti in over a century, the pervasive lack of immunity among the island's inhabitants was one factor that contributed to the rapidity of the contagion's spread. However, years of deteriorating water and sanitation systems in addition to minimal water management and subpar health care continue to impede the dissolution of the Haitian cholera crisis.

ISSUES WITH PRESENT HAITIAN WATER MANAGEMENT AND HEALTH CARE

Though many people blame the Nepalese peacekeepers and the U.N. for the introduction of the El Tor cholera to Haiti and have consequently sought legal reparations, the Haitian government is not entirely irreproachable either; socioeconomic and political problems have long plagued this island nation, compromising the quality of water, sanitation, and health care. In the past, citizens were forced to depend on private companies for water distribution and the maintenance of the limited sanitation facilities in Haiti [10]. The lack of a public agency for overseeing water use and distribution has led to the squandering of precious natural water resources. Moreover, Haiti is located in a highly natural-disaster-prone area and has been struck by countless storms, hurricanes, and floods over the past few decades. Frequent flooding, in turn, has resulted in the contamination of many rainwater collection sources [11]. Additionally, widespread deforestation and uneven rainfall between the different regions in Haiti have led to watershed problems that contribute to the flooding. Moreover, vandalism of water systems, water theft, and contamination by human and animal waste have all further exacerbated the water management issue [12].

The quality of health care in Haiti has also suffered dramatically as a consequence of the 2010 earthquake. As a result, many cases of cholera have not been treated in time, and transmission and mortality rates remain above optimal considering the disease's high preventability and treatability. Currently, health services in Haiti are largely privately owned and mostly localized in urban areas [13]. Overall, Haiti has become highly dependent on foreign aid, particularly in health care. For instance, NGOs currently provide about 70% of the health services in Haiti and focus primarily on the rural areas which usually have more limited health care resources compared to urban areas [14]. The limited amount of centralized governmental oversight for the health sector has undoubtedly weakened Haiti's response to the onset of cholera. For instance, at the start of the outbreak, the mortality rate among diagnosed cases was over 7%, among the highest in the recent history of cholera epidemics [15]. Mortality has since declined to approximately 2% overall, but even after three years since the outbreak, certain health facilities continue to have mortality rates of 4%. When diagnosed early, patients with cholera can be readily cured through antibiotics and rehydration salts, and therefore mortality rates above 1% are nowadays considered "unacceptable" under international health standards [16].

The presence of a plethora of NGOs with disparate agendas from the Haitian government has made it difficult to coordinate relief efforts. Over the past few decades, Haiti has largely relied on foreign aid to fund its national budget [17]. For instance, according to Washington D.C. foreign correspondents Majorie Valbrun and Roland Flamini, more than half of the Haitian government's funds are supplied by foreign sources and international relief efforts including U.S. donors, the World Bank, the Inter-American Development Bank, and the E.U. NGOs were able to help the Haitian government upgrade the Ministry of Public Health and Population for overseeing the development of health care infrastructure [18]. Nevertheless, Haiti continues to have the second highest per capita NGO population, behind only India; indeed, around 13,000 NGOs have participated in the delivery of relief efforts to Haiti, complicating coordination efforts [19]. According to Valbrun and Flamini, the current system in place for modernizing Haiti is essentially "cut and paste development" that only aims to fix issues on a short-term level.

Currently Haiti possesses two main agencies for the oversight of water use and management: Centrale Autonome Metropolitaine d'Eau Potable (CAMEP) which services mostly urban areas and Service National d'Eau Potable (SNEP) which supplies rural areas and secondary towns [20]. These government agencies also work alongside other organizations such as the Cooperative for American Relief to Everywhere (CARE), the United Nations Development Program (UNDP), and the U.S.-based Southern Baptist Convention (SBC). These organizations mainly attend to improving irrigation systems, wells, springs, and storm drainage and to educating locals on maintaining these systems [21]. Multiple NGOs and groups of charities like the Millennium Water Association have also gathered together to form the Regional Coalition on Water and Sanitation to Eliminate Cholera Transmission, an alliance that aims to improve water and sanitation issues in Haiti [22]. The transient stays of many of these NGOs, however, compromise the progress of water and sanitation initiatives in Haiti. As a result, Haiti currently ranks as the most water-insecure country in the Water Poverty Index out of nearly 150 nations [23].

Since the outbreak, the Haitian government and various NGOs have invested in educating the population by initiating cholera campaigns via radio and other media [24]. Many NGOs have also distributed handouts of water purification tables, soap, and oral rehydration kits. From a 2010 study of Haitian households, researchers at the U.S. Centers for Disease Control and Prevention in Port-Au-Prince found that household water treatments increased from approximately 30% to nearly 75% after these public health service activities [25]. Thus, these public health messages have at the very least proven effective in improving the Haitian population's recognition of symptoms, modes of transmission, and prevention mechanisms when dealing with cholera.

USING A MAXIMALIST POLICY TO APPROACH THE DISEASE IN HAITI

Policymakers attempting to mitigate Haiti's cholera crisis usually

align toward either the minimalist or maximalist approach. Minimalists are more cautious with respect to spending and express greater concerns over the costs of medical resources and over Haiti's lingering dependence on foreign aid. Such policymakers aim to resolve the cholera outbreak in more immediate terms, focusing on strategies such as the distribution of rehydration kits and antibiotics to treat the symptoms of the disease itself [26]. Although distributing rehydration and antibiotic kits indeed contributes to the reduction of cholera cases and mortalities, minimalist policies approach the disease with mostly short-term goals in mind, and therefore, these measures do not provide any safeguards against future outbreaks of cholera or other water-borne diseases. Given the frequency of natural disasters in Haiti in recent decades, the recurrence of a cholera epidemic is thus a major concern. Using a minimalist approach to the disease may seem favorable due to its cost-cutting advantages, but in the long-term, an approach that reevaluates and builds upon national infrastructure and government policies would prove to be much more beneficial.

Thus, given the shortcomings of the minimalist approach, maximalist policy makers are eager to employ as many available resources as possible to combat the issue from multiple fronts, despite higher costs [27]. For instance, in an article in The Lancet, senior members of the NGO Partners in Health cited five important steps for resolving the Haitian cholera crisis. These steps include improving the identification of symptoms and the treatment of the disease by reinforcing the education of local health care providers and community health workers [28]. In addition, the authors recommended that NGOs and the Haitian government obtain a greater volume of supplies for treatments such as oral rehydration or intravenous resuscitation and antibiotics for extreme cases. Moreover, these authors emphasized the use of an oral cholera vaccine, which is effective for approximately 90% of treated persons and can confer immunity for up to 3 years. An oral vaccine had not been seriously considered as a tool against cholera until recent years, especially since in this situation, a vaccine would be a reactive measure rather than a preventative one, as vaccines are more commonly used. However, increasing the distribution of an oral cholera vaccine could build up herd immunity and help stem the spread of infection.

> "THE LIMITED AMOUNT OF CENTRALIZED GOVERNMENTAL OVERSIGHT FOR THE HEALTH SECTOR HAS UNDOUBTEDLY WEAKENED HAITI'S RESPONSE TO THE ONSET OF CHOLERA."

The first validation of the potential efficacy of vaccines for resolving the cholera outbreak came through a mathematical transmission model. Drs. Jason Andrews and Sanjay Basu, epidemiologists at Harvard University and the University of California at San Francisco respectively, improved upon past infection projections by including more current transmission patterns into their model and by specifying their models for Haiti's major provinces for the period of October 2010 to January 2011 [29]. In making estimations of the vaccine's effectiveness, Andrews and Basu conservatively assumed that approximately two-thirds of the population vaccinated would be fully protected. Through their simulation, they were able to predict that vaccinating just 10% of the Haitian population would avert 63,000 cases and 900 deaths. Moreover, if these vaccines are paired with an increased distribution of antibiotic medications, they predicted that an additional 9,000 cases and 1,300 deaths could be averted.

Currently, there are two major vaccines on the world market: Dukoral and Shanchol, which cost approximately $6 and $1.85 per dose to produce respectively [30]. However, both require the two injections spread about two weeks apart, complicating the delivery of these vaccines. Some studies, though, have shown that even one dose can have a positive effect in building immunity [31]. But another disadvantage to the use of a vaccine is that the Dukoral injection, for example, involves the ingestion of a buffer solution that requires clean water, an already extremely limited commodity [32].

Another major concern among those who are cautious about implementing a full-scale cholera vaccine campaign in Haiti is that the introduction of vaccines could detract from efforts to improve sanitation and other infrastructure [33]. Opponents of the implementation of a vaccine plan also claim that vaccines would not be cost-effective because they only have preventative capabilities and thus would not be an effective reactionary measure. Despite this concern, however, previous studies have shown that vaccinating just 5% of the population could reduce the number of cases by 11%, a significant amount given the trends in the numbers of cases diagnosed in recent months [34]. The New Zealand Herald noted that other arguments against the implementation of a vaccine campaign include the assertion that immunity from these vaccines lasts for only three years and that directing funds to the improvement of sanitation would be more appropriate [35]. However, the author of the article also noted how Dr. Paul Farmer, the co-founder of Partners in Health, argues for a maximalist implementation of both vaccines and infrastructural improvements. Indeed, vaccinating even a fraction of Haiti's population could buy the Haitian government more time – a two- or three-year window at least – to focus on other pressing concerns such as sanitation and water system management without having more Haitians fall ill in the process. Indeed, past trials using Shanchol in the midst of an outbreak have proved successful. For instance, a study in Vietnam showed that even three to five years after vaccination, protection against cholera remained around 50% [36]. The cholera outbreak in Zimbabwe which began in 2008 also set an important precedent for appropriate courses of action in the Haitian cholera crisis; studies of the outbreak in Zimbabwe revealed that greater vaccination measures could have prevented thousands of deaths, suggesting that implementing such measures in Haiti could prove quite valuable. In fact, a study by Mukandavire, Smith, and Morris showed that 46% vaccination coverage in Haiti could reduce transmission of the disease altogether due to the establishment

of herd immunity [37].

On top of implementing a vaccine program, Ivers et al. also recommend addressing more long-term issues, such as improving water and sanitation systems and strengthening the nation's health infrastructure [38]. In particular, it is crucial that the Haitian government engages in the reassessment and improvement of its watershed management plan, which can be broken down into short-term, mid-term, and long-term goals. Short-term watershed improvement goals include repairing existing water distribution systems and stabilizing the detrimental effects of soil erosion on water retention. Mid-term goals refer to improving flood and sediment control and constructing small reservoirs for water [39]. Finally, long-term goals include reforestation to improve water retention and the formation of agencies to control the water supply [40]. In addition to reevaluating Haiti's watershed management plan, topography specialists Robert Knowles et al. recommend construction measures such as the building of small impoundments and hand pump wells to ease the distribution of water among the Haitian population [41].

Thus, it is essential to take a maximalist approach that combines a robust vaccination campaign with the improvement of cholera case treatment and the overhauling of water and sanitation systems. These measures together have the potential to significantly reduce the number of cholera cases in Haiti, eliminate fatalities from this treatable disease, and help prevent future outbreaks – a plan which was originally endorsed by the World Health Organization in March 2010 and has been increasingly supported by global health policy makers [42].

OVERCOMING CHALLENGES TO IMPLEMENTING A MAXIMALIST POLICY

With the cooperation of the Haitian government, local communities, and foreign aid groups, the deployment of a maximalist policy to approach the cholera outbreak is feasible. Currently, one roadblock to the full implementation of a maximalist policy is in obtaining adequate funds, especially since the country is already highly dependent on NGO support and foreign aid. A maximalist policy which combines investments on multiple fronts would indeed require more widespread and long-term investments than a minimalist policy. However, the minimalist policy provides only a simplistic, surface approach to the disease and neglects the systemic issues that pervade Haitian society in terms of lagging water, sanitation, and health care quality. Therefore, what remains a great challenge to the eradication of the disease in Haiti is the current entrenchment of attitudes toward the disease; though a minimalist policy will alleviate some of the stresses of the disease on the nation and is less costly, a maximalist policy can make larger strides against cholera and other water-borne diseases on a long-term scale. As a result, the Haitian government must shift from treating the immediate symptoms of the disease via a minimalist approach to focusing on long-term advancements against water-borne diseases on the whole, despite having to make potentially costly initial investments. A successful implementation of maximalist policy largely depends on the ability of Haitian government to oversee the improvement and modernization of multiple sectors including water systems, sanitation, and health care. The Haitian government has made some promising steps toward eradicating the disease through scaling up public health messages to increase public knowledge about the disease in terms of preventative and treatment measures. Haitian government agencies have also formed beneficial ties with foreign aid groups to better manage the national water supply. Nonetheless, the government must fully endorse and roll out a multi-year national cholera vaccination campaign to fully engage in a maximalist policy. Vaccines are currently being administered in some regions on small-scale levels mainly through NGO efforts; however, a cohesive, nationally backed vaccine program will be more successful in driving down the number of cholera cases and mortalities.

FUTURE WORK IN HAITI TO TREAT THE CHOLERA CRISIS AND PREVENT RECURRENCES

The inadequacy of water, sanitation, and health infrastructure in Haiti has crippled the government's ability to respond to the spread of cholera throughout the nation. Since the outbreak of the disease, several solutions have been proposed, including increasing the distribution of cholera treatment kits, repairing water systems, better educating the population about safety measures, and implementing a vaccine program. Investing in all of these measures would be worth the initial costs as the long-term benefits would include reducing cases and fatalities as well as preventing future epidemics of water-borne diseases.

Work that must be done in the near future include the mass production of oral cholera vaccines for distribution throughout Haiti. Even immunizing a fraction of the population will blunt the impact of the disease. This will ultimately allow the government to shift its focus from treatment of cases alone to the development of long-term projects such as updating the standards of the nation's water and sanitation systems to help prevent future outbreaks of cholera and other water-borne diseases.

ACKNOWLEDGEMENTS

I would like to thank Damon Halback and Susan Smith for their invaluable advice and feedback on this paper and for providing the opportunity to conduct this research.

NOTES

1. Gareth Evans, "Haiti: the Water Challenges Ahead," Water-Technology, last modified April 29, 2010, accessed March 11, 2013, http://www.water-technology.net/features/feature83743.
2. Louise C. Ivers, "A Chance to Right a Wrong in Haiti," The New York Times, February 22, 2013, accessed March 18, 2013, http://www.nytimes.com/2013/02/23/opinion/a-chance-to-right-a-wrong-in-haiti.html?_r=0.
3. "Millennium Development Goal Drinking Water Target Met," World Health Organization, last modified March 6, 2012, accessed April 1, 2013, http://www.who.int/mediacentre/news/releases/2012/drinking_water_20120306/en/index.html.
4. Ansel Herz, Water, ed. Noah Berlatsky (Farmington Hills, MI: Greenhaven Press, 2012), 247.
5. Herz, Water, 251.
6. Deborah Sontag, "In Haiti, Global Failures on a Cholera Epidemic," The New York Times, March 31, 2012, accessed March 19, 2013, http://www.nytimes.com/2012/04/01/world/americas/haitis-cholera-outraced-the-experts-and-tainted-the-un.html?pagewanted=all&_r=0.

7. Dominican Republic and Haiti: Country Studies, 3rd ed., Area Handbook (Washington, DC: U.S. Government Printing Office, 2001), 358.
8. Ibid.
9. Jonathan M. Katz, The Big Truck That Went By: How the World Came to Save Haiti and Left Behind a Disaster (New York, NY: Palgrave Macmillan, 2013), 225.
10. Robert B. Knowles et al., Water Resources Assessment of Haiti (n.p.: U.S. Army Corps of Engineers Mobile District and Topographic Engineering Center, 1999), 18, accessed March 20, 2013, http://www.gvsu.edu/cms3/assets/53940C9B-B252-5B69-626B48622A4C1002/publishedliterature/acoe_water_resources_of_haiti.pdf.
11. Knowles et al., Water Resources Assessment of Haiti, 2.
12. Knowles et al., Water Resources Assessment of Haiti, 6.
13. Dominican Republic and Haiti, 428.
14. Ibid.
15. Paul Farmer, "Cholera Vaccination in Haiti: Evidence, Ethics, Expedience," Harvard International Review 33, no. 3 (Fall 2011): 53, accessed March 11, 2013, http://puffin.harker.org:2302/ic/ovic/AcademicJournalsDetailsPage/AcademicJournalsDetailsWindow?failOverType=&query=&prodId=OVIC&windowstate=normal&contentModules=&mode=view&displayGroupName=Journals&limiter=&currPage=&disableHighlighting=false&displayGroups=&sortBy=&source=&search_within_results=&action=e&catId=&activityType=&scanId=&documentId=GALE%7CA273715812&.
16. "Haiti: Deplorable Conditions for Cholera Patients," Medecins Sans Frontieres, last modified March 12, 2013, accessed April 5, 2013, http://www.msf.org/msf/articles/2013/03/haiti-deplorable-conditions-for-cholera-patients.cfm.htm.
17. Marjorie Valbrun and Roland Flamini, "Rebuilding Haiti," CQ Global Researcher 6, no. 19 (October 2, 2012): 465, http://library.cqpress.com/globalresearcher/cqrglobal2012100200.
18. Dominican Republic and Haiti, 449.
19. Valbrun and Flamini, "Rebuilding Haiti," 467.
20. Knowles et al., Water Resources Assessment of Haiti, 5.
21. Ibid.
22. "Pan American Health Organization: Millennium Water Association Joins Coalition for Water and Sanitation to Eliminate Cholera from Haiti," India Pharma News (New Delhi, India), March 14, 2013, accessed March 18, 2013, LexisNexis Scholastic Edition.
23. Farmer, "Cholera Vaccination in Haiti," 52.
24. Valery E. M. Beau De Rochars et al., "Knowledge, Attitudes, and Practices Related to Treatment and Prevention of Cholera, Haiti, 2010," Emerging Infectious Diseases 17, no. 11 (November 2011): 2158, accessed March 21, 2013, http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3310585.
25. Beau De Rochars et al., "Knowledge, Attitudes, and Practices," 2160.
26. Sontag, "In Haiti, Global Failures."
27. Ibid.
28. Louise C. Ivers et al., "Five Complementary Interventions to Slow Cholera: Haiti," The Lancet 376, no. 9758 (December 18, 2010), accessed March 11, 2013, doi:10.1016/S0140-6736(10)62243-X.
29. Jason R. Andrews and Sanjay Basu, "Transmission Dynamics and Control of Cholera in Haiti: An Epidemic Model," The Lancet 377, no. 9773 (April 9, 2011): 1248, doi:10.1016/S0140-6736(11)60273-0.
30. Farmer, "Cholera Vaccination in Haiti," 53.
31. Andrew Jack, "Haiti Refused Cholera Vaccine, Chief Says," Financial Times (London, United Kingdom), July 10, 2011, accessed March 21, 2013, http://search.proquest.com/docview/875933375/13CCF9108AF5C42A9E8/11?accountid=618.
32. Farmer, "Cholera Vaccination in Haiti," 53.
33. Patrick Adams, "Haiti Prepares for Cholera Vaccination but Concerns Remain," The Lancet 379, no. 9810 (January 7, 2012), accessed March 21, 2013, http://search.proquest.com/docview/916303640/13CCF8CB317172ADE54/7?accountid=618.
34. Jack, "Haiti Refused Cholera Vaccine."
35. "Haiti Health Workers Give Cholera Vaccine," The New Zealand Herald, April 14, 2012, accessed March 18, 2013, LexisNexis Scholastic Edition.
36. Farmer, "Cholera Vaccination in Haiti," 54.
37. Zindoga Mukandavire, David L. Smith, and J. Glenn Morris, Jr., "Cholera in Haiti: Reproductive Numbers and Vaccination Coverage Estimates," Nature Scientific Reports, January 10, 2013, 1, accessed March 11, 2013, doi:10.1038/srep00997.
38. Knowles et al., Water Resources Assessment of Haiti, 14.
39. Ibid.
40. Knowles et al., Water Resources Assessment of Haiti, 31.
41. Ibid.
42. Farmer, "Cholera Vaccination in Haiti," 54.

REFERENCES

1. Adams, Patrick. "Haiti Prepares for Cholera Vaccination but Concerns Remain." *The Lancet* 379, no. 9810 (January 7, 2012). Accessed March 21, 2013. http://search.proquest.com/docview/916303640/13CCF8CB317172ADE54/7?accountid=618.
2. Andrews, Jason R., and Sanjay Basu. "Transmission Dynamics and Control of Cholera in Haiti: An Epidemic Model." *The Lancet* 377, no. 9773 (April 9, 2011): 1248-55. doi:10.1016/S0140-6736(11)60273-0.
3. Beau De Rochars, Valery E. M., Julie Tipret, Molly Patrick, Lara Jacobson, Kamil E. Barbour, David Berendes, Diana Bensyl, Cathie Frazier, Jean W. Domercant, Roodly Archer, Thierry Roels, Jordan W. Tappero, and Thomas Handzel. "Knowledge, Attitudes, and Practices Related to Treatment and Prevention of Cholera, Haiti, 2010." *Emerging Infectious Diseases* 17, no. 11 (November 2011): 2158-61. Accessed March 21, 2013. http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3310585.
4. *Dominican Republic and Haiti: Country Studies*. 3rd ed. Area Handbook. Washington, DC: U.S. Government Printing Office, 2001.
5. Evans, Gareth. "Haiti: the Water Challenges Ahead." Water-Technology. Last modified April 29, 2010. Accessed March 11, 2013. http://www.water-technology.net/features/feature83743.
6. Farmer, Paul. "Cholera Vaccination in Haiti: Evidence, Ethics, Expedience." *Harvard International Review* 33, no. 3 (Fall 2011): 51. Accessed March 11, 2013. http://puffin.harker.org:2302/ic/ovic/AcademicJournalsDetailsPage/AcademicJournalsDetailsWindow?failOverType=&query=&prodId=OVIC&windowstate=normal&contentModules=&mode=view&displayGroupName=Journals&limiter=&currPage=&disableHighlighting=false&displayGroups=&sortBy=&source=&search_within_results=&action=e&catId=&activityType=&scanId=&documentId=GALE%7CA273715812&.
7. "Haiti: Deplorable Conditions for Cholera Patients." Medecins Sans Frontieres. Last modified March 12, 2013. Accessed April 5, 2013. http://www.msf.org/msf/articles/2013/03/haiti-deplorable-conditions-for-cholera-patients.cfm.htm.
8. Herz, Ansel. *Water*. Edited by Noah Berlatsky. Farmington Hills, MI: Greenhaven Press, 2012.
9. *India Pharma News* (New Delhi, India). "Pan American Health Organization: Millennium Water Association Joins Coalition for Water and Sanitation to Eliminate Cholera from Haiti." March 14, 2013. Accessed March 18, 2013. LexisNexis Scholastic Edition.
10. Ivers, Louise C. "A Chance to Right a Wrong in Haiti." *The New York Times*, February 22, 2013. Accessed March 18, 2013. http://www.nytimes.com/2013/02/23/opinion/a-chance-to-right-a-wrong-in-haiti.html?_r=0.
11. Ivers, Louise C., Paul Farmer, Charles Patrick Almazor, and Fernet Leandre. "Five Complementary Interventions to Slow Cholera: Haiti." *The Lancet* 376, no. 9758 (December 18, 2010): 2048-51. Accessed March 11, 2013. doi:10.1016/S0140-6736(10)62243-X.
12. Jack, Andrew. "Haiti Refused Cholera Vaccine, Chief Says." *Financial Times* (London, United Kingdom), July 10, 2011. Accessed March 21, 2013. http://search.proquest.com/docview/875933375/13CCF9108AF5C42A9E8/11?accountid=618.
13. Katz, Jonathan M. *The Big Truck That Went By: How the World Came to Save Haiti and Left Behind a Disaster*. New York, NY: Palgrave Macmillan, 2013.
14. Knowles, Robert B., James O. Buckalew, Bruce Markley, and Laura Waite Roebuck. *Water Resources Assessment of Haiti*. N.p.: U.S. Army Corps of Engineers Mobile District and Topographic Engineering Center, 1999. Accessed March 20, 2013. http://www.gvsu.edu/cms3/assets/53940C9B-B252-5B69-626B48622A4C1002/publishedliterature/acoe_water_resources_of_haiti.pdf.
15. "Millennium Development Goal Drinking Water Target Met." World Health Organization. Last modified March 6, 2012. Accessed April 1, 2013. http://www.who.int/mediacentre/news/releases/2012/drinking_water_20120306/en/index.html.
16. Mukandavire, Zindoga, David L. Smith, and J. Glenn Morris, Jr. "Cholera in Haiti: Reproductive Numbers and Vaccination Coverage Estimates." *Nature Scientific Reports*, January 10, 2013. Accessed March 11, 2013. doi:10.1038/srep00997.
17. *The New Zealand Herald*. "Haiti Health Workers Give Cholera Vaccine."

April 14, 2012. Accessed March 18, 2013. LexisNexis Scholastic Edition.
18. Sontag, Deborah. "In Haiti, Global Failures on a Cholera Epidemic." *The New York Times*, March 31, 2012. Accessed March 19, 2013. http://www.nytimes.com/2012/04/01/world/americas/haitis-cholera-outraced-the-experts-and-tainted-the-un.html?pagewanted=all&_r=0.
19. Valbrun, Marjorie, and Roland Flamini. "Rebuilding Haiti." *CQ Global Researcher* 6, no. 19 (October 2, 2012): 449-72. http://library.cqpress.com/globalresearcher/cqrglobal2012100200.

Jenny Y. Chen is a freshman at Stanford University intending to major in Human Biology with a concentration in Health and Health Policy. A Bay Area native, she was first introduced to public policy through an international issues class and was inspired to pursue research in this field based on her interests in epidemiology and public health. In addition to her involvement in research, Jenny is a member of the Stanford Club Tennis team and the hip hop dance crew Common Origins.

From Side Effects to Solidarity

The Impact of Mass Incarceration on Black Solidarity

Brandon Hill
Stanford University

There is an urban holocaust in America. Silently, poor Black and Brown communities are being ground to dust by the gears of the American criminal justice system and the prison industrial complex. Mass incarceration is a socially engineered matrix of institutions, comprised of poorly funded urban public schools, asymmetric federal drug laws, and privately owned prison corporations. Working as a web, these three groups ensnare millions of Black and Brown bodies, dissolving not only the political and economic capacity of the men they consume but also crippling the fragile communities in which these young men are nurtured.

Scholars and activists alike argue that mass incarceration is but a reincarnation of past racial-caste systems bent on social control, adding to the legacy of slavery and Jim Crow. This paper will begin by detailing the mechanisms and effects of mass incarceration as a system on the national Black community. Later, it will analyze the historic link between oppression and strong Black identity, linking mass incarceration to a surge in African-American group solidarity.

As America celebrates a new post-racial era with the election of Barack Obama, a disturbing majority of urban young Black men are under control of the criminal justice system. Though Jim Crow legislation has been officially eradicated, new forms of racial caste have flourished, relegating ungodly proportions of the African American community into permanent second-class citizenship. Mass incarceration is the new Jim Crow and has now replaced segregationist policy and slavery as the new mode of socially engineered racial control. At incredible rates, the Black community is being warehoused in prisons and in other ways shackled by the criminal justice system. Legal scholar and criminal justice activist Michelle Alexander writes in her book, *The New Jim Crow,* "By targeting young Black men through the War on Drugs and decimating communities of color, the U.S. criminal justice system functions as a contemporary system of racial control" [1].

Of course, it is illegal to discriminate explicitly based on race. However, the scorn and disenfranchisement of Blacks once legal under Jim Crow are now simply assigned to felons, who are disproportionately African American. Denial of the right to vote; exclusion from jury duty; ineligibility for food stamps and public housing; and discrimination in education and employment are all acceptable and encouraged fates for those labeled as felons. This disenfranchising is dangerous to the political and economic power of the national Black community. Moreover, the system of mass incarceration is snatching Black brothers and fathers and husbands out of families, placing much of the Black community in jeopardy. The purpose of this essay will be threefold: to outline the effects of mass incarceration on the Black community; to analyze how mass incarceration is forging Black solidarity; and to examine how this solidarity is mobilizing to reform the criminal justice system.

THE COLLATERAL CONSEQUENCES OF MASS INCARCERATION ON THE BLACK COMMUNITY

Mass Incarceration as Racial Targeting

The system of mass incarceration is simply a racist mechanism for social control, specially engineered to target African Americans. In the criminal justice system, there is an irony of chronic injustice that leads to disparate rates of incarceration between Blacks and Whites. For example, although Blacks comprise roughly one-eighth of the American population, they represent about one-half of the national prison population [2]. More specifically, the system is tailored specifically for Black males, who suffer incarceration rates that are twenty-five times that of the average population [2]. Drug offenses are what fuel the system of mass incarceration, but the disproportionality at hand is intensified when we learn that "people of all colors use and sell drugs at remarkably similar rates" [1]. Alas, these figures point to a common root: racial targeting in the criminal justice system. More specifically, the vast numbers of people who are swept into criminal justice system are targeted by police who conduct drug operations primarily in poor communities of color [1]. Alexander recounts this process of racial selection:

> "Police are allowed to rely on race as a factor in selecting whom to stop and search (even though people of color are no more likely to be guilty of drug crimes than whites)—effectively guaranteeing that those who are swept into the system are primarily black and brown" [1].

Its evidence is incontrovertible: mass incarceration is designed to specifically ensnare communities of color, especially African Americans. The criminal justice system therefore is swallowing swaths of the Black community, which should pose an

immense and immediate threat to Black America. Because mass incarceration is zeroed-in on Black communities, its negative affects are concentrated there. Next, I will give account of its detrimental impact on the Black family, community, and nation.

The Collateral Consequences of Mass Incarceration on the Black Community

Author and Brown University Professor of Economics Glenn Loury puts the issue of mass incarceration into grave terms: "The criminal justice system is a monstrous machine that is grinding poor Black communities to dust" (Lopez 1012). The prison populations continue to be filled with Black faces, and this spells an inherent threat to the social, political, and economic advancement of the Black community.

Social Impact

Mass incarceration has contributed to the destabilization of the Black family, which directly destabilizes Black communities. First, mass incarceration has significantly reduced the rate of marriage among Blacks. Harvard Economist Adam Thomas found that going to prison reduces the likelihood of marriage by 50% for Black males following incarceration [11]. This un-marriageability is related to the fact that a disparity is growing between available male and female partners in the national Black community, which many scholars regard as an epidemic. Seventy percent of professional Black women are unmarried [1]. "'Where have all the black men gone?' is a common refrain heard among Black women frustrated in their efforts to find life partners" [1]. The notion that Black men have disappeared is rooted in reality. The gender gap in Black communities between males and females across the United States is as high as 37%, where the comparable rate for Whites is only 8% [1]. Michelle Alexander would attribute much of this startling gender ratio imbalance to the system of mass incarceration: "[Black men] did not walk out on their families voluntarily, they were taken away in handcuffs" [1]. Mass incarceration exacerbates ubiquity of single motherhood, which invariably weakens the Black community.

Family dysfunction is a natural consequence of large-scale incarceration. The void left by the incarcerated husband takes its toll on the family structure. It takes away the fathers income contribution to the nuclear family, his companionship to the wife or partner, caregiving to the children who need attention for their upbringing, and these factors cumulatively place insurmountable stress on the family, especially the single abandoned female. It also places undue burden on children, who are forced to adopt adult responsibilities—like cooking, cleaning, caregiving to younger siblings—instead of enjoying the splendors of youth and engaging the benefits of being a student. Thus, we can see why mass incarceration has damaged the life chances of Black children nationwide. Studies show that having a parent incarcerated makes a child between three and four times as likely to develop a record for juvenile delinquency [5]. This means that if a child's parent went to prison, the child will likely incur the same tragic fate. The same studies indicate that having an incarcerated parent makes a child 2.5 times as likely to develop a serious mental disorder, a consequence of the immense stress of strained family relations and increased responsibilities [5].

Mass incarceration has led to problematic heath outcomes for the Black community. Teenage births are correlated with higher rates of imprisonment in Black communities. According to sociologists James Thomas and Elizabeth Torrone, a doubling of incarceration rates in a given area will increase the incidence of teenage childbirth by 71.61 births per 100,000 [10]. Incarceration, which distorts local gender ratios, also explains some parts of the higher rate of HIV among Africa American men and women (Johnson and Raphael).

In sum, the noxious social environment makes incarceration a rite of passage for Black communities, wherein one in three Black men will be expected to encounter the criminal justice system in their lifetimes. In fact, a survey showed that in a variety of urban pockets, nearly 100% of respondents had a family member incarcerated within the previous five years. Regarding the familiarity of Blacks with the penal system, journalist and historian Paul Street remarked, "Black children are growing up with the sense that it is standard for older brothers, uncles, cousins, and perhaps themselves to be locked up by the state." This is a horrible observation, as it describes the psyche that breeds an inescapable cyclical nature of incarceration in the Black community.

Economic Impact

Mass incarceration has taken a severe toll on the economic prospects of poor urban Black communities, which affects those of greater Black America. Let us first examine the labor market. The labor market takes a hit as a result of mass imprisonment. According to Devah Pager, Harvard Sociologist and specialist on racial discrimination, "Large-scale incarceration can affect both [the supply of labor and the quality of workers], distorting labor market processes in unintended and unexpected ways [6]. In fact, increases in incarceration since 1990 have reduced the proportion of Black males in the labor force by 3-5 percent [4]. What this means for the Black community is fewer community members going to school and on to college, which reflects the decrease in quality of skills that Pagers indicates. It means fewer employable individuals to work in local black economies; there results a sad disappearance of barbershop owners, fast-food restaurant managers, and urban fashion-store employees.

As a fact, Black males after incarceration can expect a reduction in annual earnings by about one-third—this is a drastic cut from their already meager pre-prison incomes [11]. This is because, as Pager explains, that prison has a twofold damning effect on Black men's employability. First, physical or mental skills they entered with naturally deteriorated as a function of prison time. Second, regardless of ability, upon reentry, the convict label is crippling in the job search [6]. With hundreds and thousands of ex-cons returning home every year, they themselves—because of their injured economic capacities—become burdens on already financially traumatized communities. In sum, prisoners return to their home communities with no skills, no job, and no rights.

To elaborate on the strain placed on urban communities, Paul Street offers this economic analysis:

> "The lost potential earnings, savings, consumer demand, and human and social capital that result from mass incarceration cost Black communities untold millions of dollars in potential economic development, worsening an inner-city political economy already crippled by decades of capital flight and de-industrialization. The dazed, battered, and embittered products of the prison-industrial

> complex are released back into a relatively small number of predominantly Black and high-poverty zip-codes and census tracts, deepening the savage concentration of poverty, crime, and despair that is the hallmark of modern American 'hyper-segregation' by race and class" [12].

In essence, the fragile economic matrix of low-income Black communities is strained further by the reintroduction of offenders. This reintroduction burdens the community, and leads to the degeneration of economic prospects for the community's offspring. As previously explained, this creates a fertile breeding ground for future crime, and these children, nurtured in an impoverished community of felons, invariably become criminals themselves. Thus, the cycle regenerates.

The consequences of the marginalized economies of urban Black population centers have a direct impact on the national Black economy. This is because over 50% of African Americans live in inner-city environments, therefore if the urban Black community is unable to perform, then the total Black economy is only operating at half its economic potential.

Political Impact

According to Michele Alexander, more than one in seven Black men has lost the right to vote as a result of mass incarceration [1]. This toll is an underestimate of the extent of political disenfranchisement of the Black community, because in certain areas, ex-felons cannot restore voting rights without paying a fee—which, of course, almost none can afford [1]. Civil rights activist and Stanford Law professor Pamela Karlan says, "felony disenfranchisement has decimated the Black electorate" [1].

Poll exclusion is not the only means of political disempowerment of Blacks. The Census Bureau counts felons as citizens of the jurisdiction in which they are incarcerated, which overwhelmingly are rural White towns. Therefore, Black felons are transplanted from majority-Black districts to rural White voting districts, which accounts for an apparent increase in population there. This has immense consequences for redistricting. Black communities lose political representation while rural white communities inherit new representatives in the legislature. Alexander writes, "White rural communities that house prisons wind up with more people in the legislature [. . .] while poor communities of color lose representatives because it appears their population has declined" [1]. These factors pose a large threat to the political influence of both urban and national Black community, whose fates are intertwined.

Total Impact

Alas, we can see that the social, political, and economic consequences of mass incarceration are vast and grave. Because mass incarceration was engineered to target Black communities (as proven earlier in the paper), its negative side effects are intended and planned assaults on Black America. As MIT economist and philosopher Noam Chomsky puts it, mass incarceration is "basically a tool for controlling [. . .] Black people. It has nothing to do with drugs" [3]. The ensuing analysis will detail the shape of the retaliation.

THE EFFECT OF MASS INCARCERATION ON BLACK SOLIDARITY

Theories of Solidarity as a Reaction to Injustice

Black people are no strangers to racial injustice. Historically, when faced with oppression, Blacks have responded with resilience, unity, and morale. The observation is that Blacks tend to coalesce when faced with racial discrimination. This can be seen in the wake of slavery during reconstruction when a surge of Black politicians emerged, or in the heat of the Civil Rights movement when Blacks united to pass the most comprehensive political reforms in history. This observation can be captured in theory: "According to pragmatic nationalism, Blacks should unite and work together because they suffer a common oppression; and [. . .] they can make progress in overcoming or ameliorating their shared condition only if they embrace black solidarity" [8]. Today, mass incarceration presents an obstacle similar in scope to previous forms of injustice and therefore ignites public opinion and political mobilization.

Linked fate is the concept that explicitly links perceptions of self-interest to perceptions if racial group interest; where if linked fate is strong, racial group interests serve as a proxy for self-interest. Dawson found that when Blacks perceive their race to be subordinate to other groups, the concept of linked fate was strengthened. According to Dawson, for group interests to affect the political process, a significant number of African Americans must believe in linked fate, that what happens to the group affects their own lives. The unity around group interests reflects the belief that combined forces will make a strong enough impact to shape political outcomes.

Using statistics, I will suggest that data on African American consensus on certain issues proves that Blacks are united by group interest to combat the political and social construct of mass incarceration. Furthermore, the social, political, and economic threats that mass incarceration poses to Black communities ignited mobilization on the issue.

Black Public Opinion on Mass Incarceration: A Statistical Analysis

Dawson explains that "both economic and racial policies have historically been tied to African American group interests. Consequently, we would expect that perceptions of linked fate will shape all three of the issue domains". Shelby agrees, saying that black interests should be defined by the "unfair social disadvantages that certain individuals or groups face because [. . .] they are [Black]" [8]. Because mass incarceration is a matter of civil rights, and economic and racial policy, then linked fate shapes opinion on the issue.

Dawson shows that 66% of Blacks subscribe to the linked fate heuristic, and 65% felt that blacks were economically subordinated to Whites, which correlates with ideas of linked fate. We have established that a significant majority of Blacks are bound by linked fate and thus see issues that subordinate the group as issues that subordinate themselves. Now, Black consensus on given issues will be used as an index for Black unity on a range of issues that are closely related to mass incarceration. Because group opinion measures group solidarity—as defined by linked fate in the preceding discussion—these statistics on opinion will be used to measure Black Solidarity.

Death Penalty: According to a 2004 study conducted by Lawrence Bobo and Devon Johnson of Harvard University, 70% of Whites support the death penalty, whereas 56% of Blacks strongly oppose the death penalty. The Black opposition to the death penalty reflects deep-seated mistrust by Blacks of the criminal justice system, which operated the system of mass incarceration.

Crack vs. Power Cocaine: The same study found that 85% of Blacks strongly oppose harsher sentences for powder cocaine, while roughly a quarter of Whites approve of the disparity. This empirical perception of the cocaine discrepancy serves as a gauge for opinions on the war on drugs, against which Blacks are—as shown in the following data—unified in their opposition.

War on Drugs: According to Dawson's study, 82% of Blacks in 1982 supported the War on Drugs. This high support from Black communities is likely the result of the marketing that cloaked the War on Drugs as a blessing that would rid low-income Black communities of crime and poverty. Of course, this is not the case. In fact, the War on Drugs has had the opposite effect. That is why a majority of Blacks (56%) currently support the legalization, not criminalization, of marijuana [9]. This shift in Black attitudes on drug use has been catalyzed by the grand failure of the war on drugs, which a record low 10% of Americans support today.

The aforementioned cases model Black public opinion and solidarity against mass incarceration, which is strong and is still growing as awareness grows. Furthermore, the statistics point to a common Black agenda in opposition to the current system of criminal justice. As Dawson points out, linked fate leads Blacks to unite in a common group agenda. Even though mass incarceration specifically targets low-income Blacks, Dawson's linked fate argument provides that income level does not affect Black's conception of linked fate. Thus, all Blacks are united in the struggle, because what affects the few affects the whole.

This united group agenda is often represented by Black institutions and organization. Because mass incarceration poses such a social, political, and economic threat to this common Black agenda, Black institutions, which represent black community interests, are mobilizing to take down mass incarceration. This mobilization by institutions reflects the mobilization of the greater Black community.

FROM MASS INCARCERATION TO MOBILIZATION

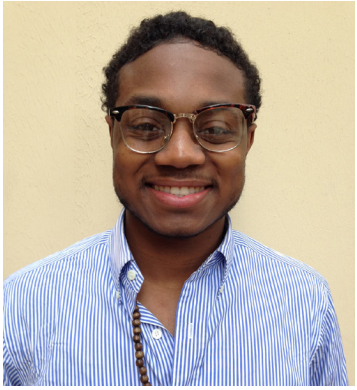
The premier Black institutions are adopting missions and campaigns to oppose mass incarceration. The NAACP, regarded as the leading civil rights organization in the nation, has developed a criminal justice reform campaign to address the issue. The Children's Defense Fund, regarded as one of the nation's most esteemed child advocacy groups, has launched an anti-'Cradle-to-Prison' pipeline to contribute to the cause. The Harlem Children Zone, often lauded as the most successful charity education organization, is founded on the mission of dismantling the pipeline. The Congressional Black Caucus, the most influential Black political body in congress, also has embraced a mission to reform the criminal justice system. This constellation of Black institutions is symbolic of Black solidarity that is being forged by the injustice of mass incarceration, just like during reconstruction and the Civil Rights movement. These efforts, however, are but a handful of the myriad movements raging against mass incarceration.

IN CONCLUSION

The Black community's solidarity is strengthened by mass incarceration, just like previous forms of discrimination have forged unity among Blacks. Mass incarceration comes with several collateral consequences: social, political, and economic. However, Black public opinion and thus Black solidarity are triggered when faced with such side effects, and the community mobilizes against them. Such is the case in the struggle to gain citizenship, to gain voting rights, to end segregation. Now, such is the case in the struggle to end mass incarceration.

REFERENCES

1. Alexander, Michelle. *The New Jim Crow: Mass Incarceration in the Age of Colorblindness*. New York: New, 2010. Print.
2. Alfred Blumstein, On the Racial Disproportionality of United States' Prison Populations, 73 J. Crim. L. & Criminology 1259 (1982)
3. Herivel, Tara, and Paul Wright. *Prison Nation: The Warehousing of America's Poor*. New York: Routledge, 2003. Print.
4. Holzer, Harry J., 2007. Collateral Costs: The Effects of Incarceration on Employment and Earnings Among Young Men. Paper presented to The Russell Sage Incarceration Policy Working Group. Russell Sage Foundation: New York, May 3.
5. Murray, Joseph and David P. Farrington. 2007. Effects of Parental Imprisonment on Children. IN Michael Tonry, ed. Crime and Justice: A Review of Research (forthcoming).
6. Pager, Devah. *Marked: Race, Crime, and Finding Work in an Era of Mass Incarceration*. Chicago: University of Chicago, 2007. Print.
7. Riggs, Mike. "HIT & RUN BLOG." *Reason.com*. N.p., n.d. Web. 11 Dec. 2012.
8. Shelby, Tommie. *We Who Are Dark: The Philosophical Foundations of Black Solidarity*. Cambridge, MA: Belknap of Harvard UP, 2005. Print.
9. Smith, Phillip. "New Poll Has Record High for Marijuana Legalization." *Raising Awareness of the Consequences of Prohibition*. N.p., n.d. Web. 10 Dec. 2012.
10. Thomas, James C., and Elizabeth Torrone. 2006. Incarceration as forced migration: effects on selected community Health outcomes. American Journal of Public Health 96(10):1–5
11. Western, Bruce. 2006. Punishment and Inequality in America. New York: Russell Sage



Brandon Hill is a sophomore at Stanford where he studies political science and African and African-American studies. A former White House intern and a presenter for TEDx, Brandon is interested in the nexus of education, policy, and entrepreneurship as a vehicle for community development and empowerment. One of the sophomore class presidents, Brandon aspires to a career in public policy and private enterprise and dreams of one day revolutionizing the nature of education.

The Cohort Effect

A Multi-Variant Study on Time, Trust, and Generational Cynicism

Breanne Deppisch
The University of Texas at Austin

Scholars have debated the decreasing levels of trust and efficacy that exist within the American political system. Researchers scramble to measure and analyze the decay of trust and efficacy in aggregate; yet no explanation seems to fully satisfy this pattern over time. Rather, it appears that this attitudinal erosion occurs at slightly different rates within each generational cohort. However, when parsing the data, these questions arise: which generational cohorts are most susceptible to political cynicism? Does political trust decrease with time, or do generations retain relatively stagnant attitudinal distributions? Through my research, I document a multi-dimensional decline of political trust over time and across generations, and examine the proposed emergence of patterns within individual cohorts.

RECENT TRENDS OF PROTRACTED DECAY

In his groundbreaking 1970s study, Arthur Miller broadly defined political trust as a "basic evaluative or affective orientation toward the government" [1, 2]. Over the past forty years, general electorate trust of the federal government has significantly plummeted—and data from the American National Election Studies (ANES) illustrate the severity of this decline. According to a study conducted in the American Political Science Review, "a situation of widespread, basic discontent and political alienation exists today" [1]. Political efficacy and political trust illustrate two dimensions of voter alienation that Miller alludes to. Public sentiment no longer regards the government as the benefit-providing, voter-protecting institution it was thought to be in the past. Rather, the mass consensus of a trusting electorate seems to have eroded over the past thirty years, giving rise to a new set of negative concepts (such as political inefficacy, scandal, and intrusion of individual rights) associated with our government [2, 3].

WHY TRUST MATTERS

Why is government trust so necessary? To best determine this answer, we must first explore the host of negative consequences that can occur as a direct result of government distrust. Decreasing levels of government trust often beget a number of damaging implications for institutional efficacy; as a study conducted in the 1960's warned, "[political] trust breeds conditions for the creation of future distrust" [3]. This increase in political distrust leads to disapproval, thus threatening the legitimacy and effectiveness of our governmental institutions. Erosion of trust is strongly correlated with lowered incumbent approval ratings, thus undermining government efficacy and problem-solving. If distrust truly breeds disapproval, and if disapproval increases the difficulty to effectively appropriate resources [4], then logically a government with lower levels of trust will simultaneously produce low levels of efficacy and institutional problem-solving abilities. Although instances of economic success or quality leadership can improve political trust within the electorate, positive institutional effects from these short-term measures have proved fleeting [2]. Jack Citrin and Donald Green [7] determined that "political trust is most strongly a function of presidential approval" [2]. Because of the president's predominance over the other two branches of government and his role in the spotlight of media coverage and public opinion, his behavior is subject to the highest levels of public scrutiny. Thus, negative or "scandalous" actions taken by the executive officer often beget high levels of public dissatisfaction, as well as lowered rates of trust and efficacy. If this were to be true, however, shouldn't the improvement of presidential job performance remedy the ill effects of previous incumbents? In a 1965 study on political support, David Easton identified criteria for two basic types of support: specific and diffuse [5]. Broadly identified, specific support entails general satisfaction with government output and the performance of political authorities. Diffuse support, in contrast, refers to the public attitude toward regime-level political authorities regardless of performance [2]. Since there seems to be little to no observed connection between improved presidential performance and increased levels of trust, it is reasonable to conclude that our electorate typically operates under a system of "diffuse support"—and additionally, a general sense of sustained low trust ultimately challenges regime legitimacy [2]. The myriad of public research devoted to levels of government trust yields the question: how does this increasing sense of cynicism come to fruition in the political preferences of younger voting groups? Are they more vulnerable to be dissuaded by scandal and controversy in office than older generations?

In my research, I demonstrate the correlation that exists between trust and efficacy levels over time, and its effect and weight in each voter cohort. I examine the decayed level of trust that has riddled the twentieth and twenty-first century American political system, and explore the effects of each cohort's attitudinal shift. Using an original time-series design

with data from the National Election studies from 1964-2008, I measure the correlations between the endogenous variables of trust and significance that political authorities hold over various generational cohorts; and address potential explanatory variables for the most radical declines in trust.

Rather than organize a measurement of political support in regimes or categories indicative of David Easton's 1965-1975 data, I chose to examine the effect political scandals play in various generational cohorts. Using 1964 as my baseline, I implemented data from several cross-sectional survey sets from the American National Election Studies (ANES) to explore several major "scandals" in the federal government, as well as examine their hypothesized effects on trust within each generation. Furthermore, I measured frequency distribution between cohorts in order to determine which generations were most susceptible to attitudinal decline. I use trust patterns as the basis of my theory, which examines differences in political behavior between different generations of the electorate. These trust patterns aid in determining which voter generations are least susceptible to decline of government trust.

Although Citrin and Miller promote the understanding of political trust as an essential element to an effectively functioning democracy, there are a number of different ways in which supporting data can be measured and analyzed. Using a time-variable model, I identified my two independent variables, voter cohort and presidential election year, as well as my dependent variable, the "trust" thermometer, offering a range of trust levels from 1-100. For purposes of a coherent research model as well as any additional allusions throughout the hypotheses, a list of generational cohorts utilized will be hereby identified as follows: 1927-1942; 1943-1958; 1959-1974; 1975-1990; 1991-present (see Appendix "B").

METHODS FOR MEASURING ATTITUDINAL SUSCEPTIBILITY WITHIN COHORTS

In Figure 1, I utilize a quasi-experimental design as a means to determine the significance of trust over time and distribution within generations. The design model I have chosen contains three elements, which I will address in the following ways. First, I test whether an overall attitudinal shift has occurred since the last presidential election. Second, I test where the shift has occurred— which generation has had the most radical change in opinion since the last cycle. For this test, if I can identify a pattern between radical changes and election years (for example, younger cohorts typically have larger margins of attitudinal shift between election years than do older generations) I can infer that certain cohorts are the most susceptible to actions taken by incumbents. In other words, one's association with a specific generational cohort could explain (with a certain amount of determinacy) potential susceptibility to attitudinal shifts as a reflection of executive behavior.

RESULTS

Upon initial examination of Figure 1, it is clear that several periods of stark decline in trust occur over the following years: 1964-1968, 1968-1972, 1972-1976, and 2004-2008. Accompanying each "trust thermometer" survey is an extreme 7-10 point erosion of trust. These numbers are sufficient to extract a trend in voter attitude. Upon further analysis, one can determine an overall decay in political trust since the mid-1960s, although this particular research model is certainly not the first to suggest this. By 1976, levels of political trust were at an all-time low—only 30% of Americans expressed their trust in the government "to do what is right" [ANES, 1976].

After extracting the levels of data in which the most significant declines occurred, I proceeded to the next step in my hypotheses: attributing these stark declines to the generational cohorts that were most strongly affected, and determining whether any overarching trends have emerged. The first quarterly drop in my analysis occurred in the 1964-1968 gap. Despite the overall 10-point decline in trust from 1964, the youngest two cohorts retained relatively high levels of trust—with both groups of respondents staying four points above the median.

This trend continues with the next quarterly drop in my data, when levels of trust reached an all-time low after the 1976 election. This drop could be attributed to the Watergate scandal, a notorious episode in American political history. Voters were also adversely affected by the deceit and debauchery that riddled the executive branch during the early 1970s. Even after taking into consideration important factors that may account for decline in voter trust, it is still necessary to determine which voting generation was most susceptible to the polluted political environment. Although public opinion and faith in the federal government had bottomed out, generational cohorts still bore semblance to the opinion distribution trend that was illustrated in the mid 1960's. The youngest surveyed cohort again retained the highest levels of positivity, while the levels of the 1927-1942 and 1943-1958 cohorts mirrored the overall data mean, offering only a 25-26% level of trust. The 1980s data set illustrates the relative unaffectedness that Watergate had over the youngest generations' vote, with the 1959-1974 trust levels actually showing a marginal three point trust increase immediately following the scandal. This immediate rebound could be accounted for by a number of variables, including perceived severity of misconduct and negative long-term consequences for the participants in the scandal (jail-time, impeachment, etc.) [3].

It is clear that within the time-series analysis I performed, some years tended to show a relatively rapid and more immediate decline than others. Levels of trust following the Watergate scandal suffered more over the course of several election years; however, not all decay seems to occur this gradually. One instance that connotes a steep and abrupt impact was the radical ten-point decrease from 2004-2008. Stemming from an upward climb in the late 1990s and the relatively positive spike in political opinion that occurred post-9/11, this ten-point drop in voter opinion marks a sharp and alarming decline. Erik Voeton noted that the cynical nature of attitudes at the time is likely attributed to the ongoing War in Iraq, as well as the financial crisis that plunged our country into a recession [8]. Upon further analysis one can again determine a similar pattern in the respondent pattern within each cohort. Similar to the levels of relative positivity observed by the youngest cohorts in previous quarterly drops, we can once again observe a marked retention of optimism compared to older generations. Although Figure 1 depicts an overall decrease of 10 points (43% to 34%) we can here observe an unequal distribution of opinion between the youngest cohort and older voters once again. The newest generation of voters

(1991-present) maintained levels of trust that were above average. Although their numbers were not extremely high compared to the overall mean, they do present startling results when viewed in contrast with other cohorts. From 2004-2008, levels of trust within the other four cohorts decreased as follows:

1975-1990: 37% to 28%
1959-1974: 36% to 23%
1943-1958: 36% to 25%
1927-1942: 36% to 30%

Each cohort suffered a 6-13 point loss in voter trust. It is important to note that in each of these respective instances, the youngest cohort remains in flux. For example, the youngest cohort in 1968 was 1943-1958; in 1980 it was 1959-1974. This then leads us to the question: *do levels of governmental cynicism increase with time and exposure to our political system?*

A plausible illustration of this trend could be found in the "diffuse system of support" proposed by Easton—one that highlights the necessity of trust within the American government. To make a strict correlation between time and distrust without accounting for any extraneous variables would be overly broad; furthermore, one should be careful not to discount the role of the economy, political socialization, and social capital in affecting these levels [3]. However, examining trust in the multi-dimensional approach which I have proposed provides new insight to the magnitude of the relationship between incumbent performance and government efficacy—thus, emphasizing possibility for its potential improvement. "Without some exogenous change that would provide for a durable increase in trust and approval, we will likely continue to operate under a system of sustained low trust." [2].

CONCLUSION

The relatively high levels of government trust we appreciated during the 1960s may never be more than a thing of the past. Although our country may never reach trust levels of 60 percentage points or more that were common in the 1960s [3], we can observe patterns and implement research to study this decline and perhaps keep it from expanding much further. By examining levels of trust in a multi-dimensional manner, as a reflection of the nature of that time period, as well as increased susceptibility to cynicism over time, we can make several important observations about voter patterns and behavior. Notably, not every political scandal guarantees a significant drop in trust—as can be concluded from observance of the Clinton scandal and trust levels of the 90s (Figure 1). Conversely, however, the absence of political scandals does not solely guarantee higher levels of trust. One potential explanation of this phenomenon could be attributed to an executive officer's misuse of government-appropriated resources; the Watergate scandal, for instance, bore direct connection to presidential duty and trust in office, whereas the infamous Clinton affair was much more personal in nature.

Since levels of political trust have only been studied since the past half-decade, there is a cap on the scope and extent to which my analysis can be accurately tested. However, if the generational trends of 1960s continue, one can expect a new, multi-faceted theory to help explain voter behavior. Although it cannot feasibly completely encompass the negativity that riddles our political system today, perhaps it can help account for generational decline and fluctuation of opinion over time.

APPENDIX A.

Table 8. Mean Cynicism by Distance From Both Parties, 1970

Distance from Republican Party	Distance from Democratic Party 0	1	2	3	4	Total
0	1.69[a] (5.9%)	1.60 (4.1)	2.50 (1.7)	1.50 (0.6)	2.10 (0.7)	1.79 (13.0%)
1	1.84 (6.1)	2.19 (18.2)	2.56 (8.4)	2.33 (3.0)	2.85 (0.9)	2.27 (36.6)
2	2.19 (4.6)	2.71 (9.8)	2.67 (11.4)	3.00 (3.1)	3.24 (1.2)	2.66 (30.1)
3	2.79 (2.4)	3.25 (4.2)	2.88 (3.5)	3.46 (2.5)	3.50 (0.6)	3.10 (13.2)
4	3.46 (2.0)	3.10 (2.1)	4.40 (1.1)	4.37 (0.9)	4.69 (1.0)	3.81 (7.1)
Total	2.16 (21.0%) Eta = .32	2.42 (38.4)	2.71 (26.1)	2.88 (10.1)	3.29 (4.4)	2.53 (100.0%) $N = 1401$

[a] Cell entries are mean cynicism values. The cynicism scale ranged from 0 = least to 5 = most.

Table 8. Mean Cynicism by Distance from Both Parties, 1970 Miller, Arthur H. 1974. "Political Issues and Trust in Government: 1964-1970." The American Political Science Review 68 (September) 951-972

"Mean Cynicism by Distance from Both Parties, 1970", used in Arthur Miller's 1974 study, demonstrate the degree to which distrust of the government increases proportionately to levels of dissatisfaction, thus supporting the aforementioned relationship between distrust and disapproval.

This provides evidence for a correlation between party identification and levels of cynicism; i.e. those who feel that neither party offers "viable solutions" will likely rank among the most distrustful, unengaged, and alienated citizens in the U.S. [1].

APPENDIX B.

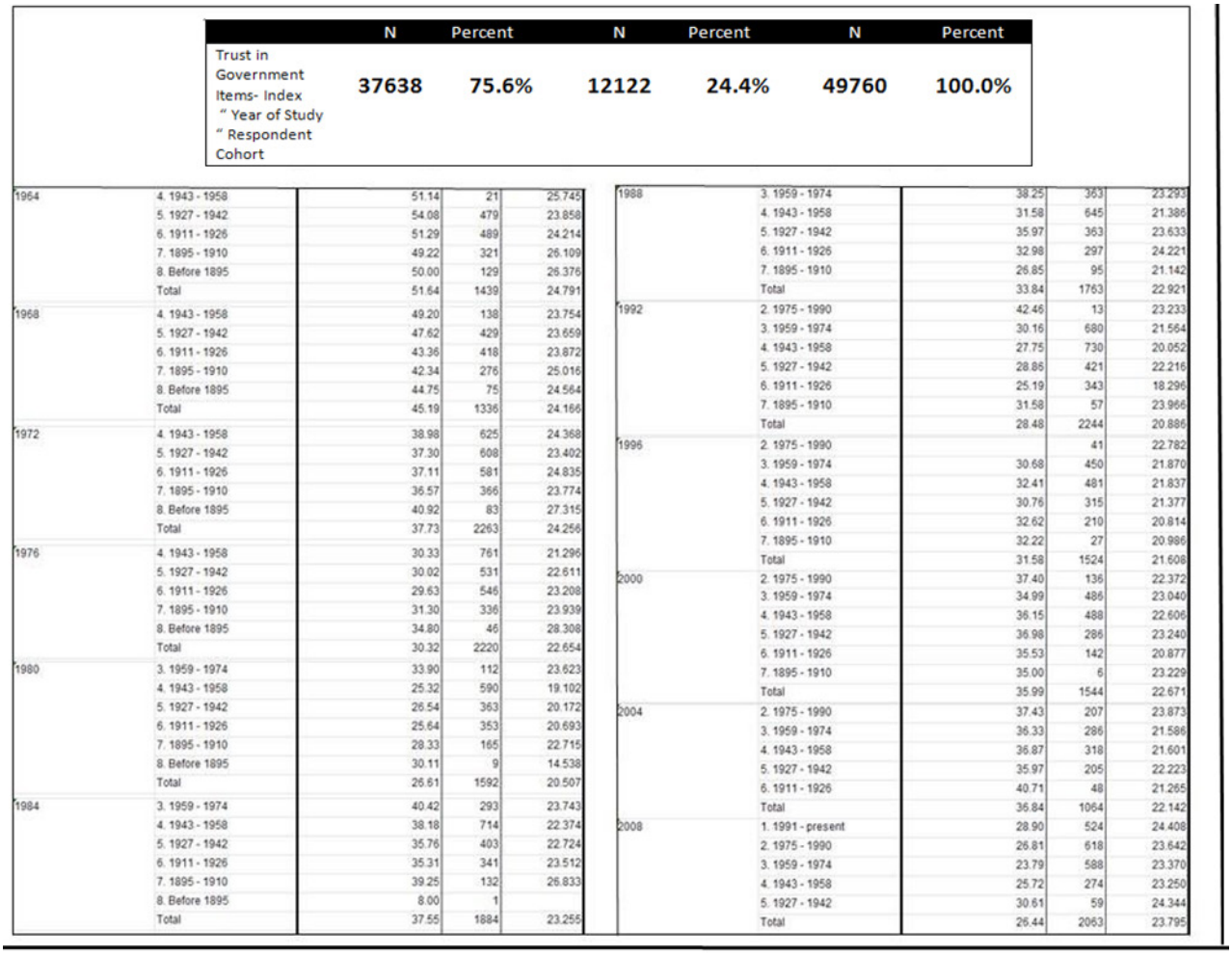
Figure One illustrates the cross-sectional model I have developed in order to extract relational patterns between feelings of trust and attitudinal distributions across cohorts. Data from the "trust" thermometer numerical scale can be found in the archived National Election Studies cumulative Time-Series report, [*Variable v0656*].

In order to examine a correlation between generational voter cohorts, it is necessary to extract a substantial sample size—thus, prompting me to choose the following five.

1927-1941
1943-1958
1959-1974
1975-1990
1991-present

Although there are other generational cohorts analyzed within the mean data set, for purposes of simplification and clarity within my range I chose the five that were most acutely present in each presidential election year from 1964-2008. By eliminating earlier generational cohorts (that were phased out by later elections in my model) an accurate trend within coherent and active voters was more likely to be found.

ANES data [*Variable v0656*] gave respondents a "feeling thermometer" to rank trust on a scale of 0-100. For purposes of coherency and consistency, I chose to analyze the mean



	N	Percent	N	Percent	N	Percent
Trust in Government Items- Index " Year of Study " Respondent Cohort	37638	75.6%	12122	24.4%	49760	100.0%

1964	4. 1943 - 1958	51.14	21	25.745
	5. 1927 - 1942	54.08	479	23.858
	6. 1911 - 1926	51.29	489	24.214
	7. 1895 - 1910	49.22	321	26.109
	8. Before 1895	50.00	129	26.376
	Total	51.64	1439	24.791
1968	4. 1943 - 1958	49.20	138	23.754
	5. 1927 - 1942	47.62	429	23.659
	6. 1911 - 1926	43.36	418	23.872
	7. 1895 - 1910	42.34	276	25.016
	8. Before 1895	44.75	75	24.564
	Total	45.19	1336	24.166
1972	4. 1943 - 1958	38.98	625	24.368
	5. 1927 - 1942	37.30	608	23.402
	6. 1911 - 1926	37.11	581	24.835
	7. 1895 - 1910	36.57	366	23.774
	8. Before 1895	40.92	83	27.315
	Total	37.73	2263	24.256
1976	4. 1943 - 1958	30.33	761	21.296
	5. 1927 - 1942	30.02	531	22.611
	6. 1911 - 1926	29.63	546	23.208
	7. 1895 - 1910	31.30	336	23.939
	8. Before 1895	34.80	46	28.308
	Total	30.32	2220	22.654
1980	3. 1959 - 1974	33.90	112	23.623
	4. 1943 - 1958	25.32	590	19.102
	5. 1927 - 1942	26.54	363	20.172
	6. 1911 - 1926	25.64	353	20.693
	7. 1895 - 1910	28.33	165	22.715
	8. Before 1895	30.11	9	14.538
	Total	26.61	1592	20.507
1984	3. 1959 - 1974	40.42	293	23.743
	4. 1943 - 1958	38.18	714	22.374
	5. 1927 - 1942	35.76	403	22.724
	6. 1911 - 1926	35.31	341	23.512
	7. 1895 - 1910	39.25	132	26.833
	8. Before 1895	8.00	1	
	Total	37.55	1884	23.255
1988	3. 1959 - 1974	38.25	363	23.293
	4. 1943 - 1958	31.58	645	21.386
	5. 1927 - 1942	35.97	363	23.633
	6. 1911 - 1926	32.98	297	24.221
	7. 1895 - 1910	26.85	95	21.142
	Total	33.84	1763	22.921
1992	2. 1975 - 1990	42.46	13	23.233
	3. 1959 - 1974	30.16	680	21.564
	4. 1943 - 1958	27.75	730	20.052
	5. 1927 - 1942	28.86	421	22.216
	6. 1911 - 1926	25.19	343	18.296
	7. 1895 - 1910	31.58	57	23.966
	Total	28.48	2244	20.886
1996	2. 1975 - 1990		41	22.782
	3. 1959 - 1974	30.68	450	21.870
	4. 1943 - 1958	32.41	481	21.837
	5. 1927 - 1942	30.76	315	21.377
	6. 1911 - 1926	32.62	210	20.814
	7. 1895 - 1910	32.22	27	20.986
	Total	31.58	1524	21.608
2000	2. 1975 - 1990	37.40	136	22.372
	3. 1959 - 1974	34.99	486	23.040
	4. 1943 - 1958	36.15	488	22.606
	5. 1927 - 1942	36.98	286	23.240
	6. 1911 - 1926	35.53	142	20.877
	7. 1895 - 1910	35.00	6	23.229
	Total	35.99	1544	22.671
2004	2. 1975 - 1990	37.43	207	23.873
	3. 1959 - 1974	36.33	286	21.586
	4. 1943 - 1958	36.87	318	21.601
	5. 1927 - 1942	35.97	205	22.223
	6. 1911 - 1926	40.71	48	21.265
	Total	36.84	1064	22.142
2008	1. 1991 - present	28.90	524	24.408
	2. 1975 - 1990	26.81	618	23.642
	3. 1959 - 1974	23.79	588	23.370
	4. 1943 - 1958	25.72	274	23.250
	5. 1927 - 1942	30.61	59	24.344
	Total	26.44	2063	23.795

Figure 1.

distribution of opinion for each presidential election year within each subset of the (5) extracted generational cohorts. Case summary items are shown in figure 1.

APPENDIX C.

I determine an overall attitudinal "shift" by examining the total mean computed at the end of each presidential election year. For each test, if there is a large increase or decrease in the time series trust thermometer, I examine which generational cohort most largely accounts for the change within the total mean. Miller, Arthur H. 1974. "Political Issues and Trust in Government: 1964-1970." The American Political Science Review 68 (September) 951-97

REFERENCES

1. Hetherington, Marc J. 1998. "The Political Relevance of Political Trust." The American Political Science Review 92 (December): 791-808
2. Dureska, Ryan, and Keele, Luke. 2007. "Scandals, Politicians, and the Decay of Government Trust." The Ohio State University (November): 1-25
3. Neustadt, Richard E. 1990. Presidential Power. New York: Macmillan.
4. Easton, David. 1965. "A Systems Analysis of Political Life." New York: Wiley.
5. Easton, David. 1975. "A Re-Assessment of the Concept of Political Support." British Journal of Political Science 5 (October): 435-457
6. Citrin, Jack, and Donald Philip Green. 1986. "Presidential Leadership and the Resurgence of Trust in Government." British Journal of Political Science 16 (October): 140-155 .
7. Voeton, Erik. 2006. "Public Opinion, the War in Iraq, and Presidential Accountability." The Journal of Conflict Resolution, 6 (December): 809-830
8. Hetherington, Marc J. 2005. "Why Trust Matters: Declining Political Trust and the Demise of American Liberalism." Princeton University Press (April): 1-35

Breanne Deppisch is a senior Government major at The University of Texas at Austin. Her love of political science is surpassed only by her love of sour candy, pug dogs, and roller blading. After graduation, she hopes to continue a career in political strategy or polling analysis.

Empirical Critique of the Existing Studies about Horizontal and Vertical Productivity Spillovers from Foreign Direct Investment in Vietnam

Anna Malinovskaya
Mount Holyoke College

This paper contributes to the literature on knowledge spillovers from foreign direct investment (FDI) in developing countries. First, I argue that the econometric approach that has been applied in the dominant literature to studying this issue in Vietnam might be inappropriate. Utilizing surveys of the Vietnamese economy and intellectual property rights literature, I show that the econometric models that have been used for Vietnam and other developing countries are not suitable because these models reflect certain built-in assumptions that are more suitable for studying the issue in developed countries. Second, I argue that the failure of existing studies to situate the analysis of knowledge spillovers from FDI into international logistics networks might affect the findings. I test these arguments by applying new econometric models that allow me to control for additional factors. Third, I demonstrate throughout the paper how estimates produced by conventional econometric models need to be interpreted with caution because they might be a result of several different knowledge spillover effects. I use firm-level panel data from the annual Vietnam Enterprise Survey, collected by the General Statistics Office of Vietnam, to estimate the models.

I. INTRODUCTION

Developing countries can acquire advanced technology through multiple channels, such as domestic innovation, licensing, trade, foreign direct investment, imitation, and piracy [1]. These channels are sometimes classified in literature into market and non-market channels. Market channels include exporting, FDI, licensing, and international joint venture. The non-market channels are imitation, movement of personnel, data in patents and test data, and temporary migration [2]. Another approach to the classification of technology transfer mechanisms is to divide them into foreign and domestic sources and then sub-divide foreign sources into two groups, namely embodied and disembodied channels. Embodied channels are imports of intermediate goods and FDI. Disembodied channels are generated by international patenting and licensing [3].

In some developing countries, such as Latin American states, national innovation systems are characterized as "open," meaning that foreign economic actors, especially transnational companies (TNCs), are the main agents of their domestic innovation processes [4]. The argument is built on intellectual property rights statistics indicating that the overwhelming majority of registered patents in Latin America are granted to foreign firms, mostly TNCs. Most developing countries, including Vietnam, would probably fit into this category, if judged by the ownership distribution of locally granted patents.

However, that fact should not be interpreted as claiming that domestic innovation in developing economies is absent. Some studies argue that domestic firms in developing countries spend significant efforts on imitative, incremental, and adaptive innovations, which are poorly captured by statistics on patents or other formal intellectual property rights [5]. The suggestion here is that FDI, trade, and licensing are only some mechanisms of technology acquisition.

In this paper, I will focus on one particular foreign source of technology transfer, namely knowledge spillovers. Rather than classifying technology transfer modes by the nature of knowledge to be transferred, I sub-divide them into two groups according to the transfer mechanism: direct and indirect technology transfer, recognizing that knowledge transfer from one partner to another may occur through different mechanisms.

Direct technology transfers through, for example, joint ventures and imports of intermediate and capital goods with embodied technology may give developing countries a shortcut to technology acquisition, enabling them to obtain advanced technology quicker than through incremental domestic innovation or technology that the country has no capacity to develop on its own.

Indirect technology transfer through knowledge spillovers from foreign to domestic firms in the same or other industries may increase domestic firms' productivity. In the international trade theory, TNCs are assumed to be more efficient than domestic firms. TNCs' superior knowledge, embodied in technology or disembodied, existing in the form of superior management and organizational knowledge, is what allows TNCs to overcome the usual barriers of entering a foreign market, such as their lack of understanding of the foreign environment and the costs of learning it, which domestic firms do not bear [6]. Domestic firms benefit from productivity spillovers that occur when a TNC cannot capture all quasi-rents due to its productive activities or to

This work contributes to the literature on knowledge spillovers from foreign direct investment in developing countries. I examine the case of Vietnam and argue that the econometric approach that has been applied in the dominant literature to studying this issue in Vietnam might be inappropriate. First, I utilize surveys of Vietnamese economy and intellectual property rights literature to suggest that the econometric models that have been used for Vietnam and other developing countries are not suitable, because these models reflect certain built-in assumptions that were originally made to study the issue in developed countries. Second, I argue that the failure of existing studies to situate the analysis of knowledge spillovers from FDI into international logistics networks might affect the findings. I then test these arguments applying new econometric models that allow me to control for additional factors. I use firm-level panel data from the annual Vietnam Enterprise Survey, collected by the General Statistics Office of Vietnam, to estimate the models.

The paper is organized as follows. Section two explains the theoretical pathways of knowledge spillovers through foreign direct investment. Section three presents a critique of the econometric approaches found in existing studies and proposes modifications to the econometric models. Section four describes the data and variables. Section five presents empirical findings. Section six discusses the results and section seven concludes. Section eight suggests further research.

II. LITERATURE REVIEW

Foreign direct investment (FDI) is expected to benefit host countries through both direct and indirect technology transfer. One strand of literature focuses on the analysis of direct technology transfer from FDI within joint ventures. A much bigger strand of literature examines how FDI facilitates indirect technology transfer from TNCs to domestic firms through knowledge spillovers. This study joins the dominant literature focus because in Vietnam most of FDI projects are 100% foreign invested firms. In 2010, 83% of foreign invested enterprises were 100% foreign invested firms [8].

Possible knowledge spillovers from FDI are often classified in literature into horizontal (intra-industry) and vertical (inter-industry) spillovers. According to literature consensus, horizontal spillovers can manifest in three ways: through a competition effect, labor turnover effect, and demonstration effect. Vertical spillovers are subdivided into forward and backward spillovers.

2.1 Horizontal spillovers

2.1.1 Labor turnover effect

Sheng et al., Lin et al., Suyanto et al., Krammer, and Xu et al. suggest that knowledge spillovers from FDI can occur through labor turnover when former workers of TNCs are employed by domestic firms or set up their own firms [9, 10, 11, 3, 12]. Some studies note, however, that this scenario is less likely for developing countries. As TNCs tend to pay higher wages, their workers may be reluctant to move to local firms [13, 14]. Unden supports this latter hypothesis for the case of Vietnam: "Being a Vietnamese worker and employed in a multinational corporation does in general imply a high market value and a higher salary compared to workers in domestic companies in the same branch […] Vietnamese workers in a MNC go directly to another MNC when changing job" [15]. Thus, the expectation is that knowledge spillovers through this channel will be insignificant, if any.

Most studies do not separate labor turnover effects from others in the horizontal spillover effects group. However, the study by Nguyen et al. on Vietnam separates the spillovers obtained through labor turnover and other horizontal spillovers by including two measures of FDI presence into the same pooled OLS model – the employment measure of FDI and the output measure. Interestingly, they find that horizontal spillovers from labor mobility, captured by the FDI employment share, are positive and significant, whereas other horizontal spillovers, captured by the FDI output share, are negative and also significant. For the service sector, the two coefficients switch signs [16], yet as our discussion below will illustrate, the pooled OLS method might be inappropriate. The same study also estimates fixed effects, random effects, and first difference model. In the corrected models, the employment measure is positive and significant and the output measure is insignificant.

2.1.2 Competition effect

The literature suggests two kinds of pressures that a TNC may exert on a host country domestic market. Most of the studies, however, consider the competition effect from only one of the two perspectives – from the perspective of technical efficiency. According to this technical efficiency hypothesis, identified by Caves, the entry of a foreign firm into the market increases competition and forces domestic firms to seek ways to utilize their resources more efficiently [7]. As a result, industry standards may rise over time [17]. Recent studies thus expect to find a rise in productivity of domestic firms in industries with higher foreign presence through this horizontal spillover effect. Some studies indeed discover that greater competition from foreign firms facilitates spillovers [11]. Others note that this effect depends on the way foreign firms organize their production and compete in host country markets [10].

However, there is also recognition in the literature that increased competition may affect productivity of domestic firms in the opposite direction if it forces local firms out of business rather than motivates local firms to be more efficient [15]. For example, Lin et al. mention both positive competition effects and the negative crowding-out effect, which may occur through two channels [10]. First, if domestic firms' sales are reduced by the entry of a TNC, they will have to spread their fixed costs over a smaller sales volume and see their productivity falling at least in the short term. Second, if foreign invested firms pay higher wages, they may raise average wages for all firms in the market, increasing production costs of domestic firms. A study by Le et al., for example, examines intra- and inter-industry wage spillovers in Vietnam and finds that wage levels in domestic private firms are higher in sectors with higher foreign presence and that domestic firms with inter-industry links to foreign firms pay higher wages [18]. Consequently, the net competition effect will depend on the interaction of the positive spillover channel and the negative crowding-out effect. Lin et al. find negative but insignificant competition effects for Chinese firms [10].

The second perspective on the competition effects, also suggested by Caves but left mostly unnoticed in recent studies

with the exception of Liu et al. [19], is what he describes as allocative efficiency [7]. He postulates that since TNCs tend to spread in industries where entry barriers are high, they may remove monopolistic distortions and improve the productivity of domestic resources through their better allocation. It follows, then, that a positive horizontal spillover effect may reflect any of the two mechanisms at work.

2.1.3 Demonstration effects

Demonstration effects are commonly cited in the literature as one of the three channels of horizontal spillovers. They are understood as direct imitation, reverse engineering, or new innovation through R&D [11]. The nature of demonstration effects makes them hard or impossible to measure, so the literature does not separate them from other horizontal spillovers.

The literature specifies that domestic firms' ability to benefit from foreign presence through demonstration effects may depend on their absorptive capacity, briefly defined as the ability to study and apply advanced technology from developed countries. To capture local firms' absorptive capacity, studies most often include into their models some or all of the following variables: technology gap, research and development (R&D) activities, and human capital quality.

The idea of a technology gap is most often defined as the difference between labor productivity in domestic and foreign firms of the same industry. Some scholars believe that the smaller the technology gap between domestic and foreign firms, the more domestic enterprises are able to benefit from horizontal FDI spillovers [20]. Tajul et al. label this idea the "catching up hypothesis" [21]. Others, however, assume that the higher the technology gap, the more catch-up local firms have to do and the more they will benefit from demonstration effects [12]. Tajul et al. label this theoretical pathway the "technological accumulation hypothesis" [21]. Some studies, such as Nguyen et al. and Crespo et al. suggest that local firms benefit from foreign presence in an industry if the technology gap exists but is not too large, implying that there is probably some threshold level [16, 13].

R&D activities by a firm are viewed not only as a function of knowledge creation but also as a function of promoting a firm's absorptive capacity [22]. More R&D in local firms, therefore, is expected to increase their ability to benefit from FDI. Most studies measure R&D by input, such as R&D expenditure [4, 12, 19, 23]. Some, however, use an output measure, such as patents or intangible assets of a firm [24]. Some research casts doubt on the idea that firms with more R&D activities are better able to benefit from FDI. Fan et al. find that firms in China with R&D activities benefit little from foreign presence if there is no foreign presence in the firms themselves, due to the difficulty of communicating with foreign firms and/or the lack of ability to digest foreign technology [23].

Human capital quality is also traditionally included in the measure of firms' absorptive capacity. The measurements of human capital quality vary. It may be proxied by the firm's average wage bill [25]; wage and training costs per employee [14]; the ratio of workers with a secondary school certificate to total employees [19]; the percentage of firm's workers with college or higher degrees [26]; or the average number of school years completed by the adult population aged 25 and over for a macro-level study [3].

Some studies also consider firm size, firm ownership structure, and firm location as significant determinants of firm's absorptive capacity. With regard to firm size, Lin et al. hypothesize that large domestic firms may be better able to absorb knowledge spillovers from FDI because they have more resources, such as formal R&D and more skilled workers, than small firms. They find that firm size may determine whether productivity spillovers from FDI are positive or negative [10]. Crespo et al. explain that small firms may not have a production scale large enough in order to be able to imitate technologies introduced by TNC [13]. Le et al. mimic this view [14]. Sinani et al. find that small firms benefit more from FDI spillovers and explain this result by small firms' ability to better benefit from knowledge created by universities and private corporations [27]. All of these explanations, however, are vague and do not provide researchers with an understanding why firm size matters for receiving productivity spillovers.

Sometimes studies control for the ownership structure of domestic firms, expecting different levels or effects of productivity spillovers from FDI. Le et al., for example, find different effects for state and private firms in Vietnam and explain this result saying that private firms are less equipped with skilled labor, technology, and capital, and therefore have weaker capacity to absorb the benefits of foreign presence. In addition, they note that private firms, due to their small size, are less able to compete with foreign firms than state enterprises [14].

Domestic firm location is often used in literature to account for differences in firms' ability to receive productivity spillovers. Damijan et al. look at geographical distance between foreign and domestic firms from the demonstration effects perspective, expecting that firms located near TNCs will have a better chance to interact with foreign enterprises [25]. Fu and Ouyang et al. discuss the notion of regional absorptive capacity [22, 28]. Ouyang et al. measure it by local industrial development proxied by the share of mining and manufacturing in GDP [28]. Fu states that region's absorptive capacity may be enhanced by the strength of its entrepreneurship, infrastructure, and presence of clusters [22]. Few studies, however, explicitly considered the impact of being located in an industrial cluster on firm's absorptive capacity.

2.2 Vertical spillovers

2.2.1 Backward linkages

Vertical spillovers are theorized to occur through TNCs' linkages with domestic firms in which domestic firms serve as suppliers to (backward linkages) or customers of (forward linkages) TNCs. Some studies, such as Lin et al. [assert that there may actually be a greater scope for vertical than horizontal spillovers if TNCs try to prevent the leakage of knowledge to their competitors [10]. Others, such as Wei et al., however, caution against this view [24]. The literature implicitly recognizes two ways for domestic suppliers to TNCs to benefit from the relationship. I label the two pathways as (1) the direct backward spillover path and (2) the indirect backward spillover path. The direct path is in effect when foreign firms and their local suppliers formally cooperate. This cooperation is theorized in the literature to take various forms. For example, Lin et al. point out that knowledge spillovers may occur if (1) TNCs help domestic suppliers set up production capacities; (2) TNCs provide local suppliers with technical assistance to raise the quality of suppliers' products; and (3) TNCs provide

trade associations or industry organizations for the purpose of information sharing [15]. Giroud conducts an empirical investigation of formal cooperation between TNCs and their domestic suppliers in Vietnam and Malaysia and finds that the cooperation modes include TNCs engaging into joint design of inputs and joint settlement of operations requirements with local suppliers [29]. Giroud collects foreign firm managers' perceptions on t he performance of their domestic suppliers and finds that domestic suppliers do improve various aspects of their production and service delivery because of partnering the foreign firms.

The indirect path refers to the idea that domestic firms supplying to TNCs may raise their productivity due to competition pressures. Unden calls this hypothesis "forced linkage effects" [15]. When foreign firms set higher requirements for product quality and on-time delivery, local suppliers are forced to improve their production processes [17]. However, this competition effect on suppliers may well be negative. If the market for supplying inputs to TNCs is competitive, foreign firms will probably take advantage of the competition and undercut prices [12]. Giroud also warns that in some contexts, the foreign buyer may hold the dominant position, enabling it to dictate the terms of trade, especially the price of inputs. Such an unequal relationship may hurt local suppliers in developing countries, who may not have large-scale production and rely solely on profit margins to finance technological upgrading [29]. Unden echoes this perspective. Unden explains that since TNCs generally focus on more value-added activities like R&D, branding, and marketing, they may shift the risk to the upstream industry, forcing local suppliers to become price-takers [15]. With regard to Vietnam, Unden argues that many foreign firms do not take into account increases in raw material prices, which leads to financial losses for local suppliers, who sometimes cannot afford to lose a contract with a powerful foreign buyer [15].

Since data limitations usually make it very difficult to separate the spillover effects occurring through the direct and indirect backward spillover paths, studies usually include one backward spillover variable in their model and if its coefficient is negative, the authors conclude that the negative impact of competitive pressures on local suppliers outweighs the positive impact of formal cooperation with foreign partners.

2.2.2 Forward linkages

Productivity spillovers from TNCs to downstream industries may occur when domestic firms gain access to better quality or less costly intermediate inputs or machinery that they would otherwise have to import or do without [10, 12, 17]. In addition, knowledge spillovers through forward linkages occur when domestic firms serve as marketing or distribution agents or customers of foreign firms. Forward linkages have been explored less in the literature than backward linkages. Damijan et al. suggest that forward linkages have received less attention in the literature because foreign firms are mostly engaged in end-user consumer goods [25]. However, there is another perspective that may explain why foreign firms do not always develop vertical (forward as well as backward) linkages in the host country. This perspective is studied in detail in the next section.

III. PROPOSITIONS

3.1 Understanding logistic networks may help to understand why spillovers occur, do not occur, or occur from only certain kinds of foreign firms

It may be helpful to situate the study of productivity spillovers from vertical and horizontal linkages into the international trade theory. When researchers estimate productivity spillovers from FDI, an implicit assumption is that all foreign firms have the same effect on local firms across various modes of industrial organization. A few studies, however, explicitly recognize that different patterns of foreign firms' industrial organization, often related to the firm's country of origin and industry in which the firm operates, may produce or prevent productivity spillovers through vertical linkages and produce horizontal spillovers opposite in sign. Such studies make a distinction between foreign-market-oriented and domestic-market-oriented foreign firms to proxy different modes of foreign firms' organization of production [10, 12, 14].

Lin et al. justify making this distinction in their study by pointing out two possible differences in the effects between the two types of foreign firms. First, they state that domestic-market-oriented foreign firms necessarily reduce market share of domestic firms. Second, export-oriented foreign firms are expected to be more closely integrated into international production networks and source their inputs from abroad. Therefore, export-oriented foreign firms are theorized to produce positive horizontal but no vertical spillovers. Yet in their study, they find no significant difference between productivity spillover effects of the two types of foreign firms [10]. Le et al. mimic this view and find that export-oriented foreign firms do not significantly affect the productivity of domestic firms in Vietnam [14]. Xu et al. find that export-oriented foreign firms in China indeed have weaker vertical linkages [12].

This distinction, however, might not be able to capture the larger picture. In this paper I argue that distinguishing foreign firms by their market orientation may be highly appropriate in some contexts like China, which offers import tariff and value-added tax exemption for imported raw materials and parts used in export processing, thus encouraging foreign firms to source their inputs and sell their outputs on international markets [12]. Yet it may be inappropriate in other contexts. It might be that foreign firms producing for the domestic market source their inputs from abroad while firms producing exports source inputs locally. Foreign firms located in North Vietnam may be more likely to import their inputs from China regardless of whether they produce for the domestic or international market. Machikita et al. analyze production networks in Hanoi and find that about 73% of TNCs that source their inputs from abroad also sell on the domestic market, and 44% of TNCs that source their inputs from Vietnam sell their output on the international market. It is clear then that there is a need for a finer distinction among TNCs engaged into different production networks in order to understand how different types of foreign firms may produce different productivity spillovers [30].

Some authors writing on FDI productivity spillovers in Vietnam do not distinguish between foreign-market-oriented and domestic-market-oriented foreign firms because of data limitations [16, 17]. Le et al., however, control for foreign firms' market orientation [14]. Their rationale for making this distinction

is the expectation that foreign-market oriented foreign firms exercise less competitive pressure on domestic firms, mitigating negative horizontal spillovers from domestic-market-oriented foreign firms. They also expect foreign-market-oriented foreign firms to have weaker vertical linkages and, consequently, produce smaller, if any, vertical spillovers. Their findings support these hypotheses [14]. No authors writing on FDI generated spillovers in Vietnam explicitly consider existing logistics networks, except for Machikita et al. who analyze process innovation in domestic firms in Hanoi through vertical linkages and exchange of professionals [30].

Another approach used in the literature to consider logistics networks is to distinguish foreign firms by investors' country of origin. Lin et al. make this distinction for China [10]. Although their division of foreign firms into two groups is rather arbitrary, since they do not confirm the rationale for such a classification empirically, they find different spillover effects for the two groups and explain this result by the difference in the two kinds of foreign firms' modes of production in the host country.

Qu et al. analyze existing international logistics networks in China and find that there are generally two modes of foreign firm organization, related to the FDI country of origin and industry [20]. FDI firms from newly developed Asian countries focus on parts processing labor-intensive production and have strong linkages with the local economy. They are export-oriented, contrary to the popular theorization in the literature that export-oriented firms maintain few linkages with the domestic economy. FDI enterprises from Europe and the US, in contrast, import capital goods from their home countries, operate in high tech industries, produce for the local market, and crowd out indigenous innovation through monopolized technology [20]. The authors also find that domestic firms are able to absorb knowledge from the first kind of enterprises but not from the second [20].

My data does not have information on foreign firms' countries of origin or their market orientation. My approach is to add to our model interaction terms between vertical spillover variables and regional dummies. In particular, I am interested in the dummy for the northern economic region of Vietnam. My assumption is that foreign firms located in the provinces bordering China are more likely than other foreign firms to source their inputs from China and export their output. Therefore, such firms are expected to develop weaker vertical linkages with the local economy and produce vertical spillovers smaller in size than vertical spillovers from foreign firms operating in other economic regions of Vietnam.

3.2 Correcting developed country assumptions

In this paper, I propose an alternative measure of domestic firms' absorptive capacity. Traditional measures of absorptive capacity—including R&D expenditure, firm ownership structure, firm size, geographical proximity, and human capital quality measured by an average wage bill—might be inappropriate in the context of a developing country.

Absorptive capacity includes four dimensions, such as firms' ability to recognize value of new knowledge, acquire it, assimilate, or transform it, and exploit it [31]. These four dimensions may be divided into realized and potential absorptive capacity, with R&D expenditure being a measure of the former. Jiménez-Barrionuevo et al. note that absorptive capacity, measured by R&D expenditure alone, is a measure that is "too simple" and "cannot reflect the richness of the construct in its totality" [31]. My claim is that R&D expenditure alone is an inappropriate measure not only because it can capture only one of the four dimensions of absorptive capacity generally accepted in the literature, but also because it fails to consider the developing country environment.

R&D expenditure is a measure of innovation inputs as opposed to innovation output, but it is also an imperfect measure of innovation inputs for firms in developing countries. Martinez-Piva points out that in poor economies, many companies spend little on purely R&D but devote significant efforts to imitative, adaptive, and incremental innovations [4]. In addition, Thoma et al. note that innovation in smaller firms is often associated with better differentiation of existing products rather than development of new ones [32]. They also note that many small firms engage in quality, efficiency, and process-related innovation that may not be considered by firms as purely R&D [32]. In Vietnam, most firms are small firms: they account for 99.9% of establishments [33].

Some studies attempt to correct this imperfect measure of firms' absorptive capacity. Fu, for example, proposes that R&D expenditure measure should also include expenditure on renewal of fixed assets, capital construction, new site construction, and related activities [22]. A study by Girma et al. measures firm's R&D by new product sales [34]. This is based on the view that a firm can increase its productivity without its own R&D through purchasing, for example, advanced technology or learning from foreign firms [34]. The study does not explain, however, how this learning can take place.

Whereas R&D expenditure is considered realized absorptive capacity, geographical proximity refers to the potential absorptive capacity (particularly, firms' ability to acquire new knowledge). This paper considers domestic firm location from two perspectives. The first perspective concerns how firm location affects its absorptive capacity with regard to demonstration effects. The second attempts to capture the impact of firm location on its ability to benefit from vertical spillovers. The literature does not distinguish clearly between these two aspects and lumps them together. The implicit assumption sometimes made is that firms located closer to TNCs geographically will automatically receive more spillovers through horizontal or vertical spillover channels, or both [25]. Yet it may be that these two aspects of domestic firm location act in opposite directions. This is possible, if, for example, being closer to TNCs means that the firm faces more competition in its industry but is also better able to benefit from foreign presence in upstream or downstream industries through vertical linkages.

In this paper, to situate local firms' absorptive capacity into a developing country context, I proxy firms' absorptive capacity using several variables. One set of variables measure "internal" absorptive capacity, that is, absorptive capacity directly related to firms' characteristics. I construct 1) two measures of firm's access to modern communication technology and 2) three measures of labor quality defined as total employee compensation divided by total labor, assuming that wages are consistent with labor quality; expenditure on personnel training defined as training budget divided by total labor; and the ratio of workers with higher education to the total number of employees and 3) technological gap. The other set measures "external" absorptive capacity and is

proxied by location in an industrial cluster. Location in a major industrial cluster will reflect firm's better ability to benefit from demonstration effects.

IV. DATA AND MEASUREMENT

4.1 Constructing panel data

I use the data collected by the General Statistics Office (GSO) of Vietnam in its annual Vietnam Enterprise Surveys (VES). I obtained the data from Department of Development Economics, University of Economics – Ho Chi Minh City. Since annual Vietnam Enterprise Surveys are national census data on all enterprises operating in Vietnam, I cannot form a pooled panel dataset from surveys from different years. I had to use either a cross-sectional dataset from some chosen year or merge the data from different years on a key variable to form a panel dataset.

Although I have data from VES 2000 through 2010, many of the data files are marred by serious problems and I cannot use them in my research. One of the problems that prevents me from creating a large panel and using the data from recent years is that starting in early 2000s, the GSO began to give enterprises identification numbers already in use, sometimes giving the same identification number to all branches of the same enterprise and sometimes to different unrelated enterprises at random. As a result, in 2006 survey data, for example, almost half of the identification numbers are duplicated. I believe that deleting the observations with duplicates might result in data biased towards enterprises with no branches (i.e., small scale firms), since it is not known and cannot be established from the data at my disposal what share of the duplicates are branches. For the years before 2007, however, each data file comes with enterprise tax codes, which I will use as enterprise identification numbers. Data files from the later years have enterprise codes too but only in the major data file for each year, making it impossible to merge data files from the same years.

Additional problems with the data include missing enterprise industry codes in data files for some years so that it is not possible to identify to what industry an enterprise belongs; missing key variables; and missing additional data files.

I will use data from the years 2004 and 2007 because only for these two years I am able to assemble complete datasets with

2-Digit industry code	Industry	Frequency	Percent	Cumulative
10	Manufacture of food products	140	0.34	0.34
11	Manufacture of tobacco products	0	0	0.34
12	Manufacture of beverages	4	0.01	0.35
13	Manufacture of textiles	213	0.52	0.87
14	Manufacture of wearing apparel	2,414	5.87	6.74
15	Manufacture of leather and related products	9,424	22.93	29.67
16	Manufacture of wood and of products of wood	40	0.10	29.76
17	Manufacture of pulp, paper and paperboard	1,537	3.74	33.50
18	Printing and service activities related to printing	2,648	6.44	39.95
19	Manufacture of coke and refined petroleum products	732	1.78	41.73
20	Manufacture of chemicals and chemical products	3,421	8.32	50.05
21	Manufacture of pharmaceuticals, medicinal chemical and botanical products	1,690	4.11	54.16
22	Manufacture of rubber and plastics products	2,617	6.37	60.53
23	Manufacture of other non-metallic mineral products	33	0.08	60.61
24	Manufacture of basic metals	1,701	4.14	64.74
25	Manufacture of fabricated metal products, except machinery and equipment	2,269	5.52	70.26
26	Manufacture of computer, electronic and optical products	3,415	8.31	78.57
27	Manufacture of electrical equipment	792	1.93	80.50
28	Manufacture of other machinery and equipment	4,911	11.95	92.45
29	Manufacture of motor vehicles; trailers and semi- trailers	1,178	2.87	95.31
30	Manufacture of other transport equipment	37	0.09	95.40
31	Manufacture of furniture	575	1.40	96.80
32	Other manufacturing	289	0.70	97.50
33	Repair and installation of machinery and equipment	131	0.32	97.82
35	Electricity, gas, steam and air conditioning supply	895	2.18	100.00

Table 1.

all the necessary variables, merge data files within each year on enterprise tax code; and merge the data files for 2004 and 2007 to form an unbalanced panel dataset.

4.2 Cleaning the data

In the process of cleaning the data, I delete firms that are joint ventures (and keep only 100% invested foreign firms and domestic firms); firms with zero output, labor, or inputs; and firms with missing industry codes.

I only keep the firms that belong to the 23 manufacturing industries (or are involved in the production of electricity and gas) in the Vietnam Standard Industrial Classification Table (VSIC) 2007 [35]. My data files contain enterprise codes at the 5-digit level. However, data files for the year 2004 contain old VSIC 1993 and new VSIC 2007 enterprise industry codes; I convert all industry codes to the 2-digit level using VSIC 2007 and keep the new VSIC 2007 code format. Although using 5-digit industry codes would make my model estimates more precise, I have to use codes at the 2-digit level to be able to convert old VSIC 1993 codes to the new 2007 format.

I also convert codes of enterprises' geographical locations into single format as GSO used different code formats for the years 2004 and 2007.

Gross industrial output is recorded in survey data in 1994 constant prices and all other values are recorded in current prices. I use Consumer Price Indices for Vietnam for the years 1996, 2004, 2007, and 2002 from the World Bank Indicators to convert all values to constant 2002 prices [36].

4.3 Summary statistics on enterprises in our panel

My data is an unbalanced panel dataset with 16,864 observations from 2004 and 24,242 observations from 2007. I note that 12,152 of enterprises, or 29.56% of enterprises in my dataset, are located in five major industrial cities.

Table 1 shows the distribution of enterprises in my dataset by industry.

4.4 Main model

In building my model and constructing my key variables, I follow conventions in literature as well as my critique in section III. The main model is as follows[1]:

$$\text{Log(output)}_{ijt} = B0 + \text{Log(capital)}_{ijt} + \text{Log(labor)}_{ijt} + \text{Log(inputs)}_{ijt} + \text{Herfindahl}_{jt} + \text{Scale}_{ijt} + \text{Tech\_gap}_{ijt} + \text{Capital\_int}_{ijt} + \text{Lab\_quality}_{ijt} + \text{Hor\_spill}_{jt} + \text{Back\_spill}_{jt} + \text{For\_spill}_{jt} + \text{regional dummies} + \text{industry dummies} + \text{year\_2004} + \varepsilon$$

V. EMPIRICAL FINDINGS

I estimate fixed effects and random effects models. I do not estimate the first difference model because I have only two time periods in my panel and in this case first difference model will be equivalent to fixed effects model. All models include regional and industry dummies, as well as a dummy for the year 2004. A star indicates significance at the 5% level.[2]

Dependent variable: log of real gross output of firm i in industry j in year t				
Variable	Fixed Effects	Fixed effects with robust standard errors	Random effects	Random effects with robust standard errors
Log(capital)				
Log(labor)				
Log(inputs)				
Herfindahl Index	-216.2196	-216.2196*	-.5484991	-.5484991
Scale	80.422	80.422*	8.03711*	8.03711*
Technological gap	-.0087346	-.0087346*	-.0001977*	-.0001977*
Capital intensity	-.012024	-.012024*	-1.41e-06	-1.41e-06
Labor quality	-.0528389	-.0528389*	.0619374*	.0619374*
Horizontal spillover	*154.2628*	*154.2628**	*-5.946195**	*-5.946195**
Forward spillover	*.0700303*	*.0700303**	*.0089363**	*.0089363**
Backward spillover	*-.2704031*	*-.2704031**	*-.0082365**	*-.0082365**
Hausman Test: Prob>chi2 = 0.0030				

Model 1. Number of observations: 13,552. We use this model as our basic model.

[1]**Editor's Note:** The chart of 'Key Variables' is available in the online addendum to this paper. Please visit http://www.stanford.edu/group/journal/cgi-bin/wordpress/?page_id=377 to view the chart.

[2]**Editor's Note:** The graphics of models 2-6 are available in the online addendum of this paper. Please visit http://www.stanford.edu/group/journal/cgi-bin/wordpress/?page_id=377 to view the tables.

VI. DISCUSSION

In Models 1 through 4, the Hausman test, which is often used to determine whether a fixed effects model or a random effects model better fits the data, indicates that a fixed effects model is preferred to the random effects one. All variables in my basic fixed effects model (Model 1) are significant at the 5% level. The Herfindahl index, scale, and technological gap have the expected signs, but labor quality and capital intensity variables have negative coefficients. One possible explanation for the negative capital intensity coefficient is that in Vietnam, most industrial enterprises are owned by the state, which is probably not very efficient in managing enterprises. Positive coefficient on the horizontal spillover indicates one of the following, in consistency with my discussion in section III: (1) greater intra-industry competition facilitates knowledge spillovers through, for example, rising industry standards; (2) presence of foreign firms in an industry means better allocation of resources; (3) transfer of skills from foreign to local firms occurs, yet it is unlikely given my discussion in section III; and (4) domestic firms are learning from foreign firms through demonstration effects. The positive coefficient on the vertical forward spillover variable means that either (1) domestic firms are learning from foreign firms, from whom they purchase intermediate inputs, through various cooperation mechanisms or (2) domestic firms are pressed to become more efficient by competition for contracts with foreign customers. The negative coefficient on backward vertical spillover variable means that foreign firms who purchase inputs from domestic firms probably take advantage of the competition on the local market.

In Model 2, I add an interaction term between horizontal spillover and labor quality, expecting that domestic firms with better quality labor are more likely to benefit from foreign firms' presence in the industry, for example, through demonstration effects. Yet I find that the coefficient on the interaction term is negative and insignificant. This might indicate that domestic firms do not benefit substantially through demonstration effects, possibly because foreign firms try to prevent knowledge spillovers to their competitors operating in the same industry.

In Model 3, I add an interaction term between horizontal spillover and scale. I expect that firms operating on a larger scale are better able to benefit from horizontal spillovers because they are, for example, better equipped to compete with foreign firms in the same industry. However, the coefficient on the interaction term is negative and significant. This might be because larger enterprises in Vietnam are owned by the government and are therefore less efficient than smaller, private enterprises.

In Model 4, I add an interaction term between horizontal spillover and firms' communication capacity. The underlying assumption is that firms' access to communication technologies is at the core of their internal absorptive capacity, as discussed in Section III. Consistent with this belief, I get a positive and significant coefficient on the interaction term.

In Model 5, I add an interaction term between horizontal spillover and location in a cluster. If a firm is located in a major industrial cluster, (1) it might be better able to learn from foreign firms through demonstration effects or labor mobility and (2) it might experience more competitive pressures from foreign firms operating in the same industry. Therefore, the coefficient on the interaction term will indicate which of the two effects outweighs the other. I find that the coefficient on the interaction term is insignificant.

In Model 6, I add interaction terms between vertical spillovers and location in the northern economic region as discussed in Section III. Here, Hausman test tells us that random effects model is preferred to fixed effects, but the coefficients on both interaction terms are insignificant in both fixed effects and random effects models.

As I add interaction terms to our basic main model, I note that the coefficient on the horizontal spillover variable is always positive and significant; the coefficient on the forward spillover variable is positive and significant in all models except for Model 6; and the coefficient on the backward spillover variable is always negative and significant in all models except for Model 6.

As demonstrated, there may be more than one explanation for the signs of coefficients on spillover variables. Thus, it is important to keep in mind all possible mechanisms at work when interpreting the coefficients. Sometimes, however, the positive or negative sign might suggest a particular channel at work.

In addition, even though my major proposition on the possible effects of international logistic networks on the sign of vertical spillovers remains unconfirmed, a better proxy for foreign firms' mode of industrial organization might produce different results.

Finally, the results also demonstrate that firms with stronger internal absorptive capacity (communication capacity) benefit more from horizontal knowledge spillovers. I deem that this measure of domestic firms' absorptive capacity in Vietnam is more appropriate than domestic firms' R&D expenditure, as discussed in section III.

VII. CONCLUSION

I find that the coefficients for the horizontal and the vertical forward spillovers are positive and the coefficient for the vertical backward spillover is negative. Rather than offering a single explanation for my findings, I claim that they might actually indicate several different mechanisms at work. Understanding local economy and the developing country context is necessary to know which mechanism produces the coefficients we observe.

I also note that in Vietnam knowledge spillovers might need to be measured differently due to the developing country context. For example, I use domestic firms' communication capacity as one of the measures of their ability to benefit from demonstration effects. As expected, I find that local firms with better communication capacity benefit more from horizontal knowledge spillovers. For another example, I claim that taking into account international logistics networks may show that the effects foreign firms have on the host economy actually vary with the ways those foreign firms organize their production. Although I find no direct proof of this proposition, I suggest that future research considers how different modes of foreign firms' industrial organization affect the linkages they develop with the local economy.

Most important, in this work I call for a new approach to studying knowledge spillovers from FDI in developing countries. The new approach would integrate both the need for new

econometric models, which fit the complexity of the different, developing country context, and the awareness of the multiplicity of knowledge spillover channels, which is important for the interpretation of findings.

VIII. FUTURE RESEARCH

First, Wei et al. point out that different measures of foreign presence may lead to different findings [24]. In this paper, I used the employment share of foreign owned enterprises when constructing my spillover variables. However, according to Wei et al., other possible FDI variables include output or value-added of FOE, capital or investment share of FOE, share of sales of FOE, and share of assets held by FOE. Thus, future research could estimate models for each of these FDI measures and compare the findings.

Second, using industry codes at the four-digit level will produce estimates that are more precise.

Third, a possible problem with my estimates involves endogeneity of input choices of enterprises. To solve endogeneity of input choices, future research should employ a semi-parametric estimation procedure (LP method) to estimate the production function and obtain consistent estimates of input coefficients, then derive total factor productivity at the firm level and use it together with new input coefficients in the main model.

Fourth, it might be that foreign firms choose to locate in industries with higher productivity. To solve this issue, future research could use an instrumental variables approach and add variables correlated with spillover variables but not the productivity of domestic firms. One example might be FDI variables for other Southeast Asian countries.

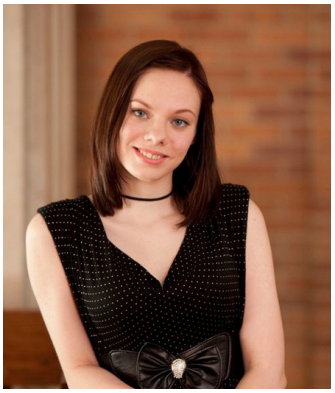
Fifth, it might be that backward and forward linkages take time to develop and all three kinds of horizontal effects take time to manifest themselves. Thus, future research could construct panel data with more time periods and estimate models with lagged spillover variables.

Finally, future research should investigate international logistics networks in Vietnam and search for patterns of foreign firms' industrial organization. Researchers could then utilize these new insights to distinguish among productivity spillovers from different kinds of foreign firms.

REFERENCES

1. Birinci Y. The role of effective protection of IP rights on economic growth. In: Aydogan N, ed. Innovation policies, business creation and economic development: a comparative approach. New York: Springer, 2009:51-61.
2. Wahab SA, Rose RC, Osman SIW. Exploring the technology transfer mechanisms by the multinational corporations: a literature review. Asian Social Science 2012; 8:142-150.
3. Krammer S. Assessing the relative importance of multiple channels for embodied and disembodied technological spillovers. Technological Forecasting & Social Change 2014; 81:272-286.
4. Martinez-Piva JM. Protection of intellectual property rights, innovation, and development. In: Martinez-Piva JM, ed. Knowledge generation and protection: intellectual property, innovation and economic development. New York: Springer, 2009:27-55.
5. Diaz A. Free trade agreements and intellectual property: impacts and challenges. In: Martinez-Piva JM, ed. Knowledge generation and protection: intellectual property, innovation and economic development. New York: Springer, 2009:117-150.
6. Caves RE. International corporations: the industrial economics of foreign investment. Economica/New Series 1971; 38:1-27.
7. Caves RE. Multinational firms, competition, and productivity in host country markets. Economica/New Series 1974; 41:176-193.
8. Statistical yearbook of Vietnam 2011. General Statistics Office, Hanoi, Vietnam.
9. Sheng Y, Xu XP. Productivity spillovers from foreign direct investment: firm-level evidence from China. World Development 2012; 20:62-74.
10. Lin P, Liu Z, Zhang Y. Do Chinese domestic firms benefit from FDI inflow? Evidence of horizontal and vertical spillovers. China Economic Review 2009; 20:677-691.
11. Suyanto RS, Bloch H. Does foreign direct investment lead to productivity spillovers? Firm level evidence from Indonesia. World Development 2009; 37:1861-1876.
12. Xu T, Zhao Z. What determines the intra-industrial technology spillovers of foreign direct investment? Economic Letters 2012; 116:562-564.
13. Crespo N, Fontoura MP. Determinant factors of FDI spillovers - what do we really know?" World Development 2007; 35:410-425.
14. Le HQ, Pomfret R. Technology spillovers from foreign direct investment in Vietnam: horizontal or vertical spillovers?" Journal of the Asia Pacific Economy 2011; 16:183-201.
15. Undén C. Multinational corporations and spillovers in Vietnam - adding corporate social responsibility. M.A. thesis, Inst of Econ, Lund Univ, Lund, 2007.
16. Nguyen NA, Nguyen T, Le DT et al. Foreign direct investment in Vietnam: is there any evidence of technological spillover effects? Development and Policies Research Center and Center for Analysis and Forecasting, Hanoi, Vietnam, Jan. 2008.
17. Anwar S, Nguyen LP. Absorptive capacity, foreign direct investment-linked spillovers and economic growth in Vietnam. Asian Business & Management 2010; 9:553-570.
18. Le QH, Pomfret R. Foreign direct investment and wage spillovers in Vietnam: evidence from firm level data. ASEAN Economic Bulletin 2010; 27:159-172.
19. Liu X, Chenggang W. Does foreign direct investment facilitate technological progress? Evidence from Chinese industries. Research Policy 2003; 32:945-953.
20. Qu T, Chen J, Li S et al. Impact of inward FDI, import on domestic innovation: evidence from China. International Journal of Business and Finance Research 2013; 7:119-136.
21. Tajul AM, Abdul HZ, Haslindar I. Spillover effects of FDI within manufacturing sector in Malaysia. Procedia - Social and Behavioral Science 2012; 58:1204-1211.
22. Fu X. Foreign direct investment, absorptive capacity and regional innovation capabilities: evidence from China. Oxford Development Studies 2008; 36:89-110.
23. Fan CS, Hu Y. Foreign direct investment and indigenous technological efforts: evidence from China. Econ Letters 2007; 96:253-258.
24. Wei Y, Liu X. Productivity spillovers from R&D, exports and FDI in China's manufacturing sector. Journal of International Business Studies 2006; 37:544-557.
25. Damijan JP, Rojec M, Majcen B et al. Impact of firm heterogeneity on direct and spillover effects of FDI: microevidence from ten transition countries. Journal of Comparative Economics 2013; 41:895-922.
26. Blalock G, Gertler PJ. How firm capabilities affect who benefits from foreign technology. Journal of Development Economics 2009; 90:192-199.
27. Sinani E, Meyer KE. Spillovers of technology transfer from FDI: the case of Estonia. Journal of Comparative Economics 2004; 32:445-466.
28. Ouyang P, Fu S. Economic growth, local industrial development and inter-regional spillovers from foreign direct investment: evidence from China. China Economic Review 2012; 23:445-460.
29. Giroud A. MNEs vertical linkages: the experience of Vietnam after Malaysia. Int Bus Review 2007; 16:159-176.
30. Machikita T, Thi Chi Binh T, Ueki Y. Technology transfer to Vietnam for process innovation through engineer exchanges under China plus one strategy: firm-level evidence. Develop and Policies Research Center, Hanoi, Vietnam, 2011.
31. Jiménez-Barrionuevo MM, García-Morales VJ, Molina LM. Validation of an instrument to measure absorptive capacity. Technovation 2011; 31:190-202.
32. Thomä J, Kilian B. To protect or not to protect? Modes of appropriability in the small enterprise sector. Research Policy 2012; 42:35-49.
33. Szirmai A, Naude WA, Goedhuys M. Entrepreneurship, innovation, and economic development: a study prepared for the World Inst for Develop Econ Research of the United Nations University (UNUWIDER) and Maastricht Econ and Social Research Inst on Innovation and Technology (UNU-MERIT)," Szirmai A, Naudé WA, Goedhuys M, Eds. Oxford:

Oxford Univ Press, 2011.
34. Girma S, Gong Y, Gorg H. Can you teach old dragons new tricks? FDI and innovation activity in Chinese state-owned enterprises. Inst for the Stud of Labor, Bonn, Germany, Aug. 2006, DP No. 2267.
35. List of Viet Nam Standard Industrial Classification 2007. General Statistics Office, Hanoi, Vietnam.
36. World Development Indicators 2013. World Bank.
37. Trinh B, Kobayashi K, Phong NV. Vietnam economic structure change based on input-output table (2000- 2007). Asian Economic and Financial Review 2012; 2:224-232.



Anna has recently graduated from Mount Holyoke College and has a long-standing interest in international development. She worked as a youth spokesperson with the International Federation of Red Cross and Red Crescent Societies. She is also a G(irls)20 Summit Ambassador for her country of origin, Russia. Anna initiated a number of community service projects with the International Research & Exchanges Board of the U.S. Department of State and Winrock International. Additionally, she served as an online columnist for the Voice of America Student Union. Recently, she has pursued independent research projects in Lalitpur, Nepal and Ho Chi Minh City, Vietnam.

engineering

Classification of Neocortical Neurons Using Unsupervised Learning Methods

Naureen Ghani, Rafael Yuste
Columbia University

ENGINEERING

Determining the circuitry of the neocortex requires an understanding of its components, making a classification of neocortical neurons necessary. The neocortex consists primarily of excitatory pyramidal neurons (~80% of neocortical neurons) and inhibitory interneurons (~20% of neocortical neurons). Though neocortical interneurons are ideally positioned to control circuit dynamics, they remain poorly understood. GABergic interneurons, in particular, largely contribute to the vast morphological and physiological variability of the cortex. A neuronal classification system is essential to organize such information and the knowledge that is derived from it. To better understand the diversity of neocortical neurons, this investigation used unsupervised learning methods to create classification schemes. First, PCA was used followed by k-means cluster analysis to classify data based on detailed anatomical and electrophysiological characterizations of 59 GFP-positive interneurons from a somatostatin-positive mouse line. Each neuron was characterized by whole-cell recordings done by patch-clamping and complete 3D anatomical reconstructions. Cluster analysis of morphological and physiological variables revealed 3 groups of cells: one comprised of Martinotti cells, the other two composed of short asymmetric axons targeting layers 2/3 and medial bending. It was revealed that each of these 3 interneuron subtypes is furthermore characterized by a unique set of morphological and electrophysiological features that may make them particularly suited for a specialized function within the neocortical circuit. Subsequently, the data set was expanded to include pyramidal neurons in addition to known interneuron subtypes. To perform a quantitative classification of this diverse set of 337 neocortical neurons, affinity propagation was used. Affinity propagation is an exemplar-based method of cluster analysis that takes a similarity measure of data points as input. It outputs a set of data points representative of the data (exemplars) and assigns all non- exemplar points to one of the exemplars, thus partitioning the data set into unique clusters.

Developmental neuroscience is an emerging field that encompasses powerful computational and mathematical techniques to model the brain. The aim of this study was to build a classification scheme of all neocortical neurons in a major effort to determine the circuitry of the neocortex. The neocortex is the top layer of the cerebral hemispheres and is about 2 to 4 mm thick. It is composed of six layers, labelled I to VI (with I being the outermost layer and VI being the innermost layer). The neocortex is one of three components of the cerebral cortex along with the archicortex and paleocortex. As it accounts for 76% of the volume of the cerebral cortex, the neocortex is one of the most significant components of the brain. It is involved in higher mental functions such as perception, memory, imagination, language, and music.

Since the neocortex participates in such a vast array of computational tasks, it is often compared to a parallel computer. Parallel computing is a form of computation in which many calculations are carried out simultaneously. The underlying principle of parallel computing is that large problems can be divided into smaller ones, which are then solved concurrently or "in parallel." In other words, many computing elements are wired together and then operate in parallel. For every computing element added, it is often necessary to add new wires from it to all the other computers. Thus, the number of wires increases much more rapidly than the number of computers: 3 wires for 3 computers, 6 wires for 4 computers, 10 wires for 5 computers, and so forth. By extending this analogy, a brain the size of a football field would be needed to encompass billions of neurons (computing elements) that are all wired together. In sharp contrast to this model, a real brain selectively wires neurons together, omitting any unneeded wires (axons) for proper function. This simple mathematical analogy hints at many aspects of the brain. For instance, as mammals grow larger with evolution, the brain similarly increases in size, and the "cost" of wires grows critical. Neuroanatomical studies have quantitatively shown that the number of axons varies greatly among different regions in the brain, where some areas are heavily linked to others with massive axon tracts, others are far less strongly linked.

Many strides have been made in understanding the formation and function of neocortical circuits. These circuits are based on detailed morphological and electrophysiological analyses of the circuit elements, or neurons. Each of these model circuits differ greatly in form and content, though, because they each have different theoretical and experimental considerations. For

historical reasons, many early neocortical circuit models were based on data from the cat primary visual cortex. In recent years, the rat somatosensory cortex has grown in prominence as a tool for understanding the cortex.

The idea of a canonical circuit has previously been applied to aspects of the vertebrate brain and spinal cord. It is highly relevant to questions of evolution, development, and homology of form and function. One illustration of this is the close similarities in basic organization across vertebrate brains. Similarly, recognizable neuronal subtypes have been found across the neocortices of different mammalian species (Ramon y Cajal 1911). Thus, it is hypothesized that a canonical cortical microcircuit may exist and this microcircuit implements a relatively simple computation.

To create a neocortical circuit model, a classification scheme must first be established to outline the components of the cortex. In the past, cell type classification was qualitative and led to inconsistent subtypes. In recent years, the standard method for classification of neurons has been quantified using unsupervised cluster analysis (Cauli et al., 1997; DeFelipe et al., 2013; Helmstaedter et al., 2009; Karagiannis et al. 2009; Karube et al., 2004; Ma et al. 2006; McGarry et al., 2010). Unsupervised cluster analysis is the classification of a set of data or objects into subsets (clusters) so that the data in each subset, ideally, shares a common feature without any prior knowledge. One popular technique has been Ward's method with hierarchical clustering. Hierarchical agglomerative clustering is a bottom-up technique. In other words, it begins by grouping the two "closest" cells as defined by the algorithm, and then continues to join the next "closest" cells and so forth. The number of clusters does not need to be specified in advance. Hierarchical clustering often generates smaller clusters that may be helpful for discovery. However, one of the main disadvantages of hierarchical clustering is that once two cells are linked, they remain joined together in the final hierarchy. Moreover, hierarchical clustering is susceptible to a chaining effect when objects may be "incorrectly" assigned to a cluster at an early stage instead of being grouped in new clusters. Additional clustering techniques include k- means analysis and k-metoids analysis. In k-means, each cluster is represented by the center of the cluster and in k-metoids analysis, each cluster is represented by one of the objects in the cluster. Affinity propagation is an innovative unsupervised clustering technique. Each data point is viewed as a node in a network, and real-valued messages are transmitted between the data points until a set of exemplars and corresponding clusters is determined by the algorithm. Thus, at any point in time, the magnitude of each message reflects the current affinity that one data point has for choosing another data point as its exemplar, which explains the name of "affinity propagation" (Frey and Deuck, 2007).

This investigation explored the application of affinity propagation to classification of neocortical neuronal subtypes. The algorithm was used to blindly classify a test dataset of four interneuron subtypes. The dataset included unlabeled cells in addition to known cells, the latter of which served as a ground truth. The dataset is comprised of 67 morphological variables and 20 electrophysiological variables describing parvalbumin-positive (PV+) basket cells (BC), PV+ chandelier cells (ChC), somatostatin-positive (SOM+) Martinotti cells (MC), and SOM+ non- Martinotti cells (non-MC) as previously described in (McGarry et al., 2010). Affinity propagation appears to generate an accurate classification in separating these four known interneuron subtypes and may be a powerful classification tool in discovering or defining neuronal cell types.

METHODS

Preparation of Brain Slices

Acute brain slices were prepared from Nkx 2.1, G42, or GIN mice, with an average of 15 postnatal days (range P13 – P25). Mice were immediately decapitated, the brain was removed and then immediately placed in a cold sucrose cutting solution (222 mM sucrose, 2.6 mM KCl, 27 mM $NaHCO_3$, 1.5 mM NaH_2P_4, 0.5 mM $CaCl_2$, 3 mM $MgSO_4$, bubbled with 95% O_2, 5% CO_2). Coronal slices of 300 μm thickness were cut using a Vibratome and then transferred to a holding chamber at room temperature with oxygenated ACSF (126 mM NaCl, 3 mM KCl, 3 mM $MgSO_4$, 1 mM $CaCl_2$, 1.1 mM NaH_2PO_4, 26 mM $NaHCO_3$, and 10 mM dextrose, bubbled with 95% O_2, 5% CO_2). The slices were left to equilibrate with the room temperature for at least 30 minutes. Slices were then transferred to a recording chamber with the perfusion of ACSF bubbled with 95% O_2, 5% CO_2.

Transgenic Mouse Lines

Three transgenic mouse lines were used to identify different types of interneurons. First, the G42 line that labels PV+ cells was used(Chattopadhyaya et al., 2007). PV+ cells are rapid spiking interneurons with basket or ChC morphology. Basket cells are distinguished from chandelier cells by their distinctive morphologies and threshold spiking responses. In addition, the chandelier cells have specially shaped axon arbors, where the axon terminals for distinct arrays called "cartridges" that can be visualized with the illumination of GFP (Woodruff et al., 2009). The Nkx2.1 line labels a population of interneurons that express the transcription factor Nkx 2.1, which includes interneurons that migrate from the medial ganglionic eminence (MGE), most notably ChCs (Xu et al., 2008). A significant proportion of the ChC cells were found at the top of layer II, close to the layer I border, in both the G42 and Nkx 2.1 lines (Woodruff et al., 2009). Finally, the GIN line was used to label SOM+ cells (Oliva et al., 2000). SOM+ cells are regular spiking interneurons with diverse morphology. In previous work, three unique subtypes of SOM+ interneurons in GIN mice were determined based on morphology and physiology: Martinotti cells and two novel subtypes (McGarry et al., 2010). As a result, Martinotti cells (MC) and two novel subtypes (non-MC) are now distinguished.

Electrophysiology Recordings

Brain slices were placed in a recording chamber at room temperature with a constant supply of oxygenated ACSF. Pipettes of 3-7 MΩ resistance were pulled from borosilicate glass. Whole cell recordings of cells were obtained using patch-clamping. Only cells with a healthy resting membrane potential (between -55 and -80 mV) were selected for recording.

Electrophysiological Analysis

20 variables were measured for each neuron by analysis of the recordings in MATLAB. The Petilla terminology scheme was used to name each variable describing firing and passive properties (Ascoli et al., 2008).

Histological Procedure

Neurons were filled with biocytin by a patch pipette. Slices were kept overnight in 4% formaldehyde in 0.1M phosphate buffer (PB) at 4°C. Slices were then rinsed three times for five minutes per rinse on a shaker in 0.1M PB. They were then placed in 30% sucrose mixture (30g sucrose dissolved in 50 ml ddH2) and 50 ml 0.24M PB per 100 ml) for 2 hours and then frozen on dry ice in tissue freezing medium. The slices were kept overnight in a -80°C freezer. The slices were defrosted. Three twenty minute rinses in 0.1M PB were done to remove tissue freezing medium. Slices were kept in 1% hydrogen peroxide in 0.1M PB for thirty minutes to pretreat the tissue. They were then rinsed twice in 0.02M potassium phosphate saline (KPBS) for twenty minutes. Afterwards, the slices were kept overnight in Avidin-Biotin-Peroxidase Complex. The slices were next rinsed three times in 0.02M KPBS for 20 minutes each. Each slice was then placed in DAB (0.7 mg/ml 3,3"- diaminobenzidine, 0.2 mg/ml urea hydrogen peroxide, 0.06M Tris buffer in 0.02M KPBS) until the slice turned a pale brown, and immediately transferred to 0.02M KPBS and transferred again to 0.02M KPBS for 20 minutes. The stained slices were rinsed in 0.02M KPBS for one final time. Each slice was observed under a light microscope and then mounted onto a slide using crystal mount.

Three-Dimensional Neuronal Reconstruction and Morphological Analysis

Three-dimensional reconstructions of successfully filled and properly stained neurons were done using Neurolucida software (MicroBrightField). The neurons were viewed with a 100x oil objective on an Olympus BX51 upright light microscope. Differential interference contrast (DIC) microscopy was employed to see otherwise invisible features of the sample. The neuron's processes were traced manually while the program recorded the coordinates of the tracing, thus creating a three-dimensional reconstruction. In addition to the neuron, the pia and white matter were drawn. The Neurolucida Explorer program was used to measure 67 morphological variables of the reconstruction describing somatic, dendritic, and axonal properties.

Affinity Propagation

Affinity propagation is a clustering algorithm based on "passing messages" between data points (Frey and Dueck, 2007). It is an innovative technique that aims to combine the advantages of affinity-based clustering and model-based clustering. Affinity propagation is similar to k- metoid clustering in that both algorithms output "exemplars" or representative data points that serve as centers of the clusters. Unlike k-metoid clustering, the exemplars are not chosen randomly in affinity propagation. Affinity propagation takes an input of real-valued similarities between data points, where the similarity *s(i,k)* indicates how appropriate a data point is to serve as an exemplar for data point *i*. Negative Euclidean distance is used to measure similarity to minimize squared error: for points x_i and x_k, $s(i,k) = -||x_i - x_k||^2$. The key advantage of affinity propagation is that it does not require the number of clusters to be specified prior to using the technique as *k-means* cluster analysis does. Instead, affinity propagation takes as input a real number *s(k,k)* for each data point *k*, such that data points with larger values of *s(k,k)* are more likely to be selected as exemplars.

There are two types of messages exchanged between data points. The first type is called the "responsibility" *r(i,k)*, sent from data point *i* to candidate exemplar point *k*, and represents how well-suited point *k* is to serve as the exemplar for point *i*, while considering all other potential exemplars for point *i*. The second type is called the "availability" *a(i, k)*, sent from candidate exemplar point *k* to point *i*, and reflects the compiled evidence for how appropriate it would be for point *i* to choose point *k* as its exemplar, taking into account the support from other points that point *k* should be an exemplar. *r(i,k)* and *a(i, k)* can be viewed as log-probability ratios. Initially, the availabilities are initialized to zero: $a(i, k) = 0$. Then, the responsibilities are computed as:

$$r(i,k) \leftarrow s(i,k) - \max' k_{k'|k' \neq k}\{a(i,k') + s(i,k')\}$$

Availabilities will eventually fall below zero as points are assigned to other exemplars. This will decrease the effective values of the input similarities, removing candidate exemplars from the competition.

Whereas the competition is data-driven for responsibilities and all the candidate exemplars compete for the ownership of a data point, the availability update gathers evidence from data points as to which candidate exemplar would make a good exemplar. The availability *a(i,k)* is set to the self-responsibility plus *r(k, k)* plus the sum of the positive responsibilities candidate exemplar *k* receives from other points:

$$a(i,k) \leftarrow \min\left\{0, r(k,k) + \sum_{i' \neq i,k} \max\{i, r(i',k)\}\right\}$$

Self-availability *a(k, k)* reflects evidence that *k* is an exemplar based on positive responsibilities sent to candidate exemplar *k* from other points:

$$a(k,k) \leftarrow \sum_{i' \neq k} \max\{0, r(i',k)\}$$

To evaluate the quality of a clustering produced by affinity propagation, two aspects were considered: (1) the number of points that are correctly classified and (2) the number of clusters. With affinity propagation, there is the assumption that the identities of the exemplars are known, so the number of correctly classified points may artificially increase. Therefore, the classification accuracy is computed as the ratio between correctly classified points (excluding the exemplars) and the total number of points (excluding the exemplars). In addition, the number of clusters is preferred to be only a few.

RESULTS

Database of three known interneuron subtypes

This investigation used affinity propagation to classify neocortical interneurons based on their morphological and physiological properties. A dataset where the identities of the neurons were known from previous studies was used to test the affinity propagation algorithm (McGarry et al., 2010; Packer and Yuste, 2011; Woodruff et al., 2011). More specifically, a physiology database that contained 337 interneurons distributed as: 57 somatostatin-positive cells (SOM+), 87 chandelier cells (ChC), 193 parvalbu-

min-positive cells (PV+) was used. The morphology database consisted of 111 interneurons distributed as: 24 ChC, 55 SOM+ and 32 PV+. Lastly, there were 51 neurons in a database that consisted of both morphology and physiology variables, formed by an intersection of the two databases. Its distribution was: 12 PV+, 16 SOM+ and 23 ChC.

Affinity propagation classification of interneuron morphologies

The analysis of the morphology database resulted in 2 clusters. The first cluster consists of 84 neurons while the second cluster had 27 neurons. The first cluster had an exemplar of member 42, a SOM+ interneuron. It consists of: 22 ChC, 32 SOM+ and 30 PV+. Even though this cluster has an exemplar of SOM+ subtype, this cluster encompasses all the PV neurons with an accuracy of 93.75%. However, it only classifies 26.19% of the SOM+ interneurons correctly. The second cluster consists of: 2 CC, 23 SOM+ and 2 PV+. Its exemplar is member 83, a SOM+ neuron. Thus, 85.9% of the SOM+ neurons were correctly classified. This suggests that the second cluster with an exemplar of member 83 is truly representative of SOM+ neuronal subtype while the first cluster is representative of the PV subtype.

Affinity propagation classification of interneuron physiologies

The analysis of the physiology database revealed 3 distinct clusters. The first cluster consisted of 57 SOM+ neurons with an exemplar of member 21, also a member of the SOM+ subtype. Hence, 100% of the SOM+ neurons were correctly classified by the affinity propagation algorithm. The second cluster consisted of 8 PV+ and 87 CC neurons, where the exemplar was a part of the CC class (member 322). It correctly classified 90.80% of the CC neurons. The third cluster consisted of 142 CC and 51 PV+ and had an exemplar of 326, a member of the PV class. While the exemplar was representative of the PV+ subtype, only 26.42% of the PV+ neurons were correctly classified.

Affinity propagation classification of interneuron joint databases

The analysis of the interneuron joint database resulted in 2 clusters. The first cluster consisted of: 6 PV, 8 SOM, and 17 CC. The exemplar was member 4, a member of the PV subtype. It correctly classified a mere 19.37% of PV neurons. The second cluster consisted of: 6 PV, 8 SOM, and 6 CC. The exemplar was member 38, a member of the CC subtype. Only 30% of the CC neurons were correctly classified.

DISCUSSION

Affinity propagation: An Exploratory Tool for Classification of Neural Data

This study has explored the use of a new algorithm, affinity propagation, for the classification of neuronal data using a database of 337 neocortical GABAergic interneurons. The interneurons that had been previously known to belong to identified cell subtypes served as a ground truth, and acted as a measure for how accurate the algorithm was. The data was based on a collection of morphological and physiological data for each neuron. The classification accuracy found was 0.56 for the Physiology database, 0.45 for the Morphology database, and 0.40 for the combined Morphology + Physiology database. The accuracy consistently decreased with a smaller data set, containing less information on neurons. The affinity propagation algorithm is able to clearly distinguish somatostatin neurons as a unique class consistently among all three databases. However, the real trouble arises when the algorithm is asked to differentiate between chandelier cells and parvalbumin cells. In the Morphology database and the combined database, the cells were grouped into a single cluster consisting of large components of both chandelier cells and parvalbumin cells as opposed to two distinct clusters. After simplifying the database to include only chandelier cells and parvalbumin cells, thus excluding somatostatin-positive cells, the affinity propagation algorithm is able to separate the cells into two clusters but with low accuracy (< 0.50 for Physiology Database, the largest database was used).

The inability of the affinity propagation algorithm to separate chandelier cells and parvalbumin cells may be due to a number of potential reasons. One reason is that the chandelier cells and parvalbumin cells are closely similar to each other based on morphology and physiology. Recent research shows that chandelier neurons or chandelier cells are a subset of GABA-ergic cortical interneurons that are said to be parvalbumin-containing and fast-spiking to distinguish them from other GABAergic neurons, when done by immunostaining. Even though chandelier cells are truly distinct from other GABAergic neurons based on morphology with their unique axonal arbors, the affinity propagation algorithm correctly picks up this assumption. This may also be explained by potential sources of error. With a smaller dataset, the affinity propagation algorithm greatly decreased in accuracy. An additional error found in the dataset was that some of the measurements of input resistance were incorrectly normalized. This may also be explained by potential sources of error. With a smaller dataset, the affinity propagation algorithm greatly decreased in accuracy. An additional error found in the dataset was that some of the measurements of input resistance were incorrectly normalized. Resistance values less than 1 are reported in Giga Ohms while resistance values greater than 20 are reported in Mega Ohms.

Despite the moderate success of affinity propagation algorithm, there is insufficient evidence to reject it as an exploratory tool for neuronal classification. With a large enough dataset with a much greater number of interneuron subtypes, there may be improved classification by affinity propagation. Such a dataset is in progress. Manipulation of the Windows Application Programming Interface (API) may allow automatation of the extraction of morphological data from Neuroexplorer. Currently, the clicks necessary to obtain the data of 67 morphological variables from a single neuron has been automated but further work is required to implement this for the entire extraction process. The goal is to create a dataset encompassing an estimated 1000 neurons of diverse interneuron subtypes, each characterized by an anatomical 3D reconstruction and whole-cell patch-clamp recording.

One great issue with classification schemes of neocortical neurons is that many markers, often transcription factors, exist to label interneuron subtypes while there are few known markers to label pyramidal neurons. Moreover, pyramidal neurons account for a much larger percentage of the neocortex compared to interneurons (~ 4x as much). The next step would be to create an algorithm that separates pyramidal neurons from interneurons

and will continue to research ways to classify pyramidal neurons. Affinity propagation and other unsupervised learning methods serve as exploratory tools to build such neuronal classification schemes.

APPENDIX

Appendix Variable	Description
Rheobase (pA)	Threshold current
Resting membrane potential (mV)	Stable membrane potential when no current applied
AP1 amplitude (mV)	Amplitude of the 1st action potential (AP)
AP1 duration (ms)	Time from onset of 1st AP, calculated as an increase >1 mV/100 ms, to offset, calculated as return to same voltage as before AP onset
AP1 half-width (ms)	Time from half-amplitude during rise to half-amplitude during fall of 1st AP
AP1 rise time (ms)	Time from onset to peak of 1st AP
AP1 fall time (ms)	Time from peak to offset of 1st AP
AP1 rise rate (mV/ms)	AP1 amplitude / AP1 rise time
AP1 fall rate (mV/ms)	AP1 amplitude / AP1 fall time
AP drop (mV)	AP1 amplitude - AP2 amplitude
Spike frequency adaptation	t2/t1, where t1 is the time between the peaks of the first two APs (interspike interval) and t2 is the last interspike interval
Index of Accommodation	Average of the difference in length of two consecutive interspike intervals normalized by the summed duration of these two interspike intervals

Table 1. Electrophysiological Variables. Action potential properties measured from response to twice threshold, 500-ms current injection from first action potential (AP1) and second action potential (AP2). AP2 variables not listed as the same measurements were made for AP2 as listed for AP1.

REFERENCES

1. Ascoli, G. A., Alonso-Nanclares, L., Anderson, S. A., Barrionuevo, G., Benavides-Piccione, R., Burkhalter, A., et al. (2008). Petilla terminology: nomenclature of features of GABAergic interneurons of the cerebral cortex. Nat. Rev. Neurosci. 9, 557–568. doi: 10.1038/nrn2402
2. Cauli, B., Audinat, E., Lambolez, B., Angulo, M. C., Ropert, N., Tsuzuki, K., et al. (1997). Molecular and physiological diversity of cortical nonpyramidal cells. J. Neurosci. 17, 3894–3906.
3. Chattopadhyaya, B., Di Cristo, G., Wu, C. Z., Knott, G., Kuhlman, S., Fu, Y., et al. (2007). GAD67-mediated GABA synthesis and signaling regulate inhibitory synaptic innervation in the visual cortex. Neuron 54, 889–903. doi: 10.1016/j.neuron.2007.05.015
4. DeFelipe,J., López-Cruz,P., Benavides-Piccione,R., Bielza,C., Larrañaga,P., Anderson,S.,et al.(2013).New insights in the classification and nomenclature of cortical GABAergic interneurons. Nat. Rev. Neurosci. 14, 202–216.doi: 10.1038/nrn3444
5. Douglas, R.J., and Martin, K.C. (2004). Neuronal Circuits of the Neocortex. Annual Reviews of Neuroscience 27, 419-451 doi: 10.1146/annurev.neuro.27.070203.144152
6. Frey, B. J., and Dueck, D. (2007). Clustering by passing messages between data points. Science 315, 972–976. doi: 10.1126/science.1136800
7. Helmstaedter, M.,Sakmann,B., and Feldmeyer,D.(2009). L2/3 Interneuron groups defined by multiparameter analysis of axonal projection, dendritic geometry, and electrical excitability. Cereb.Cortex 19, 951–962. doi:10.1093/cer- cor/bhn130
8. Karagiannis, A., Gallopin, T., David, C., Battaglia, D., Geoffroy, H., Rossier, J., et al. (2009). Classification of NPYexpressing neocortical interneurons. J. Neurosci. 29, 3642–3659. doi: 10.1523/JNEUROSCI.0058-09.2009
9. Karube, F., Kubota, Y., and Kawaguchi, Y. (2004). Axon branching and synaptic bouton phenotypes in GABAergic nonpyramidal cell subtypes. J. Neurosci. 24, 2853–2865. doi: 10.1523/JNEUROSCI.4814-03.2004
10. Liu, J.H., Hansen, D.V., Kriegstein, A.R., Development and Evolution of the Human Neocortex. Cell. 2011 July 8. 146(I): 18-36. doi: 10.1016/j.cell.2011.06.030
11. Ma,Y.,Hu, H.,Berrebi,A.S., Mathers,P.H., and Agmon,A.(2006). Distinct subtypes of somatostatin-containing neocortical interneurons revealed in trans- genic mice. J. Neurosci. 26, 5069–5082. doi:10.1523/JNEUROSCI.0661-06.2006
12. McGarry, L. M., Packer, A. M., Fino, E., Nikolenko, V., Sippy, T., and Yuste, R. (2010). Quantitative classification of somatostatin-positive neocortical interneurons identifies three interneuron subtypes. Front. Neural Circuits 4:12. doi: 10.3389/fncir.2010.00012
13. Packer, A. M., and Yuste, R. (2011). Dense, unspecific connectivity of neocortical parvalbumin-positive interneurons: a canonical microcircuit for inhibition. J. Neurosci. 31, 13260–13271. doi: 10.1523/JNEUROSCI.3131-11.2011
14. Oliva, A. A. Jr., Jiang, M., Lam, T., Smith, K. L., and Swann, J. W. (2000). Novel hippocampal interneuronal subtypes identified using transgenic mice that express green fluorescent protein in GABAergic interneurons. J. Neurosci. 20, 3354–3368.
15. Ramon y Cajal S. 1911. Histologie du Systeme Nerveux de l'Homme et des Vertebres. Vol. 2. Paris: Maloine
16. Taniguchi H, Lu J, Huang ZJ (January 2013). "The Spatial and Temporal Origin of Chandelier Cells in Mouse Neocortex". Science 339 (6115) doi:10.1126/science.1227622. PMID 23180771.
17. Woodruff, A., Xu, Q., Anderson, S. A., and Yuste, R. (2009). Depolarizing effect of neocortical chandelier neurons. Front. Neural Circuits 3:15. doi: 10.3389/neuro.04.015.2009

Naureen is a third-year student at Columbia University studying Biomedical Engineering. She has worked directly under the mentorship of Professor Rafael Yuste at Columbia University, a facility participating in the BRAIN Initiative under President Barack Obama. She is from Brooklyn, NY and loves to paint watercolors during her free time.

A Novel EZH2 Histone Methyltransferase Inhibitor: Potential Advancement in Epigenetic Cancer Therapy

Aashna Mago
Stanford University

Enhancer of Zeste Homolog 2 (EZH2) is a histone methyltransferase that di- and trimethylates Lysine-27 on histone H3 (H3K27). This silences key tumor suppressor and differentiation genes, promoting unrestricted cell proliferation and thus maintaining stem/progenitor cells. However, extensive literature links over-expressed EZH2 to development and invasiveness of many cancers. Despite an urgent need, no direct inhibitors of EZH2 have existed to normalize EZH2 levels without interfering with other, essential methylation.

SQ037, a new synthetic peptide that in vitro is a direct inhibitor of EZH2, was evaluated during in-nucleo reactions. Baseline methylation in HeLa cell culture was labeled before the nuclei were isolated. Isolated nuclei could not control histone methylation, and "light," exogenous S-adenosyl methionine, EZH2's cofactor, was added to over-stimulate the enzyme, modeling the excess, uncontrolled activity found in cancers. Nuclei were divided into three samples; SQ037 was added into one, a scrambled sequence control peptide into another. Mass spectrometry distinguished preexisting, "heavy" from new, "light" methylation, which occurred after the addition of SQ037 or a control peptide. Reverse-phase high performance liquid chromatography and tandem mass spectrometry were used to identify and quantify peptide species with different numbers of heavy/ light methyl groups.

SQ037 significantly reduced new H3K27 di- and trimethylation in this cell-based setting compared to the control peptide indicating both efficacy and specificity in inhibiting EZH2. Importantly, SQ037 did not also inhibit histone methylation at other, unrelated sites. Inhibition of EZH2 by SQ037 shows promise for further evaluation as a novel epigenetic treatment for cancer.

Epigenetic changes are stable, heritable changes in gene expression other than those caused by mutations in DNA sequence. Post-translational modifications (PTMs) to the N-terminal tails of histone proteins have recently been recognized as important mechanisms of epigenetic regulation [1], [2]. They control gene expression by establishing chromatin configurations in specific cells [3] and by increasing or decreasing nearby chromatin's affinity for transcription factors and other chromatin-associated proteins [4]. Histone PTMs facilitate essential processes such as cellular differentiation, but also contribute to the aberrant cell behavior seen in various diseases, in particular, cancers [5]–[7]. Thus, epigenetic mechanisms such as histone PTMs have the potential to explain phenomena that genetics alone could not account for [3].

This study focuses on a protein called the Enhancer of Zeste Homolog 2 (EZH2), which is the SET-domain-containing catalytic subunit of the Polycomb repressive complex 2 (PRC2) and di- and trimethylates Lysine-27 on histone H3, or H3K27 [8], [9]. This methylation of H3K27 represses the transcription of surrounding chromatin, which can result in X- chromosome inactivation as well as the silencing of homeotic, differentiation, and tumor suppressor genes [10]—[12]. EZH2 is responsible for maintaining stem cells and their ability, along with that of adult pluripotent[1] and progenitor cells, to self-renew [10], [13], [14]. Overexpression of EZH2, however, is thought to directly promote tumor formation and oncogenesis via hypermethylation of H3K27 as well as the recruitment and overstimulation of other enzymes that work with EZH2 to silence tumor suppressor and differentiation genes [11], [15]—[18]. Excess EZH2 has been associated with the silencing of more than 100 genes in prostate cell lines, including several important tumor suppressors, and was found to be the single most up- regulated gene in metastatic compared to clinically localized prostate cancer [19]. Many other types of cancerous tissues, in the liver, lungs, breasts, etc., have been found to contain abnormally high levels of EZH2 compared to the corresponding normal tissues, and overabundant EZH2 has been linked to the invasiveness of breast and prostate cancers [20]—[22]. This makes inhibition of EZH2 an important strategy

[1] Pluripotent cells are stem cells that have the ability to differentiate into cells from any of the three germ layers (endoderm, mesoderm, and ectoderm).

to focus on for treatment of many cancer types.

While the role of EZH2 in cancer and its interactions with H3K27 and with other enzymes are well characterized, there have not been any successful attempts thus far to down-regulate excessive EZH2 activity for the purpose of a drug treatment. While over-expression of EZH2 leads to uncontrolled cell proliferation and tumor invasion, moderate amounts of EZH2 are necessary for key biological functions. Thus, restoring the enzyme's activity to normal levels using an inhibitor could lead to the development of a novel drug treatment. EZH2's histone methyltransferase activity is limited to one site [12], H3K27. Hence, direct inhibition of the enzyme when it is overactive should not disrupt normal biological processes, making it an especially suitable target for epigenetic therapy. Until recently, however, most attempts to implement small molecule inhibitors of EZH2 were successful as they blocked EZH2 activity by indirect mechanisms, thus interfering with normal methylation events [23], [24]. For example, a previously used inhibitor called deazaneplanocin A (DZNep) prevents hydrolysis of S-adenosylhomocysteine (Ado-Hcy), consequently blocking the activity of all SAM-dependent methyltransferases [24]. Thus, while DZNep was fairly effective at inhibiting EZH2, it was impractical because it interfered with essential, normal methylation as well. Studies show that DZNep may be used, often in combination with other drugs, to treat non-small cell lung cancers, medulloblastoma tumors, acute myelodia leukemia and possibly other cancers in the lab [25]—[28].

After studying EZH2's structure and catalytic properties, a collaborating lab produced a synthetic peptide inhibitor called SQ037 designed to bind specifically to EZH2 and shown in vitro to inhibit the enzyme's activity as a histone methyltransferase because it has a significantly higher binding affinity to EZH2 than the histone H3 peptide [29]. This investigation aimed to confirm the efficacy of SQ037 in nuclei harvested from mammalian cells in order to assess its future potential for epigenetic therapy.

Testing the inhibitor on native EZH2 in the nucleus was important for several reasons. Firstly, EZH2 activity is greatly enhanced when histone H1 and several other PRC2 subunits are present [8], [12]. Secondly, it was important to ensure that SQ037 did not disrupt other methylation reactions required for normal cellular functions [30]—[33]. It was predicted a) that use of SQ037, a direct inhibitor of EZH2, would substantially reduce levels of H3K27 di- and trimethylation in conditions where EZH2 overexpression or up-regulation was simulated, b) that this inhibition would be specific to SQ037 (compared to a control peptide), and c) that SQ037 would not affect methylation at other histone sites.

MATERIALS AND METHODS

Overview of the Experiment

Methyl groups are transferred by EZH2 to the H3K27 substrate from a cofactor called S-(5'Adenosyl)-L-methionine (SAM) [34], [35]. To differentiate between pre-existing methylation and methylation occurring in the presence of the inhibitor, heavy methyl Stable Isotope Labeling by Amino Acids in Cell Culture (SILAC) was used. As shown in Fig. 1, HeLa cells were first grown in "heavy M" medium, which was methionine-depleted and supplemented with L-methionine-methyl-13CD3 ("heavy" methionine; Sigma-Aldrich, St. Louis, MO, USA). SAM produced by these cells and incorporated as methyl groups added in vivo

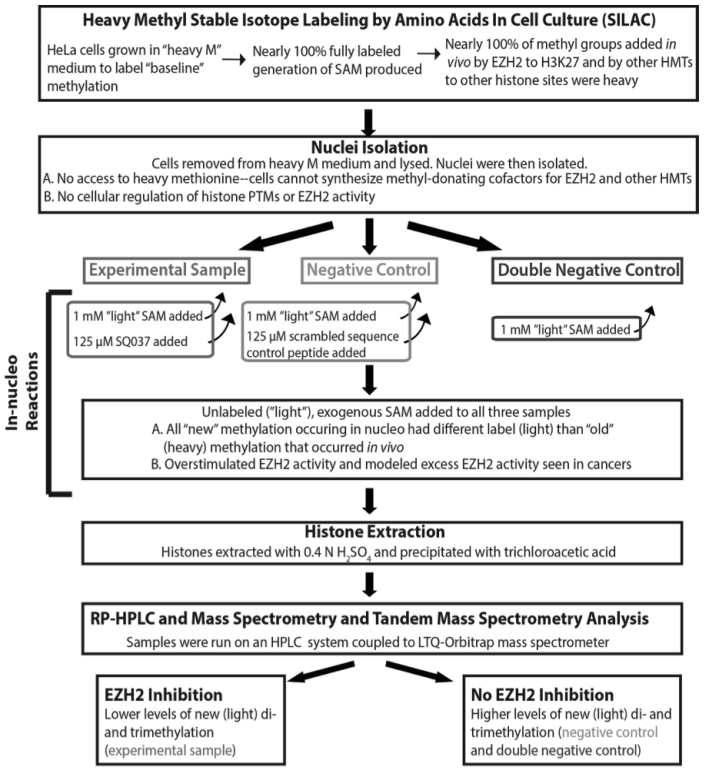


Figure 1. Methods flowchart

by EZH2 to H3K27 was nearly 100% heavy. In fact, almost all histone methylation that occurred in this cell culture was heavy-labeled, because almost all methyl-donating cofactors originated from the heavy-labeled methionine added to the medium [36]. The +18 Dalton (Da) shift conferred by each heavy methyl group added in vivo was used as a label for "old" methylation present before the addition of SQ037.

The nuclei were then isolated, and unlabeled, exogenous, "light" SAM was added to them during "in-nucleo" reactions [37]. The nuclei were divided into: 1) an experimental sample containing light SAM and the experimental inhibitor, SQ037; 2) a negative control containing light SAM and a scrambled sequence control peptide; and 3) a double negative control containing only light SAM (Fig. 1). The light SAM was added to the nuclei when they were no longer in the heavy M medium and had no means of synthesizing their own methyl-containing cofactors to donate methyl groups to EZH2 and other histone methyltransferases. This new methylation was identified by a +14 Da shift per methyl group.

Excess di- and trimethylation of H3K27 could be expected in the presence of a substantial quantity of SAM, EZH2's methyl donor, because the isolated nuclei lacked normal cellular regulation of enzymatic activity. The in-nucleo reactions mimicked the increased EZH2 activity that is found in cancer cells. The amounts of preexisting, heavy methylation and new, light methylation were measured using quantitative mass spectrometry, and their proportions were compared in the three samples (Fig. 1).

Two initial biological replicates from the lab found promising results, but the objective was to conduct a more definitive experiment, optimizing procedures and analyses. Statistical analyses were then conducted on the combined data set.

Cell Culture

HeLa cells grown in "heavy M" minimum essential modified Joklik's medium (ThermoScientific HyClone, Logan, UT, USA) with $^{13}CD_3$-methionine added as a reagent to label histone methylation occurring in the cells. The culture was maintained and harvested as previously described [38]. Harvested cells were washed in phosphate-buffered saline, flash-frozen, and stored at -80ºC.

Isolation of Nuclei

HeLa cells grown in "heavy M" minimum essential modified Joklik's medium (ThermoScientific HyClone, Logan, UT, USA) with $^{13}CD_3$-methionine added as a reagent to label histone methylation occurring in the cells. The culture was maintained and harvested as previously described [38]. Harvested cells were washed in phosphate-buffered saline, flash-frozen, and stored at -80ºC.

In-Nucleo Reactions

All preparation for the in-nucleo reactions was done at 4ºC. "NM" buffer was prepared based on the recipe suggested by Fischle [37], except that 1mM stable S-(5'-Adenosyl)-L- methionine chloride (Sigma-Aldrich) was substituted for radioactive SAM [37] and protease inhibitors (200 µM AEBSF and 10 mM sodium butyrate) were added. See Appendix for full in-nucleo protocols used.

Histone Extraction

The nuclei were thawed immediately, and acid extraction of the histones followed as described by von Holt et al. [39]. Briefly, the histones were extracted from the nuclei with 0.4 N H_2SO_4 and precipitated with trichloroacetic acid. They were then washed in acetone to remove any salts that could interfere with the subsequent trypsin digestion [40], air-dried, and resuspended in water.

Histone Preparation for Mass Spectrometry Analysis

50 µg of histone from the bulk extract allocated for later mass spectrometry (MS) analysis. Two rounds of propionic anhydride derivatization were performed as per Garcia et al. [41] with the exception that the reagent was prepared with a 3:1 ratio of isopropanol to propionic anhydride (Sigma-Aldrich) rather than 3:1 methanol:propionic anhydride. Propionic anhydride derivatization blocked digestion by trypsin at lysines and neutralized the charges at N-termini and at unmodified and monomethylated residues. After propionylation, the histones were incubated with trypsin at 37ºC as previously described [40], but at a substrate:enzyme ratio of 10:1 rather than 20:1. Two additional rounds of propionylation were performed to neutralize the charges at newly created N-termini, making the histone peptides less hydrophilic and leading to better separation and resolution in reverse-phase high performance liquid chromatography (RP-HPLC). In- house made C18 STAGE tips [42] were used for a final purification step.

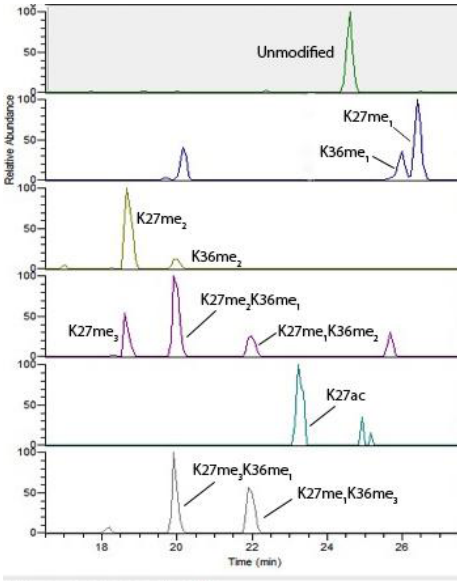


Figure 2. RP-HPLC trace of H3 27-40 peptides separated by hydrophobicity.

From top to bottom:

- The unmodified peptide was used as a point of reference.
- Monomethylated peptides (me1) were more hydrophobic than the unmodified peptide due to presence of propionyl groups, and, as shown, eluated after it.
- Dimethylated (me2) trimethylated (me3), and acetylated peptides (ac), as well as peptides with combinatorial PTMs, were more hydrophilic and eluted earlier.
- The four peaks in pink show peptides of identical mass (due to each containing a total of three methyl groups) that separated based on differences in hydrophobicity.

Mass Spectrometry and Tandem Mass Spectrometry

Samples were run on an HPLC system coupled to an LTQ-Orbitrap mass spectrometer (ThermoFisher Scientific, Carlsbad, CA, USA) as previously described [38], [43]. Peptides were separated by RP-HPLC on a fused silica microcapillary column packed with 10 cm of C18 reverse phase resin (Magic C18, 5µm particles, 200Å pore size; Michrom BioResources Inc., Auburn, CA, USA). Each full scan MS spectrum taken in the Orbitrap was followed by seven tandem mass spectrometry spectra (MS/MS spectra) in the ion trap of the seven most abundant peptides produced by collision-induced dissociation.

Detection of New Methylation

"Heavy" methyl groups added to lysine residues conferred mass shifts of +18, +36, and +54 Da from the unmodified state for mono-, di-, and trimethylation, respectively. Addition of "light" methyl groups induced mass shifts of +14, +28, and +42 Da from the unmodified state for mono-, di-, and trimethylation. Rather than the absolute masses of peptides, however, mass spectrometry detected their mass/charge ratios (m/z). The H3 27-40 peptide (see below) is generally found most abundantly as a 3+ charged peptide, so the actual m/z shifts detected were

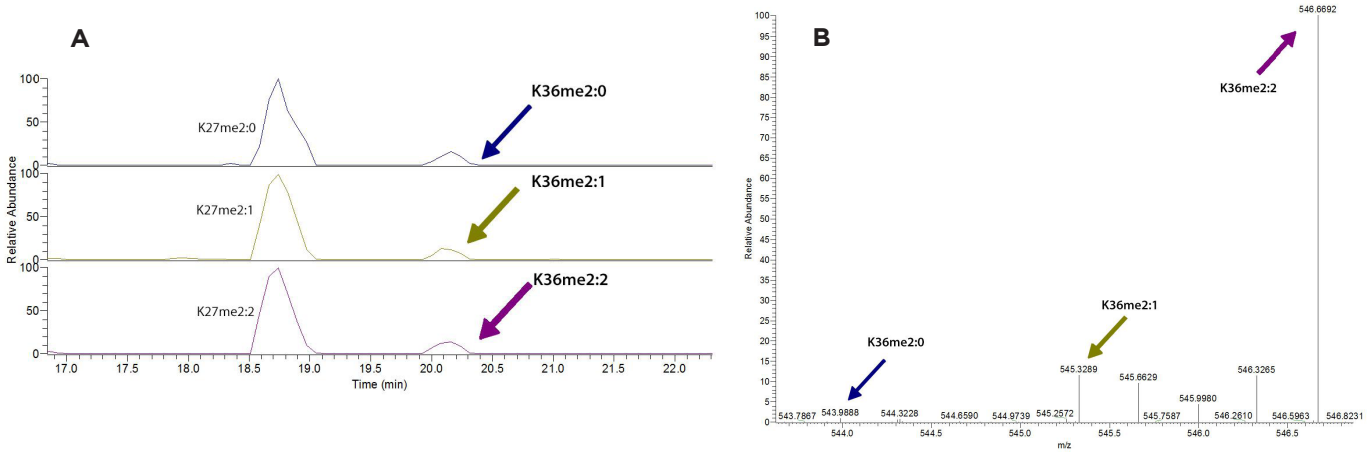


Figure 3. (A) *Extracted ion chromatogram:* Isotopic variations of the 2+ charged H3K36me2 peptide (labeled intermediates in the same methylation state) are see to co-elute. **(B)** *Full MS spectra of the same H3K36me2 peptides:* The different labeled intermediates are differentiated by their m/z ratios here. K36me2:1 has a peak at 545.33 and K36me2:2 has a peak at 546.67.

a third of those mentioned above. An additional mass shift of +56 Da was also taken into account for unmodified lysines, monomethylated lysines, and N-termini, which carried one propionyl group each from previous chemical derivatization [44].

Nomenclature

Organization of peptide forms for data analysis was based on previous studies [39], [43]. Due to digestion of the propionylated bulk histone extract with trypsin, H3K27 was found on a variety of post-translationally modified forms (species) of a peptide corresponding to residues 27-40 on histone H3. Those that were unmodified, had acetylated H3K27, or had some degree of lysine methylation were examined and were said to have distinct "methylation states" [38]. Specifically, they differed in whether H3K27 and/or H3K36 was unmodified, mono-, di-, or trimethylated (me1, me2, me3, respectively) [38]. H3K27 and H3K36 PTMs occurred alone and in combination with one another.

Organization of peptide forms for data analysis was based on previous studies [39], [43]. Due to digestion of the propionylated bulk histone extract with trypsin, H3K27 was found on a variety of post-translationally modified forms (species) of a peptide corresponding to residues 27-40 on histone H3. Those that were unmodified, had acetylated H3K27, or had some degree of lysine methylation were examined and were said to have distinct "methylation states" [38]. Specifically, they differed in whether H3K27 and/or H3K36 was unmodified, mono-, di-, or trimethylated (me1, me2, me3, respectively) [38]. H3K27 and H3K36 PTMs occurred alone and in combination with one another.

Statistical Analyses

Relative distributions of various labeled intermediates in the experimental sample were normalized to the corresponding relative distributions in the negative control sample. Stata 11.0 was used to conduct two-sample t-tests to compare these normalized distributions between the negative control and experimental sample, each in triplicate.

RESULTS

Peptide Quantification and Data Interpretation

In comparing the three samples, abundances of H3K27 di- and trimethylation were examined along with other lysine methylation

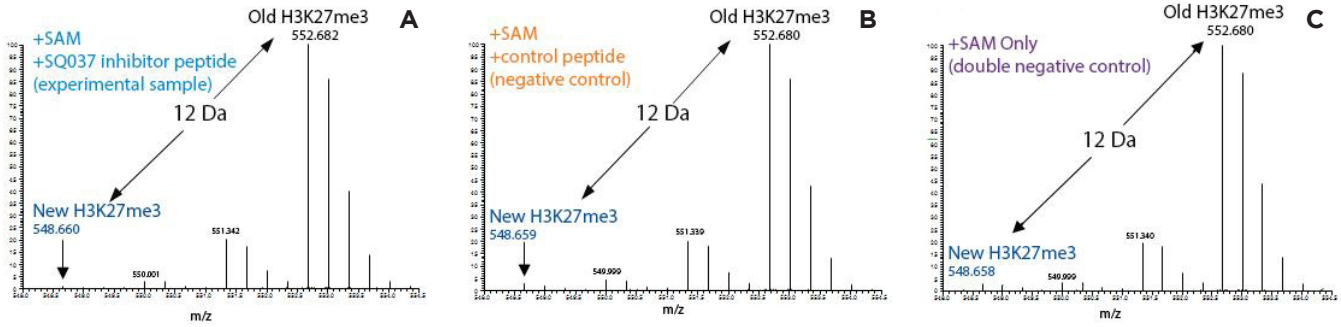


Figure 4. Full mass spectra from each of the three samples. Difference between H3K27me3:3 (old) and new H3K27me3:0 (new) was 12 Da (m/z ratio of 4). As shown, little new H3K27 trimethylation is generated during the in-nucleo reactions due to slow histone turnover rates. The relative abundance of new H3K27me3 (H3K27me3:0) seems to differ little between the negative control (B) and double negative control (C), suggesting that the control peptide did not interfere with formation of new H3K27 trimethylation. New H3K27me3 in the experimental sample (A) however, is noticeably less abundant than in either control. (Image adapted from our paper on this project) [45].

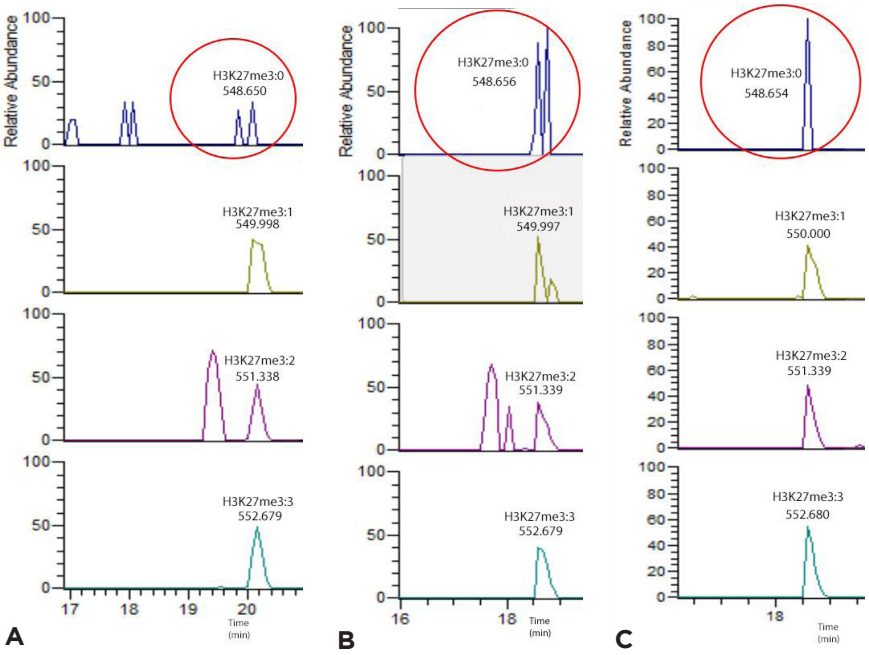


Figure 5. XIC traces of H3K27me3 intermediates that co-eluted. (A) *Experimental sample (SQ037).* While the peaks for H3K27me3:1, H3K27me3:2, and H3K27me3:3 were similar in height and quality to the peaks in the two controls, H3K27me3:0 peak was represented by a short double peak (low abundance). **(B)** *Negative control (control peptide).* Heights of peaks were comparable to those in the double negative contro, i.e., control peptide had no substantial effect on abundances of H3K27me3 intermediates. **(C)** *Double negative control (SAM only).* Each intermediate is represented by a clear, tall, single peak.

on the histone H3 27-40 peptide (e.g., K36) and on several other histones. The abundances of all relevant methylated species were quantified with Xcalibur Qual Browser software (version 2.0.7; Thermo Scientific) by manual chromatographic peak integration. Species with different degrees of methylation were identified by their HPLC retention times relative to the unmodified peptide (Fig. 2) since elution patterns are well known [44].

Different isotopes of the same peptide species co-eluted (Fig. 3A), so labeled intermediates within each methylation states were distinguished from one another by their masses (Fig. 3B). Once the abundances of every unique peptide species had been quantified, the abundances of each individual modification were calculated. This was done by combining the abundances of all peptides containing the modification of interest regardless of any modifications that were present on other residues of the peptide. For example, the total abundance for H3K27me3:3[2], an individual modification, was calculated by adding the abundances of all peptide species containing H3K27me3:3, i.e., the H3K27me3:3, H3K27me3:3K36me1:0, H3K27me3:3K36me1:1, H3K27me3:3K36me2:0, H3K27me3:3K36me2:1, and H3K27me3:3K36me2:2 peptide species.

To compare the amounts of light and heavy methylation that occurred within each sample, the relative abundance of each methylated intermediate for a particular residue was calculated with respect to all of the intermediates in that methylation state. This value was termed the "relative distribution" [38]. For example, the relative distribution of H3K27me3:0 was calculated as follows:

$$\frac{\text{(Total abundance of H3K27me3:0)}}{\text{(Total abundance of H3K27me3:0) + (Total abundance of H3K27me3:1) + (Total abundance of H3K27me3:2) + (Total abundance of H3K27me3:3).}}$$

Since the in-nucleo reactions were only performed for a short period and new methylation occurs slowly [38], the abundances of peptides with light di- and trimethylation were lower than abundances of peptides with preexisting heavy di-

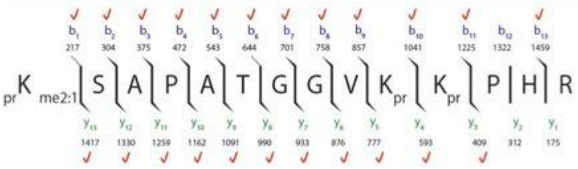
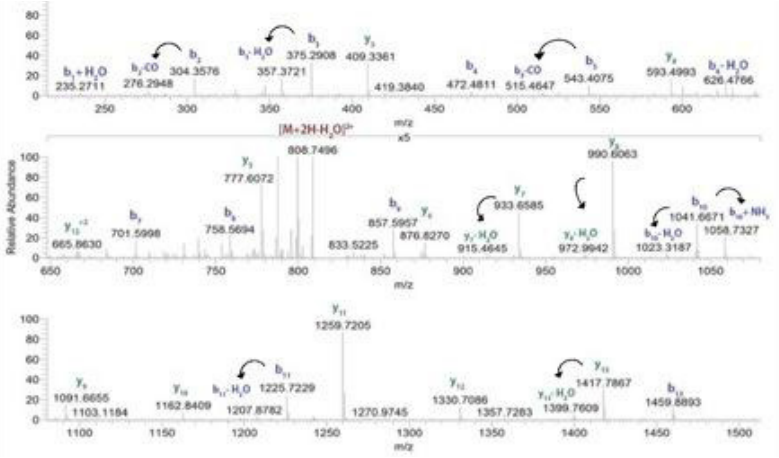


Figure 6. MS/MS spectra for the H3K27me2:1 peptide (+2 charge state; pr=propionyl). The sequence of the peptide H3 27-40 is shown at the top. b ions are shown in blue with masses written above the peptide. y ions are shown in green with masses written below the peptide. Check marks above the amino acids show that fragment was detected in the MS/MS spectra. $[M+2H-H_2O]^{2+}$ denotes the entire H3K27me2:1 peptide in the 2+ charged state with loss of water.



[2] H3K27me3:3 refers to a modification of K27 on the H3 27-40 peptide with 3 methyl groups on K27, all of which are heavy.

and tri methylation not only in the experimental sample, but also in both of the controls. For example, the most abundant labeled intermediate peptide species in the H3K27me3 methylation state were, in decreasing order, H3K27me3:3, H3K27me3:2, H3K27me3:1, and H3K27me3:0. In all three samples, the 552.679 m/z peak representing the 3+ charged H3K27me3:3 peptide was higher than the 551.339, 549.999, and 548.658 m/z signals that represented the H3K27me3:2, H3K27me3:1, and H3K27me3:0 peptides respectively (Fig. 4).

The height and quality of extracted ion chromatogram (XIC) peak indicated when peptide species were more abundant in one sample than in another (Fig. 5). A comparison of Fig. 5A to Figs. 5B and 5C shows that the peak for H3K27me3:0 peptide was found to be lower in the experimental sample.

To sequence the peptides and confirm their identities, spectra obtained through tandem mass spectrometry (MS/MS) were manually inspected. MS/MS fragments certain peptides detected in full MS and yields shorter peptides of various lengths, which are detected in the ion trap and recorded according to m/z ratio and abundance. These fragments are termed 'b' or 'y' ions depending on whether they are from the N-terminus or C-terminus, respectively, of the original, intact peptide. Various PTMs to the peptide altered the masses of any fragments that include the modified residue and could be identified because they yielded different m/z peaks for those particular fragments. As an example, Fig. 6 shows MS/MS data used to confirm the identity of the H3K27me2:1 peptide.

SQ037 reduced H3K27 trimethylation significantly compared to the control peptide

Importantly, the relative distributions of labeled intermediates in the H3K27me3 methylation state were markedly different between the samples. The relative distribution of H3K27me3:0 was 72.3% lower when SQ037 was added (experimental sample) than when the control peptide was added (negative control; *P=0.014, Figs. 7, 8).

Correspondingly, H3K27me3:3 comprised 38.0% more of the H3K27 trimethylation state in the experimental sample (SQ037) than it did in the negative control (control peptide; Fig. 8).

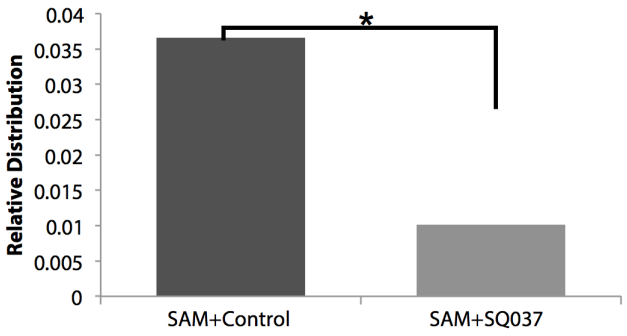


Figure 7. H3K27me3:0 relative distribution. The relative distribution of new H3K27 trimethylation was approximately 73% lower in the presence of SQ037 than in the presence of the control peptide. Data averaged from all 3 replicates.

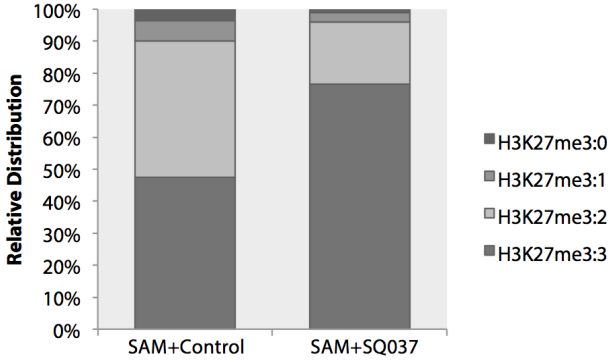


Figure 8. Relative distributions in the H3K27me3 state. The relative distributions of labeled intermediates in the H3K27 trimethylation state differed when SQ037 was added from when the control peptide was added. When SQ037 was added, the H3K27me3 state was distributed more towards old methylation and when the control peptide was added, new methylation was more substantial. Data averaged from all 3 replicates.

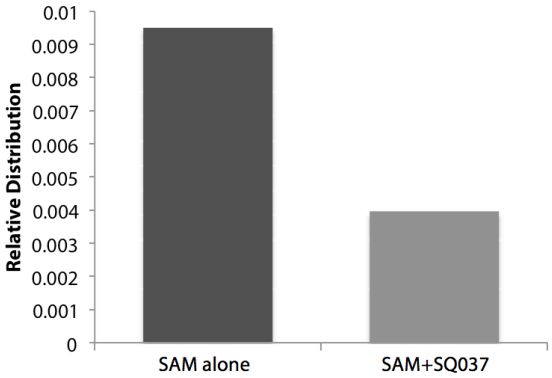


Figure 9. H3K27me3:0 relative distribution. The relative distribution of new H3K27 trimethylation was more than 58% lower in the presence of SQ037 than in the presence of SAM alone. Data from third replicate only.

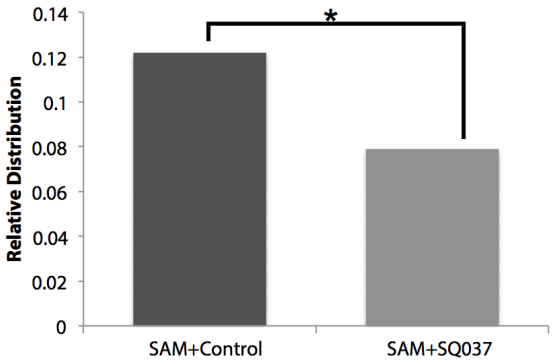


Figure 10. Relative distribution of H3K27me2:0 in the dimethylation state was about 35% lower when the inhibitory peptide was added than when the control peptide was added. Data from all three replicates. *P<0.05.

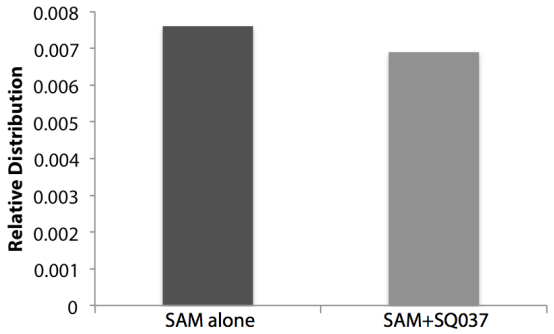


Figure 11. Relative distribution of new H3K27me2:0 in its methylation state was about 5% lower when SQ037 was added compared to when SAM alone was added to the nuclei. Data from third replicate only.

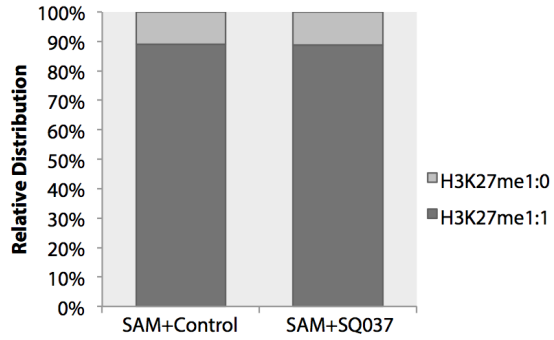


Figure 12. Relative distributions in H3K27me1 state were nearly identical when SQ037 and the control peptide were added. Data averaged from all 3 replicates.

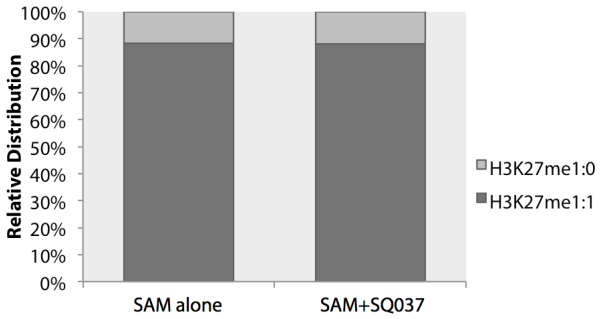


Figure 13. Relative distributions of labeled intermediates in the H3K27 monomethylation state were nearly identical when SQ037 and SAM alone were added. Data from third replicate only.

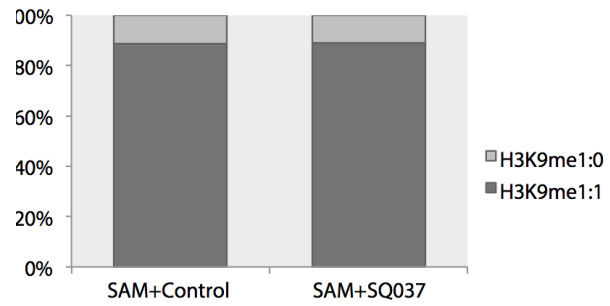


Figure 14. Relative distributions of labeled intermediates in the H3K9 monomethylation state were nearly identical when SQ037 and the control peptide were added. Data averaged from 2 replicates.

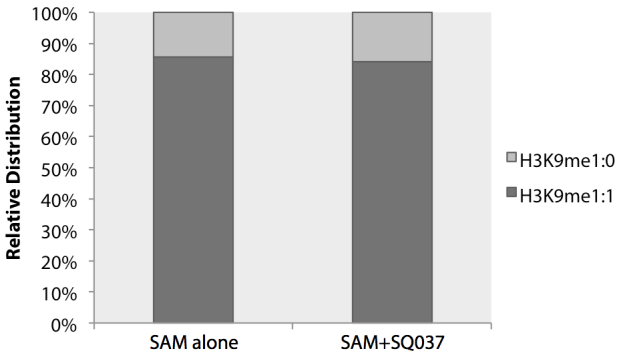


Figure 15. Relative distributions of labeled intermediates in the H3K9 monomethylation state were nearly identical when SQ037 and SAM alone were added. Data from third replicate only.

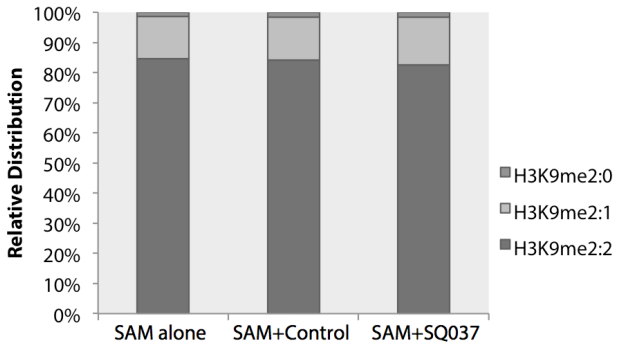


Figure 16. Relative distributions of labeled intermediates in the H3K9 dimethylation state were nearly identical when SQ037, and the control peptide, and SAM alone were added. Data from third replicate only.

H3K27 trimethylation was substantially reduced by the SQ037 inhibitor compared to "baseline" levels

The relative distribution of H3K27me3:0 was 58.3% lower in the presence of the inhibitor (experimental sample) than it was in the presence of SAM alone (Fig. 9).

H3K27 dimethylation was also reduced by the SQ037 inhibitor, but to a lesser extent

Relative distributions of labeled intermediates observed in the H3K27me2 state also differed across the three samples. The relative distribution of H3K27me2:0 was 35.2% lower in the presence of SQ037 than in the presence of the control peptide (Fig. 10). Though not as prominent as in the H3K27 trimethylation state, this difference was statistically significant (*P< 0.05).

The relative distribution of H3K27me2:0 was 5.4% lower in the presence of SQ037 than it was in the presence of SAM alone (Fig. 11).

H3K27 monomethylation was not reduced by the SQ037 inhibitor

The negative control (control peptide) and experimental sample

(SQ037) had similar relative distributions within the H3K27me1 state, as did the double negative control (SAM alone) and the experimental sample (Figs. 12, 13). All three samples had H3K-27me1:1 making up between 88% and 89% of the methylation state and H3K27me1:0 making up 11- 12% of the methylation state.

The SQ037 inhibitor did not interfere with other histone methylation

H3K9 monomethylation was found to be, on average, 89% labeled and 11% unlabeled in both the negative control (control peptide) and the experimental sample (SQ037; Fig. 14).

H3K9me1 was roughly 15% unlabeled and 85% labeled in both the double negative control (SAM alone) and the experimental sample in the third replicate (Fig 15).

H3K9me2:0 comprised 1.6%, on average, of its methylation state in all samples. (Fig. 16).

H3K9me3:3 comprised an average of 81-82% of the H3K9 trimethylation state in both the negative control (control peptide) and the experimental sample (SQ037) and made up about 84% of the methylation state in both the experimental sample and the double negative control (SAM alone) in the third replicate (Figs. 16, 17). H3K9me3:0 made up an average of about 1.5% of the H3K9 trimethylation state both when SQ037 was added and when the control peptide was added (Fig. 17). H3K9me3:0 made up less than 0.1% of the H3K9me3 methylation state in both the double negative control and the experimental sample in the third replicate (Fig. 18).

The relative distribution of H4K20 trimethylation was about 4% H4K20me3:3 in all three samples. H4K20me3:0 was not detected in the double negative control or in the experimental sample, and its relative distribution made up less than 0.1% of the methylation state in the negative control. H4K20me2:0 and H4K20me2:2 had relative distributions of 1.8% and 87%, respectively, across samples. In both the negative control (control peptide) and the experimental sample (SQ037), H4K20me1:0 made up an average of about 24% of H4K20 monomethylation, while H4K20me1:1 made up about 76% of the methylation state. In the third replicate, H4K20me1:0 made up 23% of the methylation state and H4K20me1:1 made up 76% of the methylation state in both the double negative control (SAM alone) and the experimental sample.

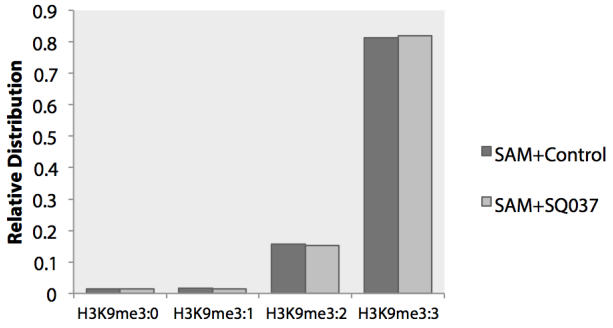


Figure 17. Relative distributions of H3K9me3 labeled intermediates did not differ when SQ037 and the control peptide were added. Data averaged from 2 replicates.

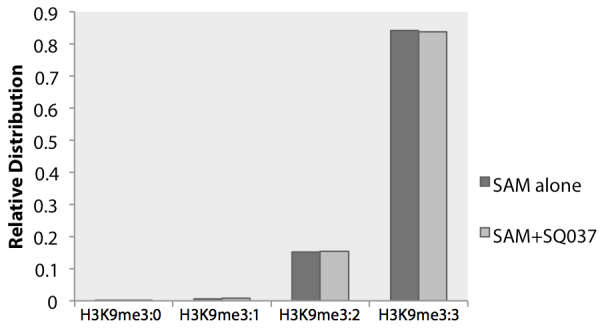


Figure 18. Relative distributions of H3K9me3 labeled intermediates did not differ when SQ037 and SAM alone were added. Data from third replicate only.

DISCUSSION

SQ037 significantly reduced H3K27 trimethylation compared the control peptide and blocked trimethylation by more than half compared to both the control peptide and SAM alone. SQ037 also significantly blocked dimethylation of H3K27 by EZH2 compared to the control peptide and substantially reduced H3K27 dimethylation compared to SAM alone, though to a lesser extent than it blocked EZH2- mediated H3K27 trimethylation. Thus, the inhibitor was effective and specific in its ability to inhibit the EZH2 histone methyltransferase.

Relative distributions of species within the H3K27 monomethylation state were similar between the experimental sample and the negative control (control peptide) and between the experimental sample and the double negative control (SAM alone), indicating that the SQ037 inhibitor peptide did not affect H3K27 monomethylation. Thus, the data are consistent with the findings of previous studies [8], [9] that EZH2 mainly trimethylates H3K27 and is also involved in some dimethylation, but not in monomethylation.

Until very recently, studies using indirect inhibition of EZH2 to lower H3K27 trimethylation and alleviate cancer phenotypes have not been successful in cell-based settings. Many researchers are now working to develop more specific EZH2 inhibitors, and some research regarding these has been published since the completion of this work [46], [47]. Analyses were performed on MS and MS/MS data from other methylation sites on histone H3 and on some sites from histone H4 to assess whether inhibition of EZH2 activity by SQ037 interfered with other, unrelated histone methylation. Since H4K20 and H3K9 are not EZH2 substrates and SQ037 is a direct inhibitor of EZH2, a significant difference in levels of mono-, di-, and trimethylation to these lysines between the experimental sample and the two controls were not expected. Methylation of H4K20 and H3K9 was found to be unaffected by the presence of SQ037, indicating that the inhibitor does not interfere with the activity of other histone methyltransferases such as SUV39H1 in nucleo and that it is specific to EZH2.

This was the first study to show that SQ037 can significantly inhibit EZH2 and reduce EZH2- mediated H3K27 di- and trimethylation in a cell-based setting when the enzyme is over-stimulated by an excess of SAM. The findings suggested that SQ037 could lower H3K27 trimethylation more than two- fold in cells with excessive EZH2 activity. SQ037 had a significantly greater effect on H3K27 methylation than the scrambled sequence control peptide, indicating that its ability to inhibit EZH2 is specific. Simon and Lange [23], and Sneeringer et al.

[35] called for a direct inhibitor of EZH2 that does not interfere with other methylation. Importantly, in contrast to previous efforts at down-regulating EZH2 activity, this study showed that SQ037 does not interfere with other lysine methylation and thus seems to have high specificity for EZH2. Also, these findings are consistent with previous studies showing that EZH2 mainly trimethylates H3K27 and is also involved in some dimethylation, but not in monomethylation.

FUTURE WORK

A limitation of this study was that while it was conducted in an in-nucleo setting, it was not conducted in entire live cells. In-nucleo evaluation modeled excess EZH2 activity and was a substantial advance from in vitro testing. However, the addition of exogenous SAM to the nuclei diluted other nucleic contents, including demethylating enzymes and the DNA methyltransferases and histone deacetylases that EZH2 works closely with. Therefore, the next step will be to develop methods for uptake of SQ037 by cells, e.g, via electroporation or a virus, and evaluate its activity in entire living cells.

The implications of developing a direct and specific epigenetic drug treatment for cancer are great because current cancer treatments are not targeted or optimally effective. Many chemotherapeutic agents, for example, inhibit cell division, because rapid proliferation is a key feature of cancer cells. These agents also kill other cells in the body that multiply rapidly, without distinguishing cancer cells from dividing cells in hair follicles, bone marrow, and the gastrointestinal tract. This lack of specificity is responsible for the characteristic side effects associated with chemotherapy, including hair loss, immunosuppression, and irritation of the digestive tract. Developing inhibitors of key enzymes that contribute to cancer development, like EZH2, is necessary in the progression towards targeted cancer therapies, but unless these inhibitors are direct and specific, they will also interfere with normal cellular activities and cause side effects. This study confirmed that peptidic enzyme inhibitors engineered via computational de novo peptide design can be effective and specific in nucleo, suggesting the possibilities that this method of inhibitor development holds [45]. The efficacy of SQ037 in inhibiting EZH2 in a cell-based setting, along with its specificity for EZH2 demonstrated in this study, suggest that it is suitable for further investigation as a potential novel drug treatment for cancer.

Appendix

In-nucleo Reactions Protocol

The protocol for in-nucleo reactions published by Fischle [37] was highly modified for this study and the procedure used is described below. NM buffer was first prepared without SAM or the control and inhibitory peptides. The pellet of frozen nuclei was resuspended in this incomplete buffer and divided into three identical aliquots. One mM of unlabeled SAM and 125μM of SQ037 were added to the experimental sample and 1mM of unlabeled SAM and 125μM of a scrambled sequence control peptide were added to the negative control. Only 1mM of unlabeled SAM was added to the isolated nuclei in the double negative control, which served as a point of reference for "normal" histone methylation in the unrestricted in-nucleo setting. The samples were incubated at 37ºC and inverted briefly every 15 minutes. After 2 hours, the nuclei were centrifuged at 10,000 rpm at 4ºC. Each supernatant was removed by pipetting, and the pellets were flash-frozen to quench the reactions.

REFERENCES

1. Bannister AJ, Kouzarides T. Regulation of chromatin by histone modifications. Cell Res 2011; 21:381-395.
2. Berger SL. Histone modifications in transcriptional regulation. Curr Opin Genet Dev 2002;12:142-148.
3. Jenuwein T, Allis CD. Translating the histone code. Science 2001; 293:1074-1080.
4. Young NL, DiMaggio PA, Garcia BA. The significance, development, and progress of high-throughput combinatorial histone code analysis. Cell and Molecular Life Sciences 2010; 67:3983-4000.
5. Garcia BA, Busby SA, Shabanowitz J et al. Resetting the epigenetic histone code in MRL-lpr/lpr mouse model of lupus by histone deacetylase inhibition. Journal of Proteome Research 2005; 4:2032-2042.
6. Sadri-Vakili G, Cha JH. Mechanisms of disease: Histone modifications in Huntington's disease. Nature Clinical Practice Neurology 2006; 2:330-338.
7. Sawan C, Herceg Z. Histone modifications and cancer. Adv Genet 2010; 70:57-85.
8. Cao R, Zhang Y. The functions of E(Z)/EZH2-mediated methylation of lysine 27 in histone H3. Curr Opin Genet Dev 2004; 14:155-164.
9. Velichutina I, Shaknovich R, Geng H et al. EZH2-mediated epigenetic silencing in germinal center B cells contributes to proliferation and lymphomagenesis. Blood 2010; 116:5247-5255.
10. Boyer LA, Plath K, Zeitlinger J et al. Polycomb complexes repress developmental regulators in murine embryonic stem cells. Nature 2006; 441:349-353.
11. Cha TL, Zhou BP, Xia W et al. Akt-mediated phosphorylation of EZH2 suppresses methylation of lysine 27 in histone H3. Science 2005; 310:306-310.
12. Martin C, Cao R, Zhang Y. Substrate preferences of the EZH2 histone methyltransferase complex. J Biol Chem 2006; 281:8365-8370.
13. Lee TI, Jenner RG, Boyer LA et al. Control of developmental regulators by Polycomb in human embryonic stem cells. Cell 2006; 125:301-313.
14. O'Carroll D, Erhardt S, Pagani M et al. The polycomb-group gene Ezh2 is required for early mouse development. Mol Cell Biol 2001; 21:4330-4336.
15. Ohm JE, McGarvey KM, Yu X et al. A stem cell-like chromatin pattern may predispose tumor suppressor genes to DNA hypermethylation and heritable silencing. Nature Genetics 2007; 39:237- 242.
16. Schlesinger Y, Straussman R, Keshet I et al. Polycomb-mediated methylation of Lys27 of histone H3 pre-marks genes for de novo methylation in cancer. Nature Genetics 2007; 39:232-236.
17. Stock JK, Gladrossi S, Casanova M et al. Ring1-mediated ubiquitination of H2A restrains poised RNA polymerase II at bivalent genes in mouse ES cells. Nature Cell Biology 2007; 9:1428-1435.
18. van der Vlag J, Otte AP. Transcriptional repression mediated by the human polycomb-group protein EED involves histone deacetylation. Nature Genetics 1999; 23:474-478.
19. Varambally S, Dhanasekaran SM, Zhou M et al. The polycomb group protein EZH2 is involved in progression of prostate cancer. Nature 2002; 419:624-629.
20. Collett K, Eide GE, Arnes J et al. Expression of enhancer of zeste homologue 2 is significantly associated with increased tumor cell proliferation and is a marker of aggressive breast cancer. Clinical Cancer Research 2006; 12:1168-1174
21. Kleer CG, Cao Q, Varambally S et al. EZH2 is a marker of aggressive breast cancer and promotes neoplastic formation of breast epithelial cells. Proc Natl Acad Sci U S A 2003; 100:11606-11611.
22. Wang S, Robertson GP, Zhu J. A novel human homologue of Drosophila polycomblike gene is up-regulated in multiple cancers. Gene 2004; 343:69-78.
23. Simon JA, Lange CA. Roles of the EZH2 histone methyltransferase in cancer epigenetics. Mutat Res 2008; 647:21-29.
24. Tan J, Yang X, Zhuang L et al. Pharmacologic disruption of Polycomb-repressive complex 2-mediated gene repression selectively induces apoptosis in cancer cells. Genes Dev 2007; 21:1050-1063.
25. Alimova I, Venkataraman S, Harris P et al. Targeting the enhancer of zeste homolog 2 in medulloblastoma. Int J Cancer 2012; 131:1800-1809.
26. Chiba T, Suzuki E, Negishi M et al. 3-Deazaneplanocin A is a promising therapeutic agent for the eradication of tumor-initiating hepatocellular carcinoma cells. Int J Cancer 2012; 130:2557-2567.

27. Crea F, Fornaro L, Bocci G et al. EZH2 inhibition: targeting the crossroad of tumor invasion and angiogenesis. Cancer Metastasis Rev 2012; 31:753-761.
28. Kikuchi J, Takashina T, Kinoshita I et al. Epigenetic therapy with 3-deazaneplanocin A, an inhibitor of the histone methyltransferase EZH2, inhibits growth of non- small cell lung cancer cells. Lung Cancer 2012; 78:138-143.
29. Peterson MB. "Targeting disease with a new de novo protein design framework for drug discovery," Ph.D. dissertation, Chemistry Dept., Princeton University, Princeton, NJ, 2011.
30. Sanders SL, Portoso M, Mata J et al. Methylation of histone H4 lysine 20 controls recruitment of Crb2 to sites of DNA damage. Cell 2004; 119:603-614.
31. Tachibana M, Sugimoto K, Nozaki M et al. G9a histone methyltransferase plays a dominant role in euchromatic histone H3 lysine 9 methylation and is essential for early embryogenesis. Genes Dev 2002; 16:1779-1791.
32. Wysocka J, Swigut T, Milne TA et al. WDR5 associates with histone H3 methylated at K4 and is essential for H3 K4 methylation and vertebrate development. Cell 2005; 121:859-872
33. Zhao W, Soejima H, Higashimoto K et al. The essential role of histone H3 Lys9 di-methylation and MeCP2 binding in MGMT silencing with poor DNA methylation of the promoter CpG island. J Biol Chem 2005; 137:431-440.
34. Mousavi K, Zare H, Wang AH et al. Polycomb protein EZH1 promotes RNA Polymerase II elongation. Molecular Cell 2012; 45:255-262.
35. Sneeringer CJ, Scott MP, Kuntz KW et al. Coordinated activities of wild type plus mutant EZH2 drive tumor-associated hypertrimethylation of lysine 27 on histone H3 (H3K27) in human B-cell lymphomas. Proc Natl Acad Sci U S A 2010; 107:20980-20985.
36. Ong SE, Mann M. Identifying and quantifying sites of protein methylation by heavy methyl SILAC. Current Protocols in Protein Science 2006. URL http://onlinelibrary.wiley.com/doi/10.1002/0471140864.ps1409s46/full
37. Fischle W. In nucleo enzymatic assays for the identification and characterization of histone modifying activities. Methods 2005; 36:362-367.
38. Zee BM, Levin RS, LeRoy G et al. In vivo residue-specific histone methylation dynamics. J Biol Chem 2010; 285:3341-3350.
39. von Holt C, Brandt WF, Greyling HJ et al. Isolation and Characterization of Histones. Methods in Enzymology 1989; 170:431-523.
40. Garcia BA, Pesavento JJ, Mizzen CA et al. Pervasive combinatorial modification of histone H3 in human cells. Nature Methods 2007; 4:487-489.
41. Garcia BA, Mollah S, Ueberhide BM et al. Chemical derivatization of histones for facilitated analysis by mass spectrometry. Nature Protocols 2007; 2:933-938.
42. Rappsilber J, Ishihama Y, Mann M. Stop and go extraction tips for matrix-assisted laser desorption/ionization, nanoelectrospray, and LC/MS sample pretreatment in proteomics. Anal Chem 2004; 75:663-670.
43. Zee BM, Levin RS, DiMaggio PA et al. Global turnover of histone post-translational modifications and variants in human cells. Epigenetics & Chromatin 2010; 3. URL http://www.epigeneticsandchromatin.com/content/3/1/22.
44. Plazas-Mayorca MD, Zee BM, Young NL et al. One-pot shotgun quantitative mass spectrometry characterization of histones. Journal of Proteome Research 2009; 8:5367-5374.
45. Smadbeck J, Peterson MB, Zee BM et al. De novo protein design and validation of histone methyltransferase inhibitors. PLoS ONE 2014; 9(2).
46. Knutson SK, Wigle TJ, Warholic NM et al. A selective inhibitor of EZH2 blocks H3K27 methylation and kills mutant lymphoma cells. Nature chemical biology 2012; 8:890-896.
47. McCabe MT, Ott HM, Ganji G et al. EZH2 inhibition as a therapeutic strategy for lymphoma with EZH2- activating mutations. Nature 2012; 492:108-112.

Aashna is originally from Newtown, Pennsylvania and is currently a freshman at Stanford University. She is interested in multiple academic areas including molecular biology, artificial intelligence, and computer science. She enjoys exploring the molecular mechanisms behind disease and conducting molecular and cellular research that has clinical applications. She currently works in Dr. Or Gozani's molecular biology lab in Stanford University's Biology Department. She is also a member of Stanford's Solar Car Team.

humanities

The Case for a Citizens' Council

Giving Effectual Authority to Deliberative Bodies

Rohan Sampath
Stanford University

This paper makes use of the California State Legislature as a heuristic to demonstrate the infirmities in the existing, prevalent institutions of lawmaking and popular will formation in the United States, and sets forth a specific proposal that may go towards rectifying these deficiencies. This proposal draws upon many of the principles of the Deliberative Polling method pioneered by James S. Fishkin, Chair of the Communications Department, Stanford University.[1]

Lawmaking, as it stands today in California and the other states, is very much in the doldrums. Some of the problems it faces may, in theory, be palliated within the existing institutional framework of government; there are others, however, that economists would call "endowment" issues – flaws that are inherent to and inseparable from representative democracy as we know it. These connatural flaws require us to not just *emend*, but also *amend*, the *status quo* with respect to the way we make and promulgate laws. *Ad rem*, I address two classes of institutional problems with our democratic structure - first, the flawed manner in which the "voice of the people" is currently given utterance and parsed;[2] second, problems with lawmaking that are independent of, but compounded by, this flawed nature of public opinion.

DECIPHERING THE PEOPLE'S VOICE

George Gallup once referred to public opinion polls as "sampling referenda;" he viewed such polls as the most effective check on popularly elected governments, between elections. Fishkin, however, raises important normative questions about whether public opinion is really a valuable criterion to keep in mind when making crucial policy decisions. Drawing on those celebrated lines of James Madison in the Federalist No. 10 – "to refine and enlarge the public views," Fishkin makes a distinction between "raw" and "refined" public opinion; he infers from Madison that refined opinion is "the considered judgments that can result from the deliberations[3] of a small representative body," as opposed to "the temporary errors and delusions of public opinion that may be found outside this deliberative process."[4] The pervasive maculae in "raw" public opinion are documented extensively in his (hereunto) seminal work, *When the People Speak*; I deal briefly with three of them.

Preeminent among these three is what social scientists term "rational ignorance:"[5] the difficulty in motivating citizens *en masse* to make a concerted effort to have informed, well-reasoned, opinions. Being one voice among a multitude makes the benefits of seeking an informed opinion seem nugatory and certainly not commensurate to the dedication of time and effort involved. Abbe Sieyès cites this fact as testament to the *superiority* and *necessity* of representative government. He notes that, in commercial societies occupied with economic transactions, "citizens no longer enjoy the leisure required to attend constantly to public affairs and must therefore use election to entrust government to people who are able to devote all their time to the task;"[6] in other words, he sees the need for government to be "a special profession."[7] The problem with this rationale, of course, is that the election of "virtuous" representatives that embody the needs and concerns of their electorates is contingent upon the electorate using a metric for evaluating candidates that is not perfunctory, apathetic, and based on a paucity of information.

Next, Fishkin considers the problems of fabricated, or "phantom," opinions that form the basis for many of the results in standard public opinion polls. John Stuart Mill, employing some beautiful turn of phrase, attributes this concoction of opinion to the average voter being, "destitute of faith, but terrified at skepticism."[8] This indigence of coherent opinion, or "faith," is but the natural consequence of rational ignorance; an embarrassment to admit the same, however, is the fundamental flaw in according opinion poll results with any measure of legitimacy and validity. Prior to the 2012 General Election, for example, Jimmy Kimmel conducted a mock "survey" of voters that solicited their opinion of who won the third presidential debate – before the debate had

[1] See, e.g., Fishkin, James: *When the People Speak* (Oxford University Press: 2009) and *The Voice of the People* (Yale University Press: 1991)
[2] The lack of meaningful collective *will formation* (a term popularized by Juergen Habermas) is the wellspring wherefrom problems of "political voice" arise.
[3] Deliberation, in general, refers to careful thought and discussion of an issue among a group of people.
[4] Fishkin, *When the People Speak*, p. 16
[5] The term "rational ignorance" was coined by Anthony Downs, in *An Economic Theory of Democracy*.
[6] Manin, Bernard: *The Principles of Representative Government* (Cambridge University Press: 1997), paraphrasing Sieyès, Emmanuel: *Dire de l'abbé Sieyès, Sur La Question Du Veto Royal: A La Séance Du 7 Septembre 1789*
[7] Manin, *Principles*
[8] Mill, John Stuart. *On Liberty,* Chapter II.

even happened. Several "respondents" were recorded on camera as fabricating an opinion on the victor, and even going as far as to "quote" particular statements and segments that had been decisive in swaying them![9] Of course, a non-random, localized survey edited for comedic effect is as unscientific a "poll" as can be; nonetheless, it is symptomatic of the broader malaise that Mill alludes to and Fishkin ascribes to "raw" public opinion.

Third, we have the reluctance of people to actively consider new avenues of thought and discourse that may be discordant, or even inimical, to their own, ossified, beliefs. This translates to a "self-selectivity of sources" – a proclivity for seeking opinions, whether from other people or from the media, that comport with one's own. This, of course, is yet another consequence of rational ignorance: when people spend a marginal amount of time on discussing politics and policy, why would they want to be drawn into a potentially acrimonious verbal scrimmage? Fishkin alludes to Abbe Sieyes' characterization of modern society as "commercial;" in such a world, he says, why would someone expend valuable interpersonal and social capital by raising "flashpoints of conflict?"[10]

The upshot of rational ignorance, phantom opinions, and self-selectivity of sources, especially in the context of the almost Orwellian ubiquity of the twenty-first century media, is a largely pliant populace whose opinions are vulnerable to manipulation. This is manifest in our predilection for evaluating complex political decisions on the basis of flippant, disingenuous "sound bites," for example, or our tractability when presented with distorted and asymmetrical comparisons in the media. From all this, it is pellucid that regardless of how scientific and meticulous the processes used by opinion polls may be, they are vulnerable to distortions; hence, their value to discussions about policy and lawmaking is inherently suspect.

THE DELIBERATIVE POLL

Fishkin sees the Deliberative Poll as a potent solution to these defects. A Deliberative Poll, in its essence, is a way of tracking the metamorphosis of the opinions of a randomly selected microcosm of the population before, during, and after, the process of deliberation. It consists of a random, scientifically-selected sample of the population whose opinions are being solicited; the sample chosen is large enough to ensure balance and a meaningful heterogeneity of opinions. Fishkin believes that gauging the opinions of a microcosm on issues after they have had the opportunity to give thoughtful consideration to the various perspectives that their peers enunciate is an infinitely more substantive way of assessing the true opinions of the macrocosm, for it illustrates not what the general public does think ("raw public opinion"), but rather what it "*would* think about an issue if it were to experience better conditions for thinking about it."[11]

ADDITIONAL ISSUES WITH REFERENDA AND ELECTIONS

The implications of Fishkin's analysis – the nub of this essay – are not just applicable to public opinion polls; at a panoptic level, his critique of the political knowledge of citizens is a damning indictment of the two primary expressions of popular sovereignty in our nation – *elections* and *referenda*. Surely, a healthy democracy requires a reasonable degree of cognizance and thought to go into the actions that reify its democratic status – i.e. its citizens casting ballots, whether in an election or in a referendum.[12] As Walter Lippmann lamented, the fact that "the compounding of individual ignorance in masses of people can produce a continuous directing force on public affairs,"[13] is not semblant of a system of government that legislates prudently and governs thoughtfully.

I begin by discussing the characteristics of *referenda* that make them an abortive attempt at achieving civic participation and public empowerment. The fundamental problem with referenda, of course, is that voters are apathetic and generally exhibit a lack of knowledge (rational ignorance). Perhaps the most glaring manifestation of how this "informational vacuum"[14] could yield erroneous results that are antipodean to the general preferences of the public is the California Rent Control Initiative of 1980. Ethan Leib cites Smith and Townsend (1980, 22) as saying, "Analysis of the *Los Angeles Times'* exit poll [of the Rent Control Initiative] demonstrated that *over three-fourths* of California voters did not match their views on rent control with their vote on the proposition: twenty-three percent wanted to protect rent control but incorrectly voted "yes," and fifty-four percent were opposed to rent control but incorrectly voted "no.""[15] Stratagems such as the distribution of information pamphlets to voters have also yielded little results[16], for hardly anyone reads through such booklets. In addition, voters may choose an option at random (phantom opinions), may go with whatever their preferred newspaper had endorsed (self-selection of sources), or be manipulated by "sound bites" propagated in the media.

However, even if we were to attain that utopian ideal of a truly well-informed citizenry, our *body politic* would still be fraught with pitfalls; for example, I identify two issues with referenda that are independent of whether the public is informed. First: the disproportionate influence of money and special interests. By and large, there are two ways to get an initiative on the ballot: either by procuring a certain number of signatures within a short period of time[17], or by forcing a legislative referendum. The former requires not just popular will, but also organizational strength, which, in today's day and age, is tantamount to large expenditures of money and the involvement of special interests; the latter is virtually impossible without a concerted media campaign, which also requires funding.[18] As a result, several

[9] Fishkin, James: Lecture, *Can the People Rule?* (10/31/2012)
[10] Fishkin, *When the People Speak,* p. 3
[11] Fishkin, *When the People Speak*, p. 13.
[12] A referendum is a direct vote in which the general populace is asked to vote on a substantive policy issue (and not merely a candidate); the choice available on the ballot is usually binary (i.e. you can vote either for or against the proposal).
[13] Lippmann, Walter: *The Phantom Public* (1927)
[14] Magelby 1995
[15] Magelby 1995, 38-39, citing Smith and Townsend 1980, 22.
[16] Leib, Ethan J: *Deliberative Democracy in America: A Proposal for a Popular Branch of Government.* Pennsylvania State UP: 2004.
[17] For example, in California, the requirement is 500,000 signatures in 90 days.
[18] Leib, *Deliberative Democracy.*

issues that are widely popular, but do not enjoy the backing of moneyed interests, may never get onto the ballot. Second, I take issue with the binary choice that voters face in referenda. The purported goal of a referendum is to give import and meaning to the otherwise vacuous ideal of "supremacy of popular will," by empowering the people to enact popular legislation when faced with an intransigent legislature. However, a choice of *yes* or *no* makes a mockery of the public, for it gives special interests the ability to draft legislation, and then expects demure complaisance to the parameters set by these interest groups, and prevents the people from expressing their support for nuanced alternatives. Of course, under the *status quo*, allowing multiple possibilities for voters would compound the problems of phantom opinions and ignorance and produce even more perverse results; this dilemma is yet another indication of the need for a paradigm upheaval in our democracy. An adaptation of deliberative polling, as I set forth *ante*, is an effective way to overcome this dilemma.

I next consider the flaws in our system of representative democracy. First, there exists a locus of "bundled" or aggregated policy stances. Every candidate stands on a platform that is an aggregation of different stances on issues. A vote for a candidate, however, does not imply a voter's endorsement of the entire gambit of the candidate's policies; nevertheless, representatives, as repositories of their constituents' trust, may go ahead and pursue policies that are not in comport with the majority of their constituents. This is inherent in the trusteeship model of representation that Edmund Burke famously propounded in his speech to the electors of Bristol; he theorized that "their [the people's] wishes ought to have great weight with him [the representative]; their opinion, high respect. ….. But his *unbiased* opinion, his mature judgment, he ought not to sacrifice to you, to any man, or to any set of men living." Your representative owes you……his judgment; and he betrays, instead of serving you, if he sacrifices it to your opinion."[19] Arguably, though, claiming a "mandate" to further all policy stances that one set forth prior to an election is undemocratic, for it is almost certain that many of them do not command a majority of the electorate's support; even more suspect is justifying a policy that one *did not* run on, as an extension of this "mandate." Perhaps the most iconic example of such perfidy occurred during the New Deal with President Franklin Roosevelt's "Judicial Procedures Reform Bill of 1937," commonly referred to as the "court-packing plan." Frustrated with the repeated invalidation of several pieces of legislation that were at the core of the New Deal, President Roosevelt introduced this bill that would have allowed him to appoint as many as six more justices to the Supreme Court, and thus immediately tilt the balance of the Court in his favor.[20] Apart from his flimsy justification of the justices being "overworked," the broader claim that Roosevelt made was that his landslide victory in the Presidential Election of 1936[21] gave him a popular mandate to ensure the implementation of his New Deal Policies, whatever that may entail. Strong public opinion against the bill ultimately killed it, for the public conflated an attack on the U.S. Supreme Court with an affront to the Constitution, and therefore saw this action as an unscrupulous power-grab masquerading under *bona fide* credentials. How was public opinion solicited and measured, though? Through Gallup's public opinion poll, and as I have set forth in some detail, these polls of raw opinion are not the indicia that should guide a nation's lawmaking. Furthermore, even if one were to ignore all the flaws of raw public opinion, the business of government involves several pieces of legislation that do not attract media attention, but may nevertheless impact individuals in subtle, yet significant ways. These bills may never even be polled on; if they are, their obscurity would increase the incidence of "phantom opinions," thus making the results of the poll even more untrustworthy.

I group the next set of problems with representative democracy under the umbrella term "gaming the system;" this includes all the malapropos ways in which our legislators make laws, including pork-barrel spending, the capitulation of legislators to lobbyists and other moneyed interests, and other behavior that is, as Leib puts it, "not kosher."[22] There are two fundamental reasons which perpetuate these underhand happenings in our legislatures; the first is rooted in human behavior, the second in the evolution of elections in the modern era. The first is the unequal impact that bills have on different classes of people; as a result, a *summum bonum*[23] maximizing bill may be defeated at the hands of a small, influential minority. For example, the elimination of tariffs on grapefruits may benefit consumers and the economy as a whole; however, the benefit to each consumer may be far less than the harm to a few grapefruit producers. If the grapefruit producers can effectively mobilize and get the backing of influential interests, it is possible for this bill to be defeated, for although the total benefit to all consumers is significant, the marginal benefit to each consumer does not make lobbying for the bill practicable. This is not to say that the interests of minorities such as grapefruit growers should be overlooked; what is undemocratic, however, is that the disproportionate influence that certain groups have may hold the legislature, and thus the people, hostage. Further, the second factor exacerbates the problems engendered by the first. In the modern era, fundraising is an essential component to winning elections – one needs money to mobilize grassroots support and pay for advertisements. As a result, there is even more of an impetus for legislators to "receive impressions favorable"[24] to such special interest groups, for it is quite possible that their sojourn in office depends on such capitulation. Thus, there is a palpable disconnect between the interests of the people and the will of the legislature.

The final and most ironic defect with representative democracy is its lack of representativeness. Certain demographics continue to be underrepresented in our legislatures – whether it be women, racial minorities, the disabled, religious minorities, or any other class of people. These discommodities are further augmented by the pursuit of gerrymandering and other policies expressly targeted at undermining the voting power of certain groups.

[19] Rakove, Jack: Lecture, *Can the People Rule?* (October 3, 2012)
[20] Caldeira, Gregory A.: *Public Opinion and The U.S. Supreme Court: FDR's Court-Packing Plan* (1987)
[21] In 1936, Roosevelt won 60.8% of the popular vote, and 98.5% of the electoral vote. Both of these remain the highest percentages received by a candidate since the (uncontested) election of 1820.
[22] Leib, *Deliberative Democracy*, p. 2
[23] summum bonum is a Latin expression meaning "the highest good"; in this context, it refers to maximizing the net benefit to society.
[24] The Federal Farmer, Letters
[25] Fishkin, *When the People Speak*

MY PROPOSAL

Fishkin expressly characterizes the results from his Deliberative Polls to be counterfactual, because "the public will rarely, if ever, be motivated to become as informed and engaged"[25] as the microcosms that participate in deliberation. However, he notes the success that these polls have had in influencing policy: he mentions the polls on Wind Energy in Texas, the polls on Public Works Projects in local government in China, and others, as exemplars that have actually shaped policy. I believe, however, that the systematic failings of our existing democratic setup call for a deliberative body whose findings are not just recommendatory, but have the force of law. My proposal, at its heart, entails the formation of a *popular* Council in California, randomly selected from the people, and constituted along the lines of Fishkin's microcosms. This Council shall be a *legislative body not subject to dissolution* (i.e., permanently in session), and shall be treated on par with the other legislative bodies.

This is the rudimentary palimpsest upon which I construct, step-by-step, a comprehensive proposal that shall rectify, or at least have a palliative effect on, several of the deficiencies that I enumerated *ante*. First, though, a disclaimer: the existing literature critiquing deliberative democracy is extensive and substantial; there are numerous legitimate arguments against the concept of deliberative democracy as a whole, as well as against Fishkin's Deliberative Poll in particular; for example, Cohen and Sabel claim that deliberative polling is a façade for "pluralistic logocracy" – rule of government by words.[26] With an eye toward the constraints of space and the specific objective of this paper, however, those arguments are not dealt with. Instead, I only deal with the additional concerns engendered as a result of my enlargement and application of Fishkin's non-binding, consultative model to an effectual, legislative one.

> "EVEN IF WE WERE TO ATTAIN THAT UTOPIAN IDEAL OF A TRULY WELL-INFORMED CITIZENRY, OUR BODY POLITIC WOULD STILL BE FRAUGHT WITH PITFALLS."

A FEW PARTICULARS

Size

In the Federalist No. 10, Madison theorizes that the size of a representative body should be neither too small nor too large, to guard against both "the cabals of a few," and "the confusions of a multitude."[27] A Council of around 405 citizens, for example, composed into twenty-seven groups of fifteen citizens each, would ensure requisite demographic representativeness while at the same preventing the body from becoming large and unwieldy during the process of deliberation as a whole.

Term of Office

The term of each Council shall be as short as is feasible – a tenure of four weeks would be ideal.

EFFECTIVENESS

In order to be effective in countering the problems mentioned ante, the Council needs three characteristics:

Accountability

A principal argument against the conferral of legislative authority upon a deliberative body is the latter's complete lack of accountability; after all, following the completion of their term, councilors may go back to civil society without any liability for their actions as legislators. The Greek principle of "*ho boulemenous*," or possible criminal action for the suggestion of frivolous laws, would end up threatening or intimidating councilors. How, then, may we be assured that the councilors will not go rogue?

Empirical evidence from numerous Deliberative Polls conducted by Fishkin have yielded almost no exceptions to productive, measured debate and deliberation; this is testament to the inherent rectitude of civil society when placed in a deliberative setup, and is a motif that points towards the feasibility of my reform. However, critics may be assuaged with a broader security – that of the covenant of rotating governance. The caveat of a short, one-month tenure enlivens a principle that was thought, both by the Athenians and the Founding Fathers, to be essential to liberty: an alternating role between the governors and the governed. Manin quotes Aristotle as saying that a cornerstone of liberty is *en merei archesthai kai archein*, or "to rule and be ruled in turns." A similar sentiment was expressed by Madison, who says, "Where annual elections end, tyranny begins." Councilors make laws that they themselves must obey; there can be no surer shibboleth that will ensure their honesty. Further, the quick turnover of councilors means that councilors would be wary of passing frivolous or damaging laws and thus setting a precedent which one day may be to their own, severe, detriment.

Curtailing the influence of money and special interests on elections.

Many of the most odious contrivances of representative democracy stem from candidates' claims of an election mandate, and the need for candidates to seek reelection; these do not apply to members of civil society randomly chosen. What may

[26] Cohen and Sabel.
[27] Publius (Madison, James): *The Federalist Papers*, No. 10.
[28] Publius (Madison, James): *The Federalist Papers*, No. 53.
[29] Warren and Pearse, Chapter V.
[30] Fishkin, Lecture: *Can the People Rule?*
[31] Fishkin, *When the People Speak*, p. 125
[32] Ibid.
[33] de Tocqueville: *Democracy*, p. 598.

apply, however, is the possibility of money and special interests influencing councilors; this may be prevented by rigorous oversight, for this will significantly curtail the possibility of duplicitous, unfair influence of moneyed interests and lobby groups on the councilors during their short term of office.

I propose the formation of a State Council Oversight Committee (SCOC), built along the lines of the Redistricting Commission of California, whose efforts in 2011 were met with bipartisan approbation. A body of fourteen citizens is chosen from the public, with no more than five members from one party, and no less than four members unaffiliated with any party. These seats are filled through application solicited from the public. This body shall perform the functions performed by the Centre for Deliberative Democracy in Deliberative Polls: ensuring that the random selection process is not tampered with, ensuring neutrality of briefing materials, coordinating question and answer sessions with experts, and preventing special interests from gaining an ascendancy over any councilor.

Representativeness

Fishkin's model uses "stratified random samples" to ensure heterogeneity. Additionally, he devotes significant effort to ensuring that the sample selected is representative of the original sample that had first responded; I shall adopt a similar model. With reference to the British Columbia Citizens' Assembly, Michael Rabinder James avers the selfsame principle; he says that one of the primary sources of legitimacy of a deliberative body consisting of randomly chosen citizens is *the descriptive similarity to the electorate.*

CONCLUSION

In an eponymous novel, James Fishkin and Bruce Ackerman propound the concept of a "Deliberation Day" as a possible mechanism to facilitate the permeation of deliberation to the macrocosm. They propose a biennial holiday (instead of Presidents' Day, for example), occurring just prior to Election Day, on which the entire voting-age population is encouraged, through monetary incentives, to attend neighborhood deliberation sessions – a throwback to the town-halls of New England, lauded as "schools of public spirit" by John Stuart Mill. However, Fishkin himself accepts the inherent problems in bringing deliberation to the macrocosm: the reappearance of rational ignorance, for example, and the impossibility of ensuring good quality moderators. What is important to note, though, is that the desideratum of Fishkin's advocacy of deliberative democracy goes beyond the need for good policy *per se*; at its heart, it is an agency for molding people into better citizens.

Fishkin reminisces about the gratitude he received from the spouse of a deliberative poll participant who had been astonished by the vicissitudes in her husband, from a man who abhorred intellectual inquiry and pursuit, to one who became an inveterate reader of newspapers and other sources of political information. He describes the sociocultural metamorphosis that the 1996 National Issues Convention wrought on a "white male conservative." On the first day of deliberations, the man displayed brashness and an attitude of condescension towards his fellow participants, and, in particular, to an African-American woman; on the final day, he was overheard as asking this woman, "What are the three most important words in the English language? They are 'I was wrong.'" Thus, Fishkin's deliberative polls fostered cross-racial understanding in a way that few other social experiments may have.

With reference to the jury system – a novelty for a man brought up in the philosophy of the civil law system – Tocqueville famously said, "I do not know whether a jury is useful to the parties involved, but I am sure it is very good for those who have to decide the case." I *do* believe that a Citizens' Council will effectuate laws that are thoughtful, balanced, and empathetic, but that is perhaps debatable. The inestimable value of the Council in fostering a versant, engaged citizen body, however, is indubitable, for "the only way opinions and ideas can be renewed, hearts enlarged, and human minds developed is through the reciprocal influence of men upon each other."

I have detailed one possible prescription for the shape the Citizens' Council could take – this includes details such as remuneration, voting rules, oversight commissioners, and other procedures of the Council. Please do take the time to visit <http://www.stanford.edu/group/journal/cgi-bin/wordpress/?page_id=369> and go to the appendix to see this detailed proposal.

Rohan Sampath is a second-year undergraduate student at Stanford University studying Economics, possibly Computer Science (and, of course, the Mysteries of Life). He has always been fascinated with the electoral mechanisms and institutions that constitute the bedrock of democracy, and often experiences eureka moments in his quest to come up with the wildest of ideas on how these institutions can be strengthened. His other interests include tennis, hiking, playing cards, and any game in which he can claim underdog status.

"Exactly What I Would Have Done - Barbra"

Glee and the Performance of Jewish Womanhood

Leow Hui Min Annabeth
Stanford University

In 1983, Rachel Adler, drawing on an earlier essay by Cynthia Ozick, wrote about the exclusion of women from scripture, halakha, and liturgy. Adler's essay makes a demand for knowledge which forms the conceptual core of Jewish feminist identity: "Have we ever had a covenant in the first place? Are women Jews?" (Adler 1983, 22) Her latter question, *are women Jews*, calls into question the categories of *women* and *Jews*, both of which are identities grounded in the repetition of ritual acts. This theory of affirming identity through the performance of social acts was a framework proposed only five years after Adler's essay appeared in *Moment* magazine, by Judith Butler, a fellow Jew and feminist.

Butler proposes that "to be a woman is to have to *become* a woman, to compel the body to conform to a historical idea of 'woman,' to induce the body to become a cultural sign, to materialize oneself in obedience to an historically delimited possibility, and to do this as a sustained and repeated corporeal project" (Butler 1988, 522). Such a description adequately models both the process of being recognized as a woman, and being recognized as a Jew. Within Butler's framework, it is apparent that the social acts which render a body into a Jewish woman occur on two levels. First is the performance of "women's mitzvot." Second is the creation of a separate category of people ("women") via exclusion from the normative mitzvot, i.e. men's . Or, as Shaye Cohen puts it: "The contrast between Jewish men and women is not one of essence but of function. Men are obligated to observe all the commandments; women are not" (Cohen 2005, 126–27).

Yet what makes certain mitzvot *women's* mitzvot, if not essence? The alternative to this performative model of gendered difference is the inversion of Cohen's phrasing: difference as the product not of function but of essence. Haviva Ner-David, an ordained Modern Orthodox woman rabbi, argues that it is not the exclusion of women that makes certain mitzvot "male." Instead, she writes, the belief is that "as a woman, I am so *essentially* different from men … that women are not obligated in certain mitzvot because they are *inherently* male mitzvot" (Ner-David 2000, 56 [emphasis mine]). Describing her mother, Ner-David writes that "the idea that there are certain mitzvot that only men perform flowed naturally from her gendered worldview. *This is what religious Jewish men do*, she undoubtedly believed, without thinking these words consciously" (103).

The tension over Jewish women's identities, however, is not limited to religious practice. Beyond the question of whether women are Jews is the idea of what Jewish women are not. Menachem Kaiser, in an essay for the *Los Angeles Review of Books,* picks apart at the portrayal of "shikse" in the culture of contemporary Jewish America. As a sexual object forbidden to Jewish men by customary prohibitions of intermarriage, Kaiser notes, the notion of the shiksa as a seductress turns her into what a Jewish woman cannot be: "a symbol of temptation, not of classism or segregation. She inspires disgust, fascination, obsession, sin; she is sexual in that religious way that doesn't necessarily have anything to do with sex: she is constantly and thoroughly moralized" (Kaiser 2013, n. pag.).

This distinction cuts both ways. There is a strange parallel between the Yiddish shtetl culture's construction of the shiksa, and European Christendom's construction of the Jewess. Kaiser observes that this emerges out of an attitude that is more about embodied racial traits than it is about religious practice—in the 1950s, "the Jew-Gentile fault line was shifting away from a religious-based binary and towards *characteristics*, or types. … The underlying conceit is that it is no longer religion or even ethnicity that separates Jews and Gentiles" (Kaiser 2013, n. pag.). Outside of a religious Judaism, the act of being a Jewish woman is also historically delimited by socio-cultural stereotypes in secular life: "Jewish women are beautiful, alluring, exotic. … European literature is full of Jewish heroines who are too beautiful, too noble, and too attractive to remain within the Jewish fold" (Cohen 2005, 159). Even this beauty, though, holds a note of falseness, under which lie accusations "that the anti-Semitic spectacle of Jewish femininity was always already staged and unnatural" (Pellegrini 1997, 130).

This was the charge brought against nineteenth-century French actress Sarah Bernhardt by anti-Semitic detractors—that her dark hair and dark eyes rendered her unmistakably Jewish, and separate from Christian Europe (129). This is the charge made, also, by Jewish Americans who claim Barbra Streisand as one of their own; Rachel Kranson calls it "a representation of American Judaism from when Jewish ethnicity truly seemed like the stuff of difference, when Jews seemed to experience a pain and passion that no one else could understand, when Jews seemed like the liberal conscience of white America and the true martyrs of history" (Kranson 2001, 36). All this occurs within a history of discursive dichotomization—between men and women inside and outside Jewish society, and around the position of Jewish society vis-à-vis the Christian West.

These religious and cultural constructs of Jewish womanhood do not circulate in a vacuum. Rather, they find their place shaping, and being shaped, by the arts and popular culture. Letty Cottin Pogrebin devotes an entire chapter of her memoirs, *Deborah, Golda, and Me*, to analysing the influence of Hollywood movies on her Jewish New York girlhood. Of the female characters in movies about Jewish American culture, she writes:

> Not one of them could be considered a complete Jewish heroine, a fully realized, positive, well-balanced, successful, or admirable Jewish woman. … Instead the best I could

> find were women who made the least intolerable trade-offs, those who did not have to pay too high a price for being themselves.
>
> For instance, Choose or Die: *If you had to live your life as Marjorie Morningstar or Yentl, which would you be?* … Take your pick: Marjorie's bourgeois subordination or Yentl's lonely rebellion. Some choice! (Pogrebin 1991, 258)

Pogrebin classifies these popular representations of Jewish women as falling into four categories: the Jewish-American Princess, the Jewish Mother, the Jewish Big Mouth, and the Jewish Man's Burden. All lack happy-ever-afters; all make it to the end credits unfulfilled.

Kaiser examines how portrayals of the shiksa have evolved over time in secular U.S. American culture—a culture that has also been produced through the assimilation of Jewish Americans. How have representations of the shiksa's Jewish cousin similarly evolved? In 2001, Kranson wrote in *Lilith*, in the wake of Streisand's fading celebrity: "What really connected us Jewish women—with our widely varied noses, personalities, concerns—to her? What made her stand out as the quintessential Jewish star? And, as the real Barbra retreats into myth, will we find anyone to take her place?" (Kranson 2001, 36) Eight years later, an answer seemed apparent in Lea Michele, who stars as Rachel Berry on the musical high school drama Glee (2009–). Though Kranson acknowledges that the cultural ideal of *the* essential Jewish woman is non-existent, she also recognizes the appeal of the myth that "Jewish women are passionate, outspoken, liberal, talented, Semitic looking, and from Brooklyn" (*ibid.*), which Streisand embodied and upon which the character of Rachel has been modeled.

Set in the fictional William McKinley High School in the real-life city of Lima, Ohio, *Glee* follows the progress of a struggling high school show choir en route to competing in nationals. In its choir of misfits are two characters explicitly written as Jewish—Michele's Rachel, and Noah "Puck" Puckerman (Mark Salling). Two more are suggested, based on their names, to be Jewish—Tina Cohen-Chang (Jenna Ushkowitz), who is Asian American, and wheelchair-using Artie Abrams (Kevin McHale). Glee has been lauded for the diversity of its casting, even going so far as to have been presented with the Multicultural Motion Picture Association's diverse ensemble cast award in its debut year. At the same time, it has also come under fire for trading in stereotypes—the tragic cripple (played by an able-bodied actor), the promiscuous lesbian Latina cheerleader, the fat sassy Black girl. Its portrayal of minority characters veers into tokenism, sometimes caricature. Belonging to a minority group becomes a critical part of each character's shtick, but little more. In this way, Puck is first of all depicted as the working-class son of a single mother, Artie as disabled, Tina as Asian.

What it means to be Jewish thus becomes complicated by the manner in which characters' minority identities are foregrounded. Class, dis/ability, and race supersede Jewish identity within Glee's narrative—which correspondingly implies that the normative Jew is middle-class, abled, and Ashkenazi. Only Rachel is depicted as definitely and definitively the show's Jewish star. Yet, as mentioned, her Jewishness is portrayed problematically. On the one hand, she is firmly entrenched as the spiritual successor to Barbra Streisand—she idolizes the actress and dreams of starring in Funny Girl on Broadway—and, as the left-leaning daughter of an interracial gay couple, she is both liberal and talented. On the other, her passion and outspokenness are characterized as obnoxious and brash; Rachel's sense of entitlement towards leading roles and solos raises the specter of the ugly stereotype of the Jewish American princess.

Rachel's Jewishness is always present in Glee, but it occurs less as a religious identity, and more—as in "Born This Way"—as the crisis over whether or not to get a nose job. Her Jewish identity does not primarily emerge from throwaway jokes about her fathers "sitting shiva" in "Prom-o-saurus," after she fails an audition for a prestigious arts academy. As Hillary Busis notes in an article for *Jewcy*:

> Rachel's once-vital religion has been all but forgotten. In this year's emphatically denominational holiday episode, for example, Rachel presented Finn with an exorbitant Christmas list, enthusiastically participated in an homage to *The Judy Garland Christmas Special*, and cheerfully sang a bevy of Christmas carols—only remembering her heritage during a quick, barely audible "Happy Hanukkah!" shouted just as the hour ended. (Busis 2012, n. pag.)

Instead, in her characterization, her Judaism has been both thoroughly secularized, and also completely essentialized.

Rachel may "act" Jewish, in her appropriation of Streisand's legacy, and in the falseness of her identification as an actress. The very ambition of performing professionally on stage "reiterates dangerous associations between Jewishness and artifice," as Pellegrini writes of Bernhardt (127). Yet Jewishness is figured onto her body as well. Even though the Jewish woman is presented as alluring and beautiful, her feminine beauty is read as perfidiously artificial, such that "[t]he hyperbolic femininity of the *belle juive* conceals her perverse masculinity" (129). Rachel, with her "Jewish" nose and the masculine features that earn her the nickname "man hands," manifests her Jewishness in an essential physical difference. In an environment where her very body is coded as Jewish, Rachel becomes not just *the* Jewish woman, but *the* Jew—at the expense of the show's other Jewish characters, but also at the expense of her own characterization.

It is ironic that Rachel's features should be coded "Jewish," to the extent that the Jewish Women's Archive's *Jewesses With Attitude* blog trumpets Michele as Streisand's heiress, "a new, female Jewish pop icon with a nose." While Michele's father is Sephardi—her family name is Sarfati—she has been raised in her mother's Catholicism—similar to how Ushkowitz, who plays Tina, is a transnational Korean American adoptee, raised Catholic with one Jewish grandfather. Meanwhile, Quinn, who starts off the show as a devout Catholic, is played by Dianna Agron, a Russian Jew. The diversity of the actresses' experiences speaks to Kranson's statement that "Jews are many things, and can look, act and live in the most 'goyische' of ways" (Kranson

[1] The Jewish system of jurisprudence.
[2] In Judaism, a mitzvah (pl. mitzvot) is a commandment governing behaviour and obligations. Some mitzvot are traditionally restricted to only women or only men.
[3] Shiksa (pl. shikse) is a Yiddish term, sometimes pejorative, used to refer to a non-Jewish woman.

2001, 36). Nonetheless, although the show does make attempts to engage with that diversity, it is ultimately content to trade in stereotypes of Jewish womanhood.

This is evident in the show's treatment of Tina, an underutilized and marginal character despite her presence as a mainstay of the ensemble cast. In an analysis of Glee's pilot episode, blogger Gnat measures the amount of speaking time given to each of the twelve characters who formed the principal cast of the show's first season, and manipulates promotional artwork to reflect this. The result demonstrates how the show favors representation of three characters—the choir director, Will Schuester (Matthew Morrison), and football player Finn Hudson (Cory Monteith), both white and nominally Christian men, as well as Rachel. The character representation of the series premiere is no indication as to the general trend over the course of the series as it progresses, but it allows a quick reference point in its privileging of its white heterosexual characters. As Gnat observes, "I'm really curious how it would look for all the episodes, averaged out. … Like, Quinn's head would get a lot bigger, but I bet Tina's would stay the same size" (Gnat 2010, n. pag.).

Although Rachel and Tina are the only Jewish women in their high school show choir, their interaction has never been predicated on this shared identity. Instead, they have been placed into competition with each other for coveted lead roles in their choir—with Rachel always coming out on top. Tina's identity is constantly depicted as exclusively formed by her race, with the word "Asian" permanently affixed to her name whenever other characters call on her. For example, when the school principal demands that she give up her goth fashion sense in the first-season episode "Theatricality" (2010), Tina complains, "I feel like an Asian Branch Davidian." The label "Asian" is constantly used to remark on her deviation from the normative, so that she is not just a hypothetical Asian Branch Davidian. Were she to assert her Jewish identity, it seems like that too would have to be prefixed by her race. Her personality traits are designed to call to mind the image of the meek model-minority Asian American woman—she is shy, she stutters, and when she finally explodes at Rachel in "Props" (2012), her concerns are condescended to.

Rachel fiercely argues, upon being confronted, that she deserves to star in solos because she works for it. She then rattles off to Tina a list of her achievements—memorizing librettos, leading sixteen extracurricular clubs, maintaining a 3.86 GPA, and keeping her boyfriend "interested and physically satisfied." Not only do these crude stereotypes deny the show the opportunity to explore what it means to be Jewish American outside the boundaries of middle-class European ancestry, but the selfsame behavior that serves as approval and evidence of Rachel's "Jewishness"—the assertiveness and the clawing hunger for Broadway success—is demonized in Tina.

"Props" ends with Tina parroting to secondary characters the injunction to "put in the work, be a good team player, and you'll get your solos." This is a message which the script signs off on. Right after delivering that line as the episode's feel-good moral, the camera pans to Tina's boyfriend and they trade contented smiles. Rachel, however, has been depicted throughout the series as getting her way through individualism and exceptionalism. "Props" is the same episode where Tina, after accepting that Rachel has something special about her, drives all the way to Oberlin to plead Rachel's case in appealing the results of a failed audition. Whether or not those traits of female pushiness are used fairly to characterize Rachel as "Jewish," they still cannot, apparently, inscribe Jewishness atop Tina's Asian body.

The rivalry between Rachel and Quinn is more complicated. At the beginning of the first season, Quinn was a pretty, popular, blonde cheerleader, while Rachel was presented as an unattractive social misfit. Their romantic competition for Finn's affections leaves Quinn shunned for her affair and resultant pregnancy with Puck, so that by the third season, Quinn's star is in decline, while Rachel is the sensation of the glee club and engaged to Finn as well. "Prom-a-saurus" (2012), set during Rachel's and Quinn's senior prom during the third season of *Glee*, culminates in a reconciliation where Quinn is elected prom queen, but in a gesture of magnanimity stuffs the ballot boxes so that Rachel wins the title instead. In the framing of their frenemy relationship—Quinn spends the third season in a wheelchair because of a spinal injury suffered in a car accident en route to Rachel's wedding—a question arises: Is Quinn—at least subliminally—eking out a space for herself as the Jewess-who-isn't-there?

The relationship between Rachel and Quinn is tense because there is an underlying current of contrast. If Jewish women are distinguished by the fact that they are not the normative *male* Jew, then they are also distinguished, as a minority group, by the fact that they are not Gentile women either. Rachel's fiancé is Quinn's ex, and in "Props," Rachel describes her wedding plans to Tina as "a nice little church wedding performed by a rabbi with our nearest and dearest," which highlights the religious difference between Jewish Rachel, Protestant Finn, and Catholic Quinn. In the meantime, though they are not romantically attached, Quinn is indisputably marked by her status as the mother of Puck's Jewish baby. There is a sense that Rachel and Quinn are occupying each other's positions—that, as Quinn argues, she and Finn are meant to be, a power couple, popular and attractive, football star and cheerleader; and, as an early episode of *Glee* humorously suggests, that Puck and Rachel belong together as the only "out" Jews in McKinley High. However, in "Born This Way" (2011) in the second season—that is, the same episode where Rachel invokes Barbra Streisand as a role model in her refusal of a nose job—Quinn is revealed to have had one herself, as part of her transition from adolescence as an overweight brunette, ironically signifying that there is something of the closeted Jewish woman about her after all. The Jewish actress Agron is cast as Catholic Quinn, and vice versa, because it is Michele who has the Semitic "look"; but, in counterpoint to Kranson's argument as to the valid "goyische" appearance of the modern Jewish woman, the character of Quinn also destabilises the category of "shiksa."

Another way in which the distinctions between Quinn and Rachel are blurred occurs in "Prom-a-saurus," when the wheelchair-using Quinn runs for prom queen and uses her disability to garner sympathy votes, waiting for the right moment to take to the stage and dramatically reveal that she has regained the use of her legs. However, she relents and allows herself to be upstaged by the unexpected coronation of Rachel as prom queen. Unlike Tina's deference to Rachel, Quinn's act represents individual agency because she herself orchestrates the event to express her recognition that Rachel allegedly *deserves* a moment in

[4] See: http://gnatkip.dreamwidth.org/7793.html.

the spotlight too. Quinn's stunt with the wheelchair at prom is a carefully choreographed moment that calls to mind Rachel's self-aware posing of the body in "Theatricality."

"Theatricality" is the episode in which Rachel realizes that a rival glee coach, Shelby Corcoran, is her biological mother. The instance of recognition occurs, ironically enough, when Rachel witnesses the older woman performing the title song from *Funny Girl*. "Exactly what I would have done," Rachel whispers to herself. "Barbra. I could do it in my sleep." Even though Rachel is the adoptive daughter of a Jewish couple—thus removing the traditional necessity of matrilineal Jewish descent—the constant emphasis on her nose and hands and physical appearance ultimately suggests that Jewishness is biological as well. Indeed, Shelby goes on to adopt Puck and Quinn's child, stamping the infant with a sort of Jewish matrilineal seal of legitimacy. The casting of Idina Menzel as Rachel's birth mother seems to defend this line of thought—Menzel is "talented, Semitic looking," and, if not from Brooklyn, at least from Queens.3 The irony is that the show's Jewish actresses, like Agron, Menzel, and Jessalyn Gilsig, play characters with last names like Fabray, Corcoran, and Del Monico, which are not coded as Jewish family names. But in "Theatricality," Rachel recognizes her mother by the act of performance. In a self-aware line that comes close to breaking the fourth wall, Rachel observes, seated in an auditorium with her back to Shelby, that "[e]ven the way we're sitting right now is so dramatic and yet we feel so comfortable with it." A predisposition towards theatrics is once more trotted out as a hallmark of Jewish femininity.

> "RACHEL BECOMES NOT JUST THE JEWISH WOMAN, BUT THE JEW—AT THE EXPENSE OF THE SHOW'S OTHER JEWISH CHARACTERS, BUT ALSO AT THE EXPENSE OF HER OWN CHARACTERIZATION."

In the end, what necessarily constitutes being Jewish and a woman in America? Kranson's examination of Streisand's legacy and Kaiser's examination of the implications of the term "shiksa," establish that in contemporary Jewish life these demarcations and stereotypes of what Jewish womanhood *looks* and *acts* like are fluid. Both Kranson and Kaiser use actress Gwyneth Paltrow as an example of the Jewish woman as a contested identity. Kranson writes:

> While Paltrow does have a Jewish father …, she has never played a Jewish character. Her portrayals of white, Christian women (consider "Emma," "Shakespeare in Love") are not viewed as parodies. She is not marked as Jewish by her name, her look, her voice. As her pop-icon persona does not "perform" Jewishness, she can perform characters of other identities; in fact, her blonde, thin image is so stereotypically un-Jewish that having her portray a Jewish character seems like quite a stretch, If we think of her as Jewish at all, she is a Jewess in some serious WASP-y drag. (Kranson 2001, 36)

Or, as Kaiser puts it more succinctly, "Gwyneth Paltrow, despite her *yichus*, is a little bit shiksa" (Kaiser 2013, n. pag.). These labels hold little concrete meaning, and come across as arbitrary, in contemporary Jewish life—if indeed they were ever relevant, given how, as Adler describes, "[t]he most popular modern version of Jewish social history … sets up a sentimental account of pre-Holocaust Eastern European Judaism as a norm against which later Judaisms are measured and a frame through which earlier Judaisms are viewed" (Adler 1998, 110). To an extent, *Glee*'s casting of diverse actresses in diverse roles plays with the fluidity of a cultural dichotomy long portrayed as rigid, yet remains unwilling to definitively stake a claim as to whether Jewish womanhood is performative or embodied.

Ultimately, the brightest note in *Glee*'s portrayal of Jewish women is that Rachel, at the least, represents a new model of bourgeois success in terms of Jewish American media representation. Pogrebin has eviscerated the portrayal of Jewish women in Hollywood films, and remarked that "Baby hasn't come *all* the way. Today, she's going to *be* somebody—but she may not be *with* somebody. The forced choice is still there. Choose or Die. Either/Or. A great love or a full life" (Pogrebin 1991, 271). If Rachel is following in the tradition of decades of stereotypes surrounding Jewish womanhood, if she is achieving success at the cost of racial justice and ethnic authenticity, then at least—thirty years after Barbra Streisand and twenty years after Pogrebin's Jewish feminist memoirs—Rachel, daughter of an interracial gay couple from small-town Ohio, is going to have it *all*: the prom queen title, the Broadway stage, the leading man on her arm. Whether this constitutes a rounded character and a "full life," however, is still arguable.

Unfortunately, the show's writers' propensity for careless stereotyping minimizes the impact of such an endeavor. For example, the character of Tina Cohen-Chang provides an excellent opportunity to explore the intersection of race with Jewishness beyond the traditional model of what Pellegrini phrases as Freud's "boundary crises Aryan/Semite or Christian/Jew" (Pellegrini 1997, 123); but the opportunity is missed, when her Asianness restricts her Jewishness in ways that do not occur to white-coded and Christian-coded characters like Quinn Fabray and Shelby Corcoran. While *Glee* does play with the "myth of La Streisand," then, it is in the end content—through the vehicle of Rachel Berry and its co-option of Lea Michele's body—to reaffirm that myth of corporeally delimited and racially essentialist Jewish womanhood.

REFERENCES

1. Adler, Rachel. 1983. "I've Had Nothing Yet, So I Can't Take More." *Moment* 8: 22–26.
2. —. 1998. *Engendering Judaism.* Philadelphia: Jewish Publication Society.
3. Berkenwald, Leah. 2011. "Rachel Berry's nose job." *Jewesses With Attitude*, April 27. http://jwa.org/blog/rachel-berrys-nose-job.
4. Busis, Hillary. 2012. "Network Jews: Rachel Berry from FOX's 'Glee.'" *Jewcy*, May 22. http://www.jewcy.com/arts-and-culture/network-jews-rachel-berry-from-foxs-glee.
5. Butler, Judith. 1988. "Performative Acts and Gender Constitution: An Essay in Phenomenology and Feminist Theory." *Theatre Journal* 40, no. 4: 519–31.
6. Cohen, Shaye J.D. 2005. *Why Aren't Jewish Women Circumcised?: Gender and Covenant in Judaism.* Berkeley: University of California Press.

7. *Glee: The Complete First Season*. 2009–10. Beverly Hills, CA: 20th Century Fox Home Entertainment, 2010. DVD.
8. *Glee: The Complete Second Season*. 2010–11. Beverly Hills, CA: 20th Century Fox Home Entertainment, 2011. DVD.
9. *Glee: The Complete Third Season*. 2011–12. Beverly Hills, CA: 20th Century Fox Home Entertainment, 2012. DVD.
10. Gnat. 2010. "Glee art: Being a Part of Something Special (1.01 Pilot)." *Gnat*, January 31. http://gnatkip.dreamwidth.org/7793.html.
11. Ner-David, Haviva. 2000. *Life on the Fringes: A Feminist Journey Toward Traditional Rabbinic Ordination*. Needham, MA: JFL Books.
12. Kaiser, Menachem. 2013. "Anti-non-Semitism: An Investigation of the Shiksa." *Los Angeles Review of Books*, March 3. http://lareviewofbooks.org/article.php?type=&id=1461.
13. Kranson, Rachel. 2001. "The Myth of La Streisand." *Lilith* 25, no. 4: 36.
14. Pellegrini, Ann. 1997. "Whiteface Performances: 'Race,' Gender, and Jewish Bodies." *Jews and Other Differences: The New Jewish Cultural Studies*, edited by Jonathan Boyarin and Daniel Boyarin, 108-75. Minneapolis: University of Minnesota Press.
15. Pogrebin, Letty Cottin. 1991. *Deborah, Golda, and Me: Being Female and Jewish in America*. New York: Crown Publishers.

Leow Hui Min Annabeth is a second-year undergraduate at Stanford University. She is majoring in Comparative Studies in Race and Ethnicity, with a concentration in literature and ethnicity and a minor in English. Her essays have appeared in Transformative Works and Cultures, Bitch, and The WisCon Chronicles, among other publications. Her academic interests include postcolonial Anglophone literature, race and gender in science fiction and fantasy, and Chinese diasporic nationalism. She loves being, and is proud to be, part of the community in Casa Zapata.

COMT Genetics: How Scientists and the Media Promote a Heteronormative Binary

Kevin P. McPherson
Emory University

I. THESIS & PAPER INTRODUCTION

As he gripped the podium at his Nobel banquet speech in 1970, Dr. Julius Axelrod was prepared to defend science during a time when the Vietnam War was in full swing. A shadow of mistrust hung over basic science research during this era because many scientific discoveries were aiding in the construction of chemical and nuclear artillery.[1]

Dr. Axelrod, the discoverer of the catechol-O-methyltransferase (COMT) enzyme, was a fervent believer in the power of basic science research, even during times when the chants of "Make Love, Not War" echoed across the nation. In addressing Sweden's royalty and the Nobel Committee upon receiving his Nobel Prize in Physiology or Medicine, he thanked both parties for making his work highly visible to the general public. He stated, "This award comes at a time when our young and many of our most influential people believe that basic research is irrelevant or is put to evil uses." He began to ponder the impact of his discovery, saying, "[it] gives us an opportunity to show how misinformed and mistaken they are." Axelrod promised that the work recognized by the Nobel Committee would one day explain such illnesses as depression, Parkinson's disease, and drug abuse, "and lead the way to the treatment of these terrible afflictions."[2] Dr. Axelrod's hopes and aspirations for understanding COMT could most definitely be labeled as hopeful; maybe even idealistic. Axelrod's hope was not once overshadowed by a shred of doubt in science.

Dr. Axelrod proved to have quite the foresight in his prediction because as science research entered the "genomics era ,"[3] the COMT gene was identified and variations were noted. By the late 1990s, the COMT gene was seen to have two major variants, or functional translated proteins.[4] These two functional proteins differed in only one amino acid, but these functions emerged to ruffle a lot more than the lab coats of a few preeminent molecular biologists.

The COMT enzyme and the gene that regulates its two variant functions have an extensive history that is still being built upon today in laboratories across the nation. Much of what is understood about COMT and its functional variants falls under the realms of science, psychology, and modern medicine. However, if the magnifying lens is lifted, and a broader view is adopted, we can see many surprising implications for the research and the results being reported – implications that may spell more out for how we understand gender roles, illness reporting, and character traits due to genetics. Indeed, at first sight, it would seem that no real structure can be ascribed to how we understand COMT. As this paper examines, the previous statement is blatantly false. Since the advent of the COMT enzyme's discovery and the subsequent discovery of the two main gene variants (which will be described later), COMT genetics research has successfully created multiple binaries that mirror the larger, historical themes of what separates gender, what constitutes femininity versus masculinity, and what reinforces gender roles in society. In summary, COMT genetics research reinforces the idea of heteronormative roles of society through multiple avenues. Disease etiology and reporting are the foundation for an overarching dualism. Speculations of characteristic traits reinforce this dualism, and translation of the research to a simplified model such as the "warrior versus worrier" model allows popular culture and the media to feed the layperson a heternormative standard.

Dr. Axelrod was able to foresee the benefits of studying the COMT enzyme, but never could he have foreseen the general population's attachment to basic science's experimental results. Nor could have he predicted something else: the subtle and misogynistic alignment science would take with gender stereotypes put forth nearly a century earlier. Even more, how the science research he so coveted would promote and accentuate that which would come to be known as heternormativity; more specifically, the concept that people fall into two distinct genders (male or female) and these genders have specific qualities that one can term "masculine" or "feminine."

II. THE BASIC RESEARCH AND ITS ILLUSTRATIONS

The scientific literature on COMT enzyme and its genetics has grown since Axelrod's discoveries: the amount of published literature on the COMT molecule in the past 10 years alone exceeds over 2200 articles. In 1995, researchers at the National Public Health Institute in Helsinki, Finland began to research a new variant in the COMT enzyme. Originally, it was discovered that the COMT enzyme was made up of a string of more than 150

[1] Bronson, Po, and Ashley Merryman. *Top Dog: The Science of Winning and Losing*.. Kindle Edition.
[2] Ibid.
[3] The genomics era of science research is described as a period of time from 1972-present (this latter date is debatable, some researchers say we are in a post-genomic era). More information on the genomic era of research can be found at http://www.genome.gov/19016618 (Francis Collins's address).
[4] Lotta T, Vidgren J, Tilgmann C, *et al.* (April 1995). "Kinetics of human soluble and membrane-bound catechol O methyltransferase: a revised mechanism and description of the thermolabile variant of the enzyme". *Biochemistry* 34 (13): 4208
[5] Ibid, 4202.

codons or amino-acid encoding regions.[5] What makes COMT special is its ability to still function despite a single nucleotide polymorphism (SNP) in the 158th codon. This codon, which originally encodes for a methionine amino acid (a nucleotide sequence of ATG) is altered in the SNP and reflects a single nucleotide change, resulting in a GTG reading sequence. This GTG does not code for the same amino acid as ATG, but rather, it codes for a valine.[6] This one codon change results in what Bronson and Merryman call a "lazy to hardworking" switch.[7] The original codon that produces a methionine results in slow-acting COMT activity. The valine polymorphism results in fast-acting COMT activity.

How does this translate physiologically? Many studies have examined the Val158Met polymorphism in greater detail. A current search in PubMed on "Val158Met" yields more than 280 results in the last 10 years! Studies range gauging levels of personal distress to emotional empathy to prevalence of schizophrenia; some studies even predict that COMT may affect sleep cycles in older adults and autism in Egyptian children. However, if you sift through all of the literature to find the basal function of the valine polymorphism, you'll find a common theme: it is most often compared and contrasted with its wild type counterpart, the unaltered methionine product. This is because these two variants are most found in the prefrontal cortex of the brain, the main physiological region of study for COMT.

The prefrontal cortex (or PFC, for short) has always been a mysterious portion of the brain. From early on, the PFC has played a role in neurology as being the center of behavior. The seminal case in prefrontal cortex function is most certainly that of Phineas Gage, whose prefrontal cortex was destroyed when an iron rod, an inch and a fourth in diameter, was driven through his skull, near his eye socket, during a railroad maintenance accident. Gage survived the accident and was able to retain normal speech, motor skills, and memory, but his behavior had changed drastically. He was more irritable, quick-tempered, and impatient. These characteristics were not like the former Gage.[8]

Much like Gage's altered behavior, the type of COMT enzyme a person's gene encodes for can tell a lot about how their prefrontal cortex reacts with a neurotransmitter called dopamine, especially during stress response. Dopamine is the neurotransmitter associated with reward.[9] When the brain is flooded with dopamine, it acts in a way to sustain reward-activating stimuli. For instance, extraversion is linked to high sensitivity to dopamine. Although dopamine would seem like a beneficial neurotransmitter, too much is not a good thing, especially when located in the prefrontal cortex. Just like an engine that is flooded with gasoline, if the PFC is overloaded with dopamine, it tends to "meltdown," especially since dopamine is released as a stress response by the sympathetic nerve system.[10] This is where COMT enzyme comes in: the COMT enzyme is responsible for breaking down dopamine and syphoning it out of the prefrontal cortex. COMT has been pinpointed to be the primary enzyme in the PFC to do so since there are no other primary transfer proteins located in the region to break down this neurotransmitter.[11]

Although it would seem intuitive to think that an excess of dopamine in the system may help with executive tasks, the converse is actually true. If the brain can get rid of dopamine faster during times of high stress, the executive mechanisms of the PFC work more efficiently. Therefore, people who have inherited the valine polymorphism are at an advantage. They don't freeze up during high pressure situations. Their COMT enzyme is "hardworking," depriving the PFC of indulging in dopamine, while other people's COMT enzyme is "lazy," allowing the brain to drown in dopamine under high stress conditions.[12]

In addition to marked differences handling stressful situations, people who express the multitude of variants of the COMT enzyme tend to display different behavior. Take the following case study about a mother whose sons appear to live two drastically different lives:

> *"Jolene was discussing her fraternal twin sons with her primary care physician. It was amazing how different they were. Jason loved to be out¬side, excelled at downhill skating, and looked for¬ward to anything that involved thrills and spills (martial arts, roller coasters, etc.). John, on the other hand, loved reading, was superb at chess, and tried to avoid anything that involved possible injury (martial arts, roller coasters, etc.). She was quite sure that her sons had differed from birth; although she had provided them with the same home, they had developed different likes and dif¬ferent skills. She found it necessary to respond to them in entirely different ways in order to prevent the various excesses that each was prone to and to bring out the best in them." [13]*

To explain how Jason and John enjoy divergent lifestyles, scientists have claimed that those who are "thrill seeking," like Jason, seek these thrills because their COMT enzyme is overactive – it is always depriving their brain of an excess of dopamine. In turn, they seek out experiences that induce stress. On the other hand, those who have an excess of dopamine tend to have better working memory[14], are more creative[15], and usually have high IQs[16]. They tend to seek activities that engage these functions, and they are perceived as more "tame" individuals. Just as Stein et. al., has noted, parents like Jolene are learning to engage their children in different ways in order to bring out the best in them. The same goes for Noah and his mother Ms. Kathleen Muthler, who is particularly keen to Noah's response to stress (he has a

[6] Throughout the paper, I will be actively switching between the two na mes for this polymorphism: Val158Met is the common scientific way to address the polymorphism, but some authors refer to it as the 'valine polymorphism.' Both are the same, and I choose the one used in each reference based on stylistic preferences for the sentence.
[7] Bronson and Merryman, Top Dog: *the Science of Winning and Losing*, Kindle Edition.
[8] Malcolm Macmillan, *An Odd Kind of Fame: Stories of Phineas Gage* (MIT Press, 2000), pp.116-119, 307-333, esp. pp.11,333.
[9] Depue, R. A., & Collins, P. F. (1999). Neurobiology of thestructure of personality: Dopamine, facilitation of incentive motivation, and extraversion. *Behavioral and Brain Sciences*, 22, 491–517.
[10] Bronson and Merryman, Top Dog: *The Science of Winning and Losing*. Kindle Edition.
[11] Stein, Dan J., Timothy K. Newman, Jonathan Savitz, and Rajkumar Ramesar. "Warriors Versus Worriers: The Role of COMT Gene Variants." *CNS Spectrums* 11, no. 10 (2006): 745.
[12] Bronson and Merryman, Top Dog: *The Science of Winning and Losing*. Kindle Edition.
[13] Stein et. al. *Warriors Versus Worriers: The Role of COMT Gene Variants*, 4.
[14] Bronson and Merryman, Top Dog: *The Science of Winning and Losing*. Kindle Edition.
[15] Ibid.
[16] Bruder, Gerard E., John G. Keilp, Haiyan Xu, Marina Shikhman, Efrat Schori, Jack M. Gorman, and T. Conrad Gilliam. "Catechol-O-methyltransferase (COMT) genotypes and working memory: associations with differing cognitive operations." *Biological psychiatry* 58, no. 11 (2005): 902.

"lazy" COMT gene):

> *"Noah Muthler took his first state standardized test in third grade at the Spring Cove Elementary School in Roaring Spring, Pa. It was a miserable experience, said his mother, Kathleen Muthler. He was a good student in a program for gifted children. But, Muthler said, "he was crying in my arms the night before the test, saying: 'I'm not ready, Mom. They didn't teach us everything that will be on the test.' " In fourth grade, he was upset the whole week before the exam. "He manifests it physically," his mother said. "He got headaches and stomachaches. He would ask not to go to school." [17]*

Noah has since switched schools, from public to private, where standardized tests are not emphasized as part of the curriculum. Ms. Muthler notes that she has noticed a difference in his demeanor: "The pressure is off his shoulders now," his mother said. When he doesn't grasp a concept immediately, he can talk it through without any panic. "He looks forward to science class and math class again," Muthler said. Noah evenreports that he's thinking about being a nuclear engineer.[18]

It is interesting to see how this one tiny molecule can govern our choice of schools and how parents react to their kids. Even more so, how the subjects act and the traits they exhibit. In the past decade, biologists have examined COMT, and specifically the two functional phenotypes' effect on behavior. Moreover, there is a growing cache of scientific literature that studies COMT and its modulation due to sex difference (between male and female). Some of the reported results are shocking. For instance, women with high estrogen have lower COMT activity and may be more prone to stress, worry, and mental breakdown.[19] In addition, males score lower on tests that measure harm avoidance, behavior inhibition, and higher on sensation seeking when they have the Val158Met polymorphism.[20] That is to say, men with the valine polymorphism sought out harmful experiences and had a tendency not to decrease harmful behavior, while seeking out more thrills.

When surveying literature related to schizophrenia, a hot target for current COMT gene research[21], scientists have already shifted gears in terms of research participants. Wichers reports that studies with heavy participation by women are not confounded due to the "sex-specific effects of the COMT polymorphisms."[22] This has led to multiple studies in COMT-related research linking women to higher risk of schizophrenia and other psychoses.[23]

Although scientists are required to report all such results, there is particular significance to those outlined above. The results are stark in contrast to each other; not only do they transcend genetic differences, they are modulated by a belief in sex difference. As Chen et. al. writes, "Beyond the COMT gene and personality, of course, there is a growing literature of sex-dependent genetic effects on behavior."[24] Such a statement signals that there is already a scientific search to separate behavior of sexes due to genetics. Wichers's comments alone call attention to a belief in science that studies already establishing sex-difference disease etiology should not be challenged. So what does this mean for the current state of COMT genetics research? Perhaps looking a century earlier will give us the answers we most desire.

III. A HISTORICAL PERSPECTIVE: THE GENDER BINARY

Far before Dr. Julius Axelrod accepted his Nobel Prize, there were important shifts in society that were taking place; shifts that would have an impact on how we perceive the studies of COMT genetics. Between 1820 and the Civil War, the Industrial Revolution had taken grip of the United States. The Industrial Revolution, noted for its boom in scientific and technological advances, also had a profound effect upon the citizens who made up the American society and participated in the American workforce.[25]

Unlike the agrarian economy that preceded it, the industrialized world began to see a change in the participants that contributed to a family's wellbeing and income. No longer did the family have to make what it needed to survive. Industrialized job produced goods or services that the men in the family could reap benefits from, and provide for the family, while the women stayed at home and took care of the children. The middle class of America slowly started to change into families that included men as office workers, lawyers, physicians, and factory workers. This ultimately took the man away from the home for a good portion of the day, in turn separating men and women, physically. However, this separation would prove to be a much stronger symbolic illustration of new societal norms.

As the men went off to work, the male-dominant workforce helped create a view that men alone should support the family. In addition, there was a belief that surrounded the world of work, the public sphere, as one that was rough and unpredictable. In order to succeed, a man had to battle the temptations, violence, and trouble that riddled the outside world. On the other hand, women were left to succeed in the private sphere. Success at home meant dealing with a relatively unvarying and sheltered home life. The separation of where women and men were able to succeed began to reflect on the gender of women and men, individually, and soon, women were seen as the delicate and weak creatures who should not venture out into the harsh world of the

[17] Bronson and Merryman. *Why Can Some Kids Handle Pressure While Others Fall Apart?* Digital Access.
[18] Ibid.
[19] Crick, Nicki R., and Carolyn Zahn–Waxler. "The Development of Psychopathology in Females and Males: Current Progress and Future Challenges." *Development and Psychopathology* 15, no. 03 (2003): 719–742.
[20] Chen, Chunhui, Chuansheng Chen, Robert Moyzis, Qi Dong, Qinghua He, Bi Zhu, Jin Li, He Li, Jun Li, and Jared Lessard. "Sex Modulates the Associations Between the COMT Gene and Personality Traits." *Neuropsychopharmacology* 36, no. 8 (2011): 1593–1598.
[21] Shifman, Sagiv, Michal Bronstein, Meira Sternfeld, Anne Pisanté-Shalom, Efrat Lev-Lehman, Avraham Weizman, Ilya Reznik, Baruch Spivak, Nimrod Grisaru, and Leon Karp. "A Highly Significant Association Between a COMT Haplotype and Schizophrenia." *American Journal of Human Genetics* 71, no. 6 (2002): 1296.
[22] Wichers, Marieke, Mari Aguilera, Gunter Kenis, Lydia Krabbendam,Inez Myin-Germeys, Nele Jacobs, Frenk Peeters, Catherine Derom,Robert Vlietinck, and Ron Mengelers. "The catechol-O-methyl Transferase Val158Met Polymorphism and Experience of Reward in the Flow of Daily Life." *Neuropsychopharmacology* 33, no. 13(2007): 3030–3036.
[23] Leung, M. D. "Sex Differences in Schizophrenia, a Review of the Literature." *Acta Psychiatrica Scandinavica* 101, no. 401 (2000): 3–38.
[24] Chen et. al. *Sex Modulates the Associations Between the COMT Gene and Personality Traits.* 1593.
[25] Brannon, L. *Gender: Psychological Perspectives*, 152.

TABLE 7.1 *Elements of Stereotyping of Women and Men*

The Cult of True Womanhood	*Male Sex Role Identity*
Piety: True Women were naturally religious.	*No Sissy Stuff:* A stigma is attached to feminine characteristics.
Purity: True Women were sexually uninterested.	*The Big Wheel:* Men need success and status.
Submissiveness: True Women were weak, dependent, and timid.	*The Sturdy Oak:* Men should have toughness, confidence, and self-reliance.
Domesticity: True Women's domain was in the home.	*Give 'Em Hell:* Men should have an aura of aggression, daring, and violence.

Sources: Based on "The Male Sex Role: Our Culture's Blueprint of Manhood and What It's Done for Us Lately," (p. 12), by Robert Brannon, in Deborah S. David & Robert Brannon (Eds.), *The Forty-Nine Percent Majority*, 1976, Reading, MA: Addison-Wesley; and "The Cult of True Womanhood: 1820–1860," by Barbara Welter, in Michael Gordon (Ed.), *The American Family in Social-Historical Perspective* (2nd ed.). New York: St. Martin's Press.

men, lest they fall prey to all of the dangers that entrenched the public sphere.

The exclusion from either sphere by either gender created what is known as the Doctrine of Two Spheres. The doctrine espouses that men and women have interests that diverge completely – that men and women have their separate areas of influence. Women are influential over the home and the children, while men are influential in the workplace and in the outside world. These two realms have fairly little overlap and position themselves at opposite ends of one dimension, creating what is known as a binary, or a system that is defined by its reliance on two distinct definitions to describe all actions and interactions within it. This conception of a binary not only dictated social views of gender, but psychology's measurement of what embodied masculinity and femininity.[26]

Around the same period as the Industrial Revolution, the Cult of True Womanhood emerged. The Cult of True Womanhood could be seen represented everywhere in popular culture of the 19th century – from magazines to religious journals to advice books and even fiction. The Cult of True Womanhood stated, "The attributes of True Womanhood, by which a woman judged herself and was judged by her husband, her neighbors, and society could be divided into four cardinal virtues—piety, purity, submissiveness, and domesticity" The virtues set about by the Cult of True Womanhood created a new ideal for the Victorian woman to live by, promising the woman power and happiness in her new role put forth by the industrialization of the modern world. Without the Cult of True Womanhood and the characteristics it so voraciously promoted, the Victorian woman could never live a life of full meaning.[27]

Of course, such a life of meaning for women garnered consequences for the men of the new industrialized world. Men were seen as opposites of women in multiple ways: women were delicate, dependent, passive, and timid; men were active, independent, coarse, and strong. Although the Cult of True Womanhood and the virtues it was founded upon reached its height in the late 19th century, what is referred to as the Male Sex Role Identity, has roots back to the late 16th century. As men entered the industrialized 19th century, the Male Sex Role Identity became reinvigorated and redefined into its modern concept.

For instance, as society shifted to industrialization, it was believed that men were not as masculine as they once were because industrialization pressured men to find positions that helped to provide for the family. Fulfilling these roles became increasing difficult for men, thus decreasing the masculinity they once had.[28] However, the Male Sex Role Identity was revitalized when men began to seek education to become competitive in the workforce. The role of an educator, usually held by women of the time, sought to train boys to be "well-behaved pupils," or in other words, "sissies." Soon, the prohibition of being a sissy and the rejection of the feminine became a concept in what formulated masculinity. Such concepts still live on today, and a figure outlining the views of the Male Sex Role can be seen above.[29]

Just as the traits of a "true woman" were standards from which the woman lived and gained happiness, so were the concepts that constituted the Male Sex Role Identity. The closer a man conformed to the characteristics set forth by this identity, the closer he was to a "real man." Furthermore, the pressures to live up to these ideals of masculinity are strong.[30] This mirrors the similar pressure women have to live up to the ideals of being a "true woman": both are stringent in their principles and harsh in their consequences. An all-or-nothing contract with the man or woman who believes in these principles created a volatile situation for those seeking acceptance into the new, modern, industrialized society. The Cult of True Womanhood and the Male Sex Role Identity may be seen in today's modern society as artifacts of history because multiple groups identifying as transgender, homosexual, and androgyne; giving the illusion that present society appears to be past such a polarizing binary.

Although the concepts of the Cult of True Womanhood have deconstructed into new ideas about what role in society women should take, both the Cult of True Womanhood and the Male

26 Ibid, 170.
27 Ibid, 172.
28 Brannon, L. *Gender: Psychological Perspectives*, 156.
29 The figure comes from: Brannon, Linda. Gender: Psychological Perspectives. 6th ed. New Jersey: Pearson, 2010. However, as noted on the image, the ideas within the table come from another source that is cited by Brannon. (see text below the figure).
30 Ibid, 154.

Sex Role Identity were prominent markers of influence about what once constituted a social norm.[31] They are also catalysts for the emergence of gender stereotype and what it is to be a man or a woman. A significant question to ask now is: do only the categories 'man' and 'woman' exist? If not, why are we holding on to such virtues of what constitutes either? Modern feminist and social theory argue that there are now multiple genders that take up a wide spectrum of human life, but it cannot be ignored that modernity has established what is known as sexual order due to movements such as the Cult of True Womanhood and concepts like the Male Sex Role Identity.[32]

As we enter a modern age that reveals the roles of many different people of many different combinations of genders and sexualities, we begin to see a centuries-old structure upon which such people are fighting for acceptance – usually in vociferous opposition of the current order in place. The order, in which most of these people do not "fit", is known as a heteronormative structure. Heteronormativity can be defined as the lifestyle norms that hold humans to be in distinct and complementary groups, either male or female, and the natural roles of life which male or female inhabit.[33]

Heteronormativity is a fuller, more modern description of the binary set about by the Cult of True Womanhood and the Male Sex Role Identity because it describes a societal norm that only recognizes one or the other (male or female), not any mixture of both, along with traits that define masculinity and femininity. Heteronormativity is also governed by many concepts that arose in the birth of industrialization: the adherence to the nuclear family, the social contract of marriage between only a man and a woman, and finally, the roles that each of these players took within the society that surrounded their family unit. This created a social order that is still prevalent today, governed by a binary and founded upon the aforementioned gender stereotypes.[34]

In greater examination, we can see that today's society has a structure that is adherent to the heteronormative binary; the binary is prolonged by the sets of laws and social conventions like same sex marriage not being federally recognized and most states not passing laws that make same sex marriage legal, for instance. If we extend the influence of heteronormativity outward, we can begin to look at spheres of life that have been affected by the heteronormative binary or, and maybe even more compelling, are promoting the heteronormative binary by adhering to definitions of masculinity and femininity that mirror the Cult of True Womanhood or the Male Sex Role Identity.

The fields of basic science are interesting candidates to look at. In a modern society that is wrapped in the idea of genomic medicine and the power of the "script" that constitutes ones genome, basic science pushes itself to forefront. At the forefront, basic science maintains an influence in the creation of new policies of the modern society. If our goal is to be critical of a modern society that promotes a heteronormative binary, and science is a major influence in modern policy, then basic science is the most prime candidate for critical analysis.

"Muthler understands Noah's distress; more mysterious is why her son Jacob, who is in eighth grade, isn't the least bit unnerved by the same tests. He, too, is in the gifted program, but that seems to give him breezy confidence, not fear."

"The Worriers become distressed and frustrated: they become unable to switch strategies or see something in a new way."

"The Worriers score about 8% lower than the Warriors. The Worriers struggle the most on the academic subjects that tax working memory the hardest: science, social science, and math."

IV. IMPLICATIONS AND CONNECTIONS TO COMT RESEARCH

Reflecting upon which science is most worthy of critical analysis, the COMT enzyme and the study of its genetic phenotypes are even more compelling. COMT, as outlined in Section III[35], can be linked to many executive functions, mental illnesses, and characteristics. Even more importantly, it plays a very singular role in the brain, and more specifically, the prefrontal cortex. On a purely biological and philosophical level, the brain, in all its complexity and facilitative function, is what separates humans from all other animal life forms. Therefore, the analysis of genes that govern decision-making functions[36], makes the COMT gene a viable candidate for meticulous critique.

However, it is the evidence that is gathered about COMT, and the manner in which it is presented, that are of more interest to this paper, not purely the function and ultimate phenotypes of COMT itself. The results that scientists have concluded and so precisely summed up in multiple journal articles are neither immune to oblique language nor stereotypical dualities that have already been prominent in the society. As in "Sexing the Body," Anne Fausto-Sterling is concerned with the presentation of dualisms to make arguments, as she deconstructs the idea that there is only nature or nuture and gender or sex. Fausto-Sterling makes a compelling point about arguments in general. She says that for ages, dualisms are used to explain how Euro-American views are shaped. Pairs of opposing concepts, objects, or belief systems are employed to make hierarchical arguments.[37] This can most definitely be seen in the shift of COMT genetics towards sex difference modulation. With each associated behavior, male-dominant or female-dominant outcomes are assigned.

Additionally, philosopher Val Plumwood notes that the use of dualisms is intertwined with a set of culture associations and connotations. "Culture accumulates these dualisms as a store of weapons," she writes. "[These] can be mined, refined and redeployed. Old oppressions stored as dualisms facilitate and break the path for new ones."[38]

Plumwood and Fausto-Sterling's analysis of these dualities bare a significant aftermath on how we view COMT scientific experiments and the anecdotes of people with the "lazy" COMT gene versus people with the "hardworking" COMT gene. In Stein et. al's article "Warriors Versus Worriers: The Role of COMT

31 Ibid, 158.
32 Ibid, 159.
33 Ibid, 161.
34 Warner, Michael. "Introduction: Fear of a Queer Planet." *Social Text* 29 (1991): 8-10.
35 Found on pgs. 64-65.
36 Like the creation of legislation or economic decisions.
37 Fausto-Sterling, Anne. *Sexing the Body: Gender Politics and the Construction of Sexuality*. New York, NY: Basic Books, 2000.
38 Plumwood, Val. *Feminism and the Mastery of Nature*. New York, NY: Routledge, 1993.

Gene Variants," Stein proclaims his strident belief in the hard evidence science has collected. He notes, "Individual differences are not confined to super¬ficial appearances; they more likely reflect crucial differences in underlying genes, circuits, and cognitive-affective function." This statement signals surrender to the evidence at hand: genes, and what they encode, are the sole determinant of what we see. Therefore, the simplistic model Stein subscribes to makes it easy and appears logical to make the assumption that, "when it comes to contrasting warriors with worriers, a growing set of studies suggests that specific variants in the COMT gene may play a crucial role in the embodi¬ment of this distinction." It is with this surrender to the evidence that we see the duality re-deployed in science. Just like there is master and slave, day and night, there are two absolute variants of COMT that govern what Stein calls the "warriors" and the "worriers." That is, warriors are able to withstand stressors and most obstacles that stand in their way, while the worriers whimper in the shadows. Therefore, scientific researchers have adopted the most subtle of dualities, which manifests physically in the COMT variants, to make conclusions about the behavior of the wider population. Through the use of this dualism, the media is able to employ heteronormative language.[39]

In the genomics era, we are then either "warriors" or "worriers," as branded by the media and showcased throughout the general population. It also seems these two roles are destine to two totally different fates and are most influential when their phenotypes are not at a disadvantage (does this ring a historical bell?). Po and Merryman make this destiny even more explicit, outlining in their book that warriors are good for some tasks, while worriers are good for other tasks[40]:

> *"Muthler understands Noah's distress; more mysterious is why her son Jacob, who is in eighth grade, isn't the least bit unnerved by the same tests. He, too, is in the gifted program, but that seems to give him breezy confidence, not fear."*[41]
>
> *"The Worriers become distressed and frustrated: they become unable to switch strategies or see something in a new way."*[42]
>
> *"The Worriers score about 8% lower than the Warriors. The Worriers struggle the most on the academic subjects that tax working memory the hardest: science, social science, and math."*[43]

The language in the preceding excerpts that describe the "warriors" - recovery, breeziness, confidence, and better performance - is in stark contrast to the language used to describe the worriers - struggling, distressed, and frustrated. When this language is projected upon a person, without context of who the person is or what accomplishments and titles they may hold, it anthropomorphizes the gene itself.[44] The gene that encodes for "warrior" is resilient and strong, while the gene that encodes for "worrier" is *fragile* and needs some sort of aid to overcome its inherent disadvantage. Even within the names "warrior" and "worrier" themselves, it is safe to say that the terms strike some form of imagery into the reader's mind. Do we see the worrier as the one out on the front lines, muscles bulging and ready to face any challenge? Do we image the warrior at home, cowering in fear of what will happen next? Surely, the answer is no for both of these questions.

The binary then becomes a concoction of the media: those who write about the duality already established by scientists have taken the process one step further by giving human characteristics to the inanimate gene. The process of making the gene an extension of the human character is anthropomorphizing the gene. This anthropomorphization describes the gene in terms of characteristics the reader of the media relates, feels and connects to. Therefore, when connected with the human who is associated with the gene, the gene's advantages and disadvantages are projected onto the human, as Kwan concluded, "How people perceive nonhuman agents therefore utilizes the same mechanisms involved when people think about other people." By this logic, gender stereotypes can accompany such inanimate genes as with animate people.[45]

If we examine the gene's phenotypic qualities, as reported by scientific evidence, and the labels of a "warrior" versus a "worrier," we begin to see surprising familiarities. The characteristics of what it takes to fulfill the Male Sex Role Identity and the Cult of True Womanhood mime projections of the valine COMT gene (the "warriors") and the methionine COMT gene (the "worriers"), respectively. Resilient, less prone to stress, and thrill seeking, the warriors imitate that of the Male Sex Role Identity. More fragile to stress response, more prone to schizophrenia and other psychoses, and seeking of more "tame" activities, the worriers imitate that of the Cult of True Womanhood. This is not purely coincidence, but rather a carefully orchestrated pattern put forth by scientific evidence, the lack of knowledge about scientific evidence by the media, and the ultimate actions taken by the influenced laypeople.

V. CONCLUSIONS

In conclusion, it is a specific lens that is constructed by historical predispositions that influences the way scientific experiments are conducted and reported. In the case of COMT genetics, the field of research and its current shifts towards sex-dependent modulation of the gene are actively being dictated by the male/female binary created by the Cult of True Womanhood and the Male Sex Role Identity, put forth more than a century ago. If we examine the characteristics that make up both of these concepts,

[39] In Warner, Michael. "Introduction: Fear of a Queer Planet."*Social Text* 29 (1991): 5, Warner describes that social order dictates the norm language that society uses. He specifically points out that heternormative language fails to represent any gray area, but rather stick to a "black and white" spectrum of description.

[40] It is important to note that these other tasks are not so explicitly defined by Po and Merryman. This may signal an important omission from the literature based on either a) lack of information or b) lack of knowledge to assess evidence. Altogether, Po and Merryman do acknowledge that being a "worrier" is not all gloom and doom, but they do not spend as much time remediating the issue.

[41] Ibid.

[42] Ibid. This is a reference to the Taiwanese standardized tests taken by teenagers to get into the 8th

[43] It is interesting to note the reference to these subjects at this juncture. I thought about this subject more and more as the paper went on. Is it possible that these subjects, fields which are normally dominated by males, in the workforce, were mentioned specifically? I tried to create a connection through labor statistics, but the argument wasn't compelling enough for me to keep in the paper.

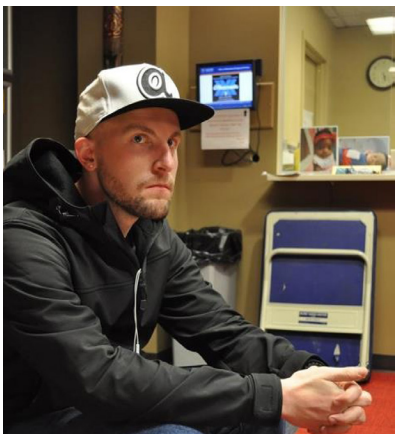
[44] Epley, Nicholas, Adam Waytz, Scott Akalis, and John T. Cacioppo. "When We Need a Human: Motivational Determinants of Anthropomorphism." *Social Cognition* 26, no. 2 (2008): 143.

[45] Ibid, 147.

we find surprising similarities to the results reported today: "warrior" phenotypes are almost unilaterally labeled with "male" characteristics, while "worrier" phenotypes are almost unilaterally labeled with "female" characteristics. As the media becomes a prime player in translating these results of basic scientific research, much can get lost in translation, and many details are propagated through the relationship the media has with science. In context of the COMT gene, media's misunderstanding and subsequent anthropomorphization of the gene only further promote the gender stereotype attached with the gene's various phenotypes. What looks like a pristine model of interaction between science and society is actually promoting a heteronormative binary onto the general population.

REFERENCES

1. Brannon, Linda. Gender: Psychological Perspectives. 6th ed. New Jersey: Pearson, 2010.
2. Bronson, Po, and Ashley Merryman. Top Dog: The Science of Winning and Losing. New York, NY: Hachette Book Group, 2013. Kindle Edition.
3. Bronson, Po, and Ashley Merryman. "Why Can Some Kids Handle Pressure While Others Fall Apart?" The New York Times, Magazine Edition, February 6, 2013.
4. Chen, Chunhui, Chuansheng Chen, Robert Moyzis, Qi Dong, Qinghua He, Bi Zhu, Jin Li, He Li, Jun Li, and Jared Lessard. "Sex Modulates the Associations Between the COMT Gene and Personality Traits." Neuropsychopharmacology 36, no. 8 (2011): 1593–1598.
5. Crick, Nicki R., and Carolyn Zahn–Waxler. "The Development of Psychopathology in Females and Males: Current Progress and Future Challenges." Development and Psychopathology 15, no. 03 (2003): 719–742.
6. Depue, R. A., & Collins, P. F. (1999). Neurobiology of the structure of personality: Dopamine, facilitation of incentive motivation, and extraversion. Behavioral and Brain Sciences, 22, 491–517.
7. Epley, Nicholas, Adam Waytz, Scott Akalis, and John T. Cacioppo. "When We Need a Human: Motivational Determinants of Anthropomorphism." Social Cognition 26, no. 2 (2008): 143–155.
8. Evans, Erv. "Tree Anatomy". Last modified May 09, 2000. http://www.ces.ncsu.edu/depts/hort/consumer/factsheets/trees-new/text/tree_anatomy.html.
9. Fausto-Sterling, Anne. Sexing the Body: Gender Politics and the Construction of Sexuality. New York, NY: Basic Books, 2000.
10. Genome Program, US Department of Energy. "How Many Genes Are In The Human Genome". Last modified September 18, 2008. http://www.ornl.gov/sci/techresources/Human_Genome/faq/genenumber.shtml.
11. Handoko, H Y, D R Nyholt, N K Hayward, D A Nertney, D E Hannah, L C Windus, C M McCormack, et al. "Separate and Interacting Effects Within the catechol-O-methyltransferase (COMT) Are Associated with Schizophrenia." Molecular Psychiatry 10, no. 6 (October 26, 2004): 589–597. doi:10.1038/sj.mp.4001606.
12. Harrison, Paul J., and Elizabeth M. Tunbridge. "Catechol-O-methyltransferase (COMT): a Gene Contributing to Sex Differences in Brain Function, and to Sexual Dimorphism in the Predisposition to Psychiatric Disorders." Neuropsychopharmacology 33, no. 13 (2007): 3037–3045.
13. Katz, Jonathan N. The Invention of Heterosexuality. 2nd ed. Chicago, Illinois: University of Chicago Press, 2007.
14. Leung, M. D. "Sex Differences in Schizophrenia, a Review of the Literature." Acta Psychiatrica Scandinavica 101, no. 401 (2000): 3–38.
15. Lotta T, Vidgren J, Tilgmann C, et al. (April 1995). "Kinetics of human soluble and membrane-bound catechol O-methyltransferase: a revised mechanism and description of the thermolabile variant of the enzyme". Biochemistry 34 (13): 4202–10.
16. Plumwood, Val. Feminism and the Mastery of Nature. New York, NY: Routledge, 1993.
17. Shifman, Sagiv, Michal Bronstein, Meira Sternfeld, Anne Pisanté-Shalom, Efrat Lev-Lehman, Avraham Weizman, Ilya Reznik, Baruch Spivak, Nimrod Grisaru, and Leon Karp. "A Highly Significant Association Between a COMT Haplotype and Schizophrenia." American Journal of Human Genetics 71, no. 6 (2002): 1296.
18. Stein, Dan J., Timothy K. Newman, Jonathan Savitz, and Rajkumar Ramesar. "Warriors Versus Worriers: The Role of COMT Gene Variants." CNS Spectrums 11, no. 10 (2006): 745.
19. Warner, Michael. "Introduction: Fear of a Queer Planet."Social Text 29 (1991): 3–17.
20. Weiss, Jillian Todd. "Gender Caste System: Identity, Privacy, and Heteronormativity, The." Law & Sexuality: Rev. Lesbian, Gay, Bisexual & Transgender Legal Issues 10 (2001): 123.
21. Wichers, Marieke, Mari Aguilera, Gunter Kenis, Lydia Krabbendam, Inez Myin-Germeys, Nele Jacobs, Frenk Peeters, Catherine Derom,Robert Vlietinck, and Ron Mengelers. "The catechol-O-methyl Transferase Val158Met Polymorphism and Experience of Reward in the Flow of Daily Life." Neuropsychopharmacology 33, no. 13(2007): 3030–3036.



Kevin McPherson is a junior at Emory University in Atlanta, Georgia majoring in biology and interdisciplinary studies with a focus on scientific racism. In addition to being a Ronald E. McNair Scholar, Kevin will be an Amgen Scholar at Stanford during the summer of 2014. Kevin enjoys connecting the sciences and the humanities and hopes that one day he can pursue an interdisciplinary PhD in science and technology studies (STS). Kevin plans to be a professor who creates bilingual science curricula while working with Native American students who are interested in science.

West Side Story and Its Place In Queer History

David Kay
Stanford University

In 1957, the United States was at a crossroads in regards to the LGBT community. Old conceptions of sexuality were beginning to be challenged in all aspects of life, with factions forming in both mainstream society and the LGBT subculture. Onto this stage stepped four queer men, whose collaboration would result in one of the most acclaimed musicals of the period: *West Side Story*. These collaborators have sometimes claimed that there is nothing gay about the show; however, I argue that it carries important messages for and about LGBT people. The queer interpretation of *West Side Story* blames society for encouraging gay people to repress their sexual identities, and encourages them to "come out" by revealing the physical and emotional detriment that results from *not* being sexually honest. In doing so the show intersects with various historical developments in the LGBT community, both responding to pre-established viewpoints and pre-dating movements still to come.

On the surface, *West Side Story* is a 1950s retelling of William Shakespeare's *Romeo and Juliet*, scored by Leonard Bernstein, with lyrics by Stephen Sondheim, a screenplay by Arthur Laurents and direction from Jerome Robbins. In the show, Shakespeare's rival families are replaced with rival street gangs in New York. Tony is an ex-member of the Jets, a white gang; his lover Maria is related to members of the Sharks, a Puerto Rican gang. The two fall in love against the approval of their gangs; this intensifies the racial rivalry, which prompts Tony to kill a Shark, and a Shark to eventually kill Tony. I argue that a more in-depth understanding of the show suggests the previously stated

VIOLENCE AND SEXUAL REPRESSION IN *WEST SIDE STORY*

The foundational element of the score to *West Side Story* is the tritone, which Bernstein uses to represent confusion regarding one's sexual identity; this concept requires a basic understanding of music theory. The term "interval" refers to the distance between notes; for instance, the first two notes of "Over the Rainbow" are separated by an octave, the largest interval, whereas the first two notes of "Für Elise" are separated by a half-step, the smallest interval in Western music. A tritone is the interval in the middle—it is one half the length of an octave—and it is often used to give a melody an anxious or troubled quality. It is lodged between two confident and content intervals—one note below the tritone is the "perfect fourth" interval (the first two notes of "Here Comes the Bride"), and directly above it is the "perfect fifth" (the first two notes of the main theme from *Star Wars*). As a result of its existence between these two confident sounding intervals, the tritone "has been considered the most ambiguous of all the intervals within the octave" [1] and a "strident dissonance that seeks resolution" [2]. The tritone "historically and harmonically represents instability" [3].

Bernstein uses the tritone to represent the sexual confusion of the gangs (in particular, the Jets); if the perfect fourth and perfect fifth represent a confidence in sexual identity, the tritone places the Jets somewhere in the middle. On one hand, they are very clearly living in a world that only accepts heterosexuality—this can be garnered from their girlfriends, white women who seem to have no significance in the show other than to affirm their boyfriends' racial purity and heterosexuality. Elizabeth Wells, associate professor of music at Mount Allison University, argues that "unlike in traditional musical comedy, [the Jets' girlfriends] don't fulfill a female pulchritude function, nor do they present their own world in relation to the men. Their perfunctory existence shows us that the Jet gang members are normal, red-blooded (i.e., heterosexual) American boys, but little more" [4]. Their insignificance in the show suggests insignificance in the lives of the show's characters; their presence is, in the eyes of the Jets, an obligatory one in a society that values heterosexuality and requires proof of it.

While the girlfriends' presence may clue modern viewers into the sexual rigidity of the period, in 1957 most audiences needed no reminding. John D'Emilio, professor of history and gender studies at the University of Chicago, writes:

> "In mid-twentieth century America, there existed a consensus that devalued homosexual behavior and justified its punishment […] In the popular mind, the homosexual and the lesbian were at best a target of ridicule and at worst a social menace […] the ostracism and punishment that almost surely followed exposure of one's sexual identity imposed a heavy burden of secrecy upon them. Condemnation of homosexuality so permeated the culture that gay men and women could not easily escape it. They too internalized negative attitudes about their sexuality" [5].

Thus the Sharks and Jets live in a society that only accepts

heterosexuality.

Conflicting with these persistent markers of heterosexual society are indications of the gangs' homoerotic desires. As filmmaker and cultural critic Frances Negrón-Muntaner argues, "The effeminacy of many dance sequences with its several numbers where Jets and Sharks pretend to fight, one on top of the other, makes spaces for ritualized same-sex physical contact, which would be otherwise impossible in a Hollywood film in 1961." [1] Confused and frustrated with their sexualities, these gang members only feel comfortable acting upon their sexual desires when they can mask them with the masculine ritual of fighting. Their indecision, resulting from the contrast between a strong desire to be heterosexual and strong impulses to be otherwise place the gangs in the ambiguous and unstable territory of the tritone, in-between the confidence of the perfect fourth and the perfect fifth the same way the gang members are in-between accepting homosexuality and accepting heterosexuality; to make the association clear, Bernstein uses the tritone extensively during the opening homoerotic fight scene (the "Prologue").

It should be noted that the homoerotic elements of the fights do not metaphorically replace the violence; two gang members are killed in the fights by the end of the show, indicating that the risks are real, and the fights are not *just* ways to hint at homosexual desires. Rather, they also serve to highlight the violence that repressed homosexuality can cause. This has a psychological basis; Richard Ryan, professor of psychology at the University of Rochester, argues, "sometimes people are threatened by gays and lesbians because they are fearing their own impulses […] they may be unaccepting of others because they cannot be accepting of themselves" [6]. Because the gang members feel uncomfortable about their own sexuality, they are prompted to take out this confusion on other gay people through violence. The fact that they indulge in homoerotic acts even as they attempt to affirm their heterosexuality by attacking other gay people is indicative of just how conflicted they are, and how strong their desire to mask their homosexuality with masculine rituals is.

ACCEPTING HOMOSEXUALITY: THE TRITONE RESOLVES

After an opening battle that is heavy in both flirtatious fighting and tritones, the audience meets Tony, an ex-Jet who is still good friends with many of the current Jets. The next song—"Something's Coming"—is a solo piece where he expresses his excitement that "something's coming, I don't know what it is / but it is / gonna be great" [7]. While Tony's portrayal is much more optimistic than the other Jets, the song is laced with tritones [8] indicating that he too is repressing his sexual orientation.

During a dance that the Jets and Sharks attend, Tony and Maria meet each other for the first time, and a key transformation takes place—Tony sings "Maria," a love song that expresses his love for her. The first two syllables of the lyric "Maria" span a tritone, but the final syllable ascends a half-step, ending on a perfect fifth. If Tony's and Maria's interracial affair represents a same-sex couple—probably two men, since most of the play occurs from male perspectives—then the evolution from the tritone to the perfect fifth indicates Tony's acceptance of his sexual orientation. By symbolically dating a man, he is acknowledging that he is gay, and in doing so achieving the confidence and stability of the perfect fifth.

Ironically, in a show that urges LGBT people to be honest about their sexuality, the creators must mask homosexual acts with interracial relationships and fight scenes. There are two potential reasons for this: first, it is very possible that the queer interpretation was never consciously intended—indeed, even though its four main creators were gay, they have publicly dismissed the idea that *West Side Story* is about homosexuality (even if this is true, it is likely that their experiences as gay men subconsciously influenced the final product). However there were also more calculated reasons for masking gay acts: censorship and reception. When *West Side Story* was released, censorship laws were just beginning to be challenged, but still strongly restricted the conservations society could have. In June of 1957 the Supreme Court ruled, "we hold that obscenity is not within the area of constitutionally protected speech or press" [9]. In addition, attitudes against homosexuality were still deeply engrained in society, so even if it could get past censorship laws and find funding, a blatant pro-gay show would have gained little exposure. For the same reason D'Emilio argues homophile organizations of the period had small memberships—"anxieties of discovery" [10] on the part of potential members—an unapologetically pro-gay musical would have attracted small audiences. The people that the queer interpretation is most tailored to—repressed gay people—would have been the least likely people to attend a play that could "out" them. Thus existing censorship laws (soon to be challenged) and societal attitudes towards LGBT people meant that whatever power was lost through the hypocrisy of using secret codes to urge honesty was worth it, in my view.

At one point, considerations of sexual orientation evaporate almost entirely from Bernstein and Sondheim's music. In a moment of shared daydreaming, Tony and Maria imagine a world where their love is accepted. The song that accompanies this dream, "Somewhere," reflects the yearning that LGBT people felt at the time for an escape from the discrimination:

> "There's a place for us,
> Somewhere a place for us.
> Peace and quiet and open air
> Wait for us
> Somewhere" [11].

These lyrics reflect the desire many LGBT people felt at the time to find a place where they could live peacefully and without fear of discrimination. Journalist Charles Kaiser writes, "the lyrics of 'Somewhere' in particular seemed to speak directly to the gay experience before the age of liberation. In 1996, it was one of the songs chosen for the first mass gay wedding of two hundred couples in San Francisco" [12]. The musical portrayal takes these ideas a step further; not only are tritones absent [13], but the few melodic perfect fourths and fifths (which represent a confident sexual identity) are not emphasized nearly as heavily as other sections of the score. This indicates the characters' desires to escape to a place where one's homosexuality is not viewed as a prominent part of one's identity. Their "somewhere" is a place

[1] While Negrón-Muntaner is discussing the 1961 film, the same could be said of the 1957 show.

where social norms have been almost completely overcome, to the point where not only is homosexuality accepted, but sexual orientation in general does not matter.

This idea of sexual identity being only a small facet of one's identity is drawn from the conservative faction of homophile groups of the period. In the mid-1950s, as *West Side Story* was being created, a rift was developing in homophile organizations like the Mattachine Society. The more radical founders argued,

> "Out of that awareness homosexuals could then evolve a 'highly ethical homosexual culture' and 'lead well-adjusted, wholesome, and socially productive lives.' The result would be a 'new pride—a pride in belonging, a pride in participating in the cultural growth and social achievements of… the homosexual minority.' And from the cohesiveness that such a self-image would create, in time the founders expected to forge a unified movement of homosexuals ready to fight against their oppression" [14].

A conservative faction was created in response to this idea. This faction argued, "the sex variant is no different from anyone else except in the object of his sexual expression" [15]. This faction did not want sexual orientation to play a role in determining one's identity; its members wanted society to not only tolerate homosexuality, but also treat and view gay people exactly as one would treat a straight person. Fantasizing of such a land—where gay people are not only tolerated, but where they are not viewed any differently than anybody else—leans strongly towards this element of the conservative faction.

This conflict within LGBT movements is still relevant today. For instance, consider the current tension between mainstream and radical LGBT movements. The former, epitomized in institutions like the Human Rights Campaign, has an agenda of inclusion; the goal of such an organization is to allow LGBT people to happily function in our current society. In contrast, more radical activists argue against inclusion, and focus instead of radically transforming society. Sarah Keenan, lecturer at the prestigious University of London, provides a critique of the "It Gets Better" video campaign (emphasis added):

> "[…] if LGBT youth wait until they're older, they will also become caught up in systems that encourage them to just focus on earning more and getting married rather than on attempting to grapple with the fact that *they might be different and that their difference could be part of a platform for a genuinely radical identity politics*" [16].

These are just two examples of conflicting ideologies within the LGBT community. *West Side Story*, with its preference for a full acceptance in society (to the point where sexual orientation is no longer a defining feature of one's identity), leans towards the more mainstream faction. According to the creators of the musical, a world where LGBT people function happily in our current society—rather than a world where LGBT activists have radically transformed society—should be the ultimate goal.

SOURCES OF HOMOPHOBIA

In stark contrast to the tritone-less calm of "Somewhere" is the sarcastic, fast-paced social commentary of "Gee, Officer Krupke," originally intended to supply some comic relief to an otherwise emotionally intense show. In the musical, the Jets sing the song as they mock police officer Krupke (who represents authorities in general) by pretending to be arrested and sent to court by Krupke. They then mimic various figures of authority—a judge, a psychologist, a social worker—as they imagine these figures attempt to understand what caused these young boys to become gang members; however, if the source of the gangs' violence is confusion over their sexuality as I have previously argued, then they are really mocking society's attempts to try to understand the cause of homosexuality. I argue that the song is really an expression of the Jets' confusion over the cause of their homosexual impulses, and that for all the mocking and sarcastic digs at authority, the song shows how negative professional opinions of homosexuality can create an internal sense of self-doubt in LGBT people that often leads to destructive behavior. For instance, in a mock-response to Krupke, a single Jet (later joined by the entire gang) sings:

> "ACTION:
> Dear kindly Sergeant Krupke,
> You gotta understand,
> It's just our bringin' up-ke
> That gets us outta hand.
> Our mothers all are junkies.
> Our fathers all are drunks.
> ALL:
> Golly Moses, natcherly we're punks!" [17].

In later verses the link between environmental "dysfunction" and gender transgressive behavior becomes even more clear—the Jets claim, "My sister wears a mustache / My brother wear a dress / Goodness gracious, that's why I'm a mess!" [18]. This framing of homosexuality makes two important points: first, by labeling themselves as "punks" the Jets acknowledge that homosexuality is a moral wrong. Second, they attribute this moral wrong to environmental problems (non-traditional or dysfunctional family members). The Jets are clearly being sarcastic; in addition to the upbeat tempo and gleeful laughter of the singers, they also end the song with a defiant "Gee, Officer Krupke / Krup you!" [19], meant to reference the phrase "fuck you!" But beneath the parody and sarcasm is a great deal of uncertainty, as musician and psychoanalyst Julie Jaffee Nagel argues:

> "The unstable tritone reminds us repeatedly throughout this jaunty song of the paradoxes and complexities in this engaging but exceedingly sober music. The tritone, as it ominously reappears before each stanza, aurally exposes the Jets' manifest boldness and latent anxiety; the interval is first sung on the words 'Dear…kindly' […] It is a sonic sarcastic attack, verbal and musical, on all authority. The tritone accentuates the Jets' boisterous, sardonic psychological explanations, their pseudo-confessions, and their abundant externalizations" [20].

The tritone is also used in terms of musical key (which, for the purposes of this paper can be understood as a grouping of notes that can be used and that is based on a single note—for instance, the key of B major is based on the note B); Nagel continues:

> "Musically paralleling their fluctuating good/bad self-assessments are […] their catchy refrain zigzags through various musical modulations [shifts from one key to another]. Of note is that in the first stanza the key of B major modulates to a key a tritone distance away—the key of F major.

> The ambiguity of the [tritone] allows the Jets to simultaneously fear and mock Officer Krupke—who, patrolling the streets silently like an absent parent, represents the family, the society […] that have failed them […] it is Bernstein's inspired use of the tritone that musically reminds us of the tenacity of the inner tensions and frustrated longings for love and acceptance that are fueled by parental failures (Lansky 1992), inept social institutions, and cultural prejudice. All these factors contribute to block self-respect […]" [21].

While Nagel argues that the Jets' sense of instability comes from their desire to reject the diagnoses of authority figures and their internal understanding that these are legitimate sources of their struggles, I argue that their sense of instability comes from their desire to reject society's moral prohibition against homosexuality and their tendency to believe society's homophobic institutions. Rather than the "parental failures, inept social institutions, and cultural prejudices" directly causing these boys to turn to violence, I argue that these societal forces are perpetuating homophobia, which causes these boys to repress their homosexual impulses, and only *then* turn to violence.

Nagel finally argues that the decision to place the tritone on the word "dear" also carries significant implications:

> "The call of the tritone beneath the word 'dear' in each stanza of *Gee, Officer Krupke* communicates the Jets' and Sharks' longing for love and their cries of despair defensively encrusted in shame, hardened cynicism, and hate. An affectionate word, 'dear,' combined with a diabolical musical interval embedded in a cancan-style platter song, highlights the bitter irony and ambiguity of the tritone and the unresolved pathos of the gangs" [22].

Thus the Jets' longing for love—homosexual love, I argue—is a crucial element in their internal struggle and confusion.

The use of mental health professionals to shame LGBT people has significant historical precedent. When the show premiered in 1957, the medical consensus was that homosexuality was a mental disease. D'Emilio writes how in the years following World War II,

> "Increasingly, Americans came to view human sexual behavior as either healthy or sick, with homosexuality falling into the latter category. Medical guides aimed at a lay audience expounded on the phenomenon of same-sex orientation and the possibilities of curing it" [23].

These ideas are clearly reflected in the song—at various points the Jets refer to their struggle as "a social disease" [24]. It was an issue the collaborators were familiar with; Bernstein reportedly sought psychoanalysis to cure his homosexual desires [25]. Moreover, it was an issue that left damaging emotional scars on LGBT individuals; medical conceptions of homosexuality "reinforced the cultural matrix that condemned and punished persons who engaged in homosexual activity […] homosexuality stigmatized an individual" [26].

Bernstein's warning against treating homosexuality as a disease was at least a predictor and at best one of the causes of efforts in the late 1960s to change the way the medical community viewed LGBT people. The new militant wing of the homophile organizations began to challenge the medical consensus; in 1963, prominent gay rights activist Jack Nichols argued that the medical understanding of homosexuality was responsible for "'untold numbers of personal tragedies and warped lives" [27]. By the end of the decade, branches of the Mattachine Society in Washington DC and New York had achieved some moderate successes in mobilizing the LGBT community against homophobic medical practices and changing the medical community's consensus, including a 1967 National Institutes of Mental Health report that challenged the notion of homosexuality as a disease [28]. This change was possibly partially due to the influence of West Side Story on gay rights activists.

"Krupke" also emphasizes the role of religion in the period as the Jets sing, "we ain't no delinquents / we're misunderstood / Deep down inside us there is good!" [29]. Nagel notes how Bernstein "employs a plagal cadence (a chord progression familiar in church hymns—also known as the 'Amen' cadence […]). When the Jets declare 'there is good' in themselves, their words are sung on this plagal cadence […]" [30]. Additionally, the Jets later make a sarcastic plea, "Gee, Officer Krupke / We're down on our knees" [31], referencing religious prayer. Thus the Jets mock religious institutions in the same way they mock medical institutions. This is especially powerful given the history of the tritone; in medieval times its emotional instability prompted musicians to call it "The Devil's Interval" [32]; if the tritone represents sexual uncertainty, Bernstein is sarcastically associating sexual deviants with the Devil. Sarcastically repenting their "devilish" ways to implied religious institutions is another way of mocking homophobic authorities; however, at the same time the Jets cannot advance beyond the tritone like Tony did, indicating that they are still not comfortable at the thought of being gay and that religion's homophobia still influences their internal struggles, just as the medical community's homophobia casts doubt over their sexualities. It should be noted that using the tritone as a sarcastic representation of devilish behavior does not mean that Bernstein affirms the relationship between homosexuality and the Devil when the tritone is used in other sequences of the score—the sarcastic link only works in the context of "Krupke," when the Jets pretend to agree with homophobic institutions for the purpose of mocking them (but at the same time wonder if these institutions are right in doubting homosexuality).

It is easy to see why Bernstein would accuse religious institutions of emotionally harming LGBT people. D'Emilio describes how "in the Judeo-Christian tradition, homosexual behavior was excoriated as a heinous sin" [33] and how "a biblical scholar who undertook in the 1950s a comprehensive reevaluation of Christian theology and homosexuality, closed his book with the thirteenth century because 'it does not appear that the tradition has undergone any significant alteration since that time'" [34]. Thus Bernstein criticizes not only medical, but also religious institutions' tendency to perpetuate homophobic ideals in society, ideals that prompt LGBT people to repress their homosexual impulses. At the same time medical experts began to shift their opinions on homosexuality, more modest shifts (but shifts nonetheless) occurred in religious communities; "although no religious institutions repudiated the moral opprobrium attached to homosexual behavior, some sectors of the religious community were willing to question its criminalization" [35]. These changes did not occur until the early 1960s, so the show's criticism of homophobia in religion pre-dated these shifts.

This is an issue that continues to haunt the LGBT community. The Human Rights Campaign has devoted a significant

effort to illegalize "gay conversion therapy," or therapy that treats homosexuality as a psychiatric disease that can be cured [36]. *West Side Story*'s strong condemnation of this view of homosexuality as a disease is just as important today as it was in the 1950s.

West Side Story also aims some criticisms at the LGBT community. The only major character that is not based on a character from *Romeo and Juliet*, a girl nicknamed Anybodys, attempts throughout the show to be accepted in the Jets as "one of the guys." Her short hair and masculine clothes indicate that she is a "butch" lesbian. Leila J. Rupp, professor of women's studies at the University of California, Santa Barbara, discusses how "by the 1950s the long tradition of utilizing masculinity as a way of proclaiming sexual interest in women had resulted in a new-style lesbian, the 'tough butch.' She dressed in typical male working-class attire […]" [37]. Despite Anybodys' continued requests to join the Jets and all the selfless acts she commits for them—for instance, after Tony impulsively kills a Shark, Anybodys tugs at his sleeve to make him aware of the approaching police sirens so he can escape police detection—the Jets not only reject her presence, but also make fun of her throughout the play, calling her "ugly" or disapproving of her gender transgressions with cruel lines like, "Ah! Go wear a skirt" [38]. Even her nickname suggests not just a lack of belonging, but also "a lack of recognition as a subject. She is an open subject—'anybody'—sitting on the sexual/gender fence" [39].

The cruel treatment that Anybodys receives serves several important functions: first, it mirrors the prejudice that gay men felt towards lesbians. In the period, "male attitudes of superiority created tensions between male and female homophile groups" [40]. Dorothy Louise Taliaferro "Del" Martin, a prominent lesbian activist, proclaimed, "lesbians are not satisfied to be auxiliary members or second-class homosexuals!" [41]. Class-based prejudices in the lesbian movement meant that even upper-class lesbians disapproved of the typically lower-class "butch" lesbians, and argued, "the kids in fly front pants and with butch haircuts and mannish manners are the worst publicity that we can get" [42]. The discrimination Anybodys receives is even more frustrating given the fact that she is as much a victim of the stifling gender norms of society as the gay males that treat her so cruelly—the only major difference is that she has the courage to act on her sexual desires, unlike the gay males. In this way, Anybodys' treatment also serves to highlight the impact of internalized prejudice; the Jets use Anybodys as an outlet through which they can show their support for traditional gender roles, to compensate for their own impulses against said gender roles (similar to their motivation for physically hurting other gay people).

Anybodys' role also shows the dangers of discrimination for the people discriminating. In the final scene, Tony, incorrectly believing Maria to be dead, walks through the city, calling for Chino, an angry Shark, to kill him. Anybodys tries to convince Tony to not give up his life, but he retorts back "You're a girl: be a girl! Beat it," which offends her enough to leave. As a result, Tony makes himself vulnerable to Chino's bullet and is shot, even though Maria is still alive. In a very direct way, the discrimination Tony shows against Anybodys causes his death, indicating the self-destructive nature of prejudice. This has implications at both an individual and a political level; perhaps the "paucity of membership" [43] in homophile organizations in the 1950s would have been higher absent prejudice within the LGBT community that divided male and female activist groups. Finally, Anybodys' inclusion serves to acknowledge the existence of lesbians in a show that otherwise presents almost all of its LGBT-related themes from the male perspective (which is perhaps to be expected when the show is created by four gay men).

In the modern age, these prejudices have shifted; they most visibly manifest themselves in gay attitudes towards transgender people. Jillian Weiss, a professor of Law and Society at Ramapo College of New Jersey, argues that the LGB community must combat its transphobia, "a task that is neither quick nor easy, and is not accomplished by adding a letter to an organization's name" [44].

RESULTS OF REPRESSION

The play ends shortly after Tony's death; a group of Sharks, Jets and adults have gathered at the scene of the murder, and a procession of Sharks and Jets carry Tony's body off stage. The last notes the orchestra plays are a somber version of "Somewhere" (without lyrics), soured by a low tritone. This suggests that while the two factions may be able to ignore their aggression for the time being and provide the "somewhere" that Tony and Maria dreamed of, the deeper, root cause of their problems—their repressed homosexuality, represented by the buried tritone—persists, and the truce will not last long. Tellingly, the last people on stage are the adults—including Officer Krupke—who represent the rest of society; this powerful decision gives them a large degree of culpability for perpetuating the homophobic aspects of society.

West Side Story urges non-heterosexuals to "come out" in order to save themselves and the ones they love from physical and emotional harm. This idea of accepting one's homosexuality to better oneself is drawn from the founders of Mattachine in the early 1950s. D'Emilio discusses how they decided,

> "With no socially approved models for their life-style, homosexuals 'mechanically superimposed the heterosexual ethic' on their own situation 'in empty imitation of dominant patterns.' The result was a daily existence predicated upon 'self-deceit, hypocrisy, and charlatanism' and a 'sense of value…disturbed, inadequate, and undesirable'" [45].

While such an interpretation suggests that acceptance of one's homosexuality is the solution to dealing with the problems of internalized hatred, this message was largely drowned out after a more conservative faction of the organization gained control and emphasized gay assimilation into heterosexual lifestyles rather than societal acceptance of gay lifestyles. It would not be until the gay liberation movement of the 1970s that an even more explicit encouragement to "come out" and embrace one's homosexuality would become mainstream in LGBT communities, making *West Side Story* ahead of its time. D'Emilio describes how,

> "Throughout the 1950s and 1960s, leaders of the homophile cause had in effect extended their coming out to the public sphere through their work in the movement. But only rarely did they counsel lesbians and homosexuals at large to follow their example, and when they did, homophile activists presented it as a selfless step taken for the benefit of others. Gay liberationists, on the other hand, recast coming out as a profoundly political act that could offer enormous personal benefits to an individual. The open avowal of one's sexual

identity […] symbolized the shedding of the self-hatred that gay men and women internalized, and consequently it promised an immediate improvement in one's life" [46].

West Side Story encourages repressed gay people to be honest about their sexual identity not just to help the LGBT community, but also to help themselves; it was a message that was both rooted in past LGBT movements and indicative of movements still to come. For instance, groups like the Human Rights Campaign celebrate a "National Coming Out Day" on October 11th each year [47]. This is meant to "promote a safe world for LGBT individuals to live truthfully and openly" [48].

In 1957, the show was also a response to the lesbian pulp fiction that was beginning to become popular. Rupp writes: "Mostly written by male authors for a heterosexual audience, such inexpensive paperbacks depicted lesbian life as pathetic and unhappy" [49]. In one such novel, a lesbian advises a sexually curious girl to not give in to the "twisted" world of homosexuality, and that "I can't let you lead a twisted, miserable life. And lesbians are miserable" [50]. While there were some rare exceptions that viewed lesbians in a positive light, in general the blame for the problems the lesbian community faced were placed on the lesbians themselves, and the solution was to conquer one's homosexual urges. *West Side Story* treats homosexuality in the opposite way; the problems of LGBT people are blamed on society more than the LGBT people themselves, and the solution is to embrace one's homosexuality, not repress it. While *West Side Story* focuses almost exclusively on gay men, in a broad sense it can be seen as a response to works like lesbian pulp fiction that encourage the repression of homosexuality.

CONCLUSION

West Side Story encourages gay people to be honest about their sexuality, while at the same time blaming societal institutions for perpetuating homophobic beliefs that make honesty about sexual identity difficult. In doing so, the show comments on various movements in the LGBT community and also endorses some ideas that would eventually come to pass with the rise of the militant homophile wing and the gay liberation organizations. The messages garnered from the show, while not necessarily intentionally placed by its creators, continue to preach important messages even in a modern context, bringing the LGBT community closer and closer to creating its "somewhere."

REFERENCES

1. Nagel, J. "Psychoanalytic and Musical Ambiguity: The Tritone in 'Gee, Officer Krupke.'" Journal of the American Psychoanalytic Association 2010: 58.1: 9-25.
2. Nagel, J. "Psychoanalytic and Musical Ambiguity: The Tritone in 'Gee, Officer Krupke.'" Journal of the American Psychoanalytic Association 2010: 58.1: 9-25.
3. Nagel, J. "Psychoanalytic and Musical Ambiguity: The Tritone in 'Gee, Officer Krupke.'" Journal of the American Psychoanalytic Association 2010: 58.1: 9-25.
4. Wells, EA. West Side Story: Cultural Perspectives on an American Musical. Lanham, MD: Scarecrow, 2011: 162-3.
5. D'Emilio, J. Sexual Politics, Sexual Communities: The Making of a Homosexual Minority in the United States, 1940-1970. Chicago: University of Chicago, 1983: 241.
6. Bryner, J. "Homophobes Might Be Hidden Homosexuals." Scientific American. 10 Apr. 2012. <http://www.scientificamerican.com/article.cfm?id=homophobes-might-be-hidden-homosexuals>.
7. Laurents A., Sondheim S., Bernstein L. "West Side Story." Aellea. <http://www.aellea.com/script/westside.txt>.
8. Simeone, N. Leonard Bernstein: West Side Story. Farnham, 2009: 96.
9. Brennan, William. "Roth v. United States." LII / Legal Information Institute. Cornell University Law School, Web. 20 May 2014.
10. D'Emilio, J. Sexual Politics, Sexual Communities: The Making of a Homosexual Minority in the United States, 1940-1970. Chicago: University of Chicago, 1983: 124.
11. Laurents A., Sondheim S., Bernstein L. "West Side Story." Aellea. <http://www.aellea.com/script/westside.txt>.
12. Kaiser, Charles. The Gay Metropolis: 1940-1996. Boston, 1997: 93.
13. Nagel, J. "Psychoanalytic and Musical Ambiguity: The Tritone in 'Gee, Officer Krupke.'" Journal of the American Psychoanalytic Association 2010: 58.1: 14.
14. D'Emilio, J. Sexual Politics, Sexual Communities: The Making of a Homosexual Minority in the United States, 1940-1970. Chicago: University of Chicago, 1983: 66.
15. Corber RJ, Freedman J, Millington RH. Hitchcock's America. New York: Oxford UP, 1999.
16. Keenan, Sarah. "It Gets Worse: The Queer Feminist Case against Inclusion." SOAS Teach-Out. 31 Oct. 2013. Speech.
17. Laurents A., Sondheim S., Bernstein L. "West Side Story." Aellea. <http://www.aellea.com/script/westside.txt>.
18. Laurents A., Sondheim S., Bernstein L. "West Side Story." Aellea. <http://www.aellea.com/script/westside.txt>.
19. Laurents A., Sondheim S., Bernstein L. "West Side Story." Aellea. <http://www.aellea.com/script/westside.txt>.
20. Nagel, J. "Psychoanalytic and Musical Ambiguity: The Tritone in 'Gee, Officer Krupke.'" Journal of the American Psychoanalytic Association 2010: 58.1: 16.
21. Nagel, J. "Psychoanalytic and Musical Ambiguity: The Tritone in 'Gee, Officer Krupke.'" Journal of the American Psychoanalytic Association 2010: 58.1: 16-18.
22. Nagel, J. "Psychoanalytic and Musical Ambiguity: The Tritone in 'Gee, Officer Krupke.'" Journal of the American Psychoanalytic Association 2010: 58.1: 19.
23. D'Emilio, J. Sexual Politics, Sexual Communities: The Making of a Homosexual Minority in the United States, 1940-1970. Chicago: University of Chicago, 1983: 17.
24. Laurents A., Sondheim S., Bernstein L. "West Side Story." Aellea. <http://www.aellea.com/script/westside.txt>.
25. Rockwell, John. "Maestro: The Leonard Bernstein Letters." The New York Times. N.p., 14 Dec. 2013. Web. 06 Mar. 2014. <http://www.nytimes.com/2013/12/15/books/review/the-leonard-bernstein-letters.html>.
26. D'Emilio, J. Sexual Politics, Sexual Communities: The Making of a Homosexual Minority in the United States, 1940-1970. Chicago: University of Chicago, 1983: 17-18.
27. Campbell JL. Jack Nichols, Gay Pioneer: "Have You Heard My Message?" New York: Harrington Park, 2007: 76.
28. D'Emilio, J. Sexual Politics, Sexual Communities: The Making of a Homosexual Minority in the United States, 1940-1970. Chicago: University of Chicago, 1983: 217.
29. Laurents A., Sondheim S., Bernstein L. "West Side Story." Aellea. <http://www.aellea.com/script/westside.txt>.
30. Nagel, J. "Psychoanalytic and Musical Ambiguity: The Tritone in 'Gee, Officer Krupke.'" Journal of the American Psychoanalytic Association 2010: 58.1: 9-25.
31. Laurents A., Sondheim S., Bernstein L. "West Side Story." Aellea. <http://www.aellea.com/script/westside.txt>.
32. Rohrer F. "The Devil's Music." BBC News. 28 Apr. 2006. <http://news.bbc.co.uk/2/hi/uk_news/magazine/4952646.stm>.
33. D'Emilio, J. Sexual Politics, Sexual Communities: The Making of a Homosexual Minority in the United States, 1940-1970. Chicago: University of Chicago, 1983: 13.
34. D'Emilio, J. Sexual Politics, Sexual Communities: The Making of a Homosexual Minority in the United States, 1940-1970. Chicago: University of Chicago, 1983: 13.
35. D'Emilio, J. Sexual Politics, Sexual Communities: The Making of a Homosexual Minority in the United States, 1940-1970. Chicago: University of Chicago, 1983: 215.
36. "The Lies and Dangers of Efforts to Change Sexual Orientation or Gender Identity." Human Rights Campaign. N.p., n.d. Web. 05 Mar. 2014. <http://www.hrc.org/resources/entry/the-lies-and-dangers-of-reparative-therapy>.
37. Rupp, Leila J. A Desired Past: A Short History of Same-sex Love in America. Chicago, 1999: 150.
38. Laurents A., Sondheim S., Bernstein L. "West Side Story." Aellea. <http://www.aellea.com/script/westside.txt>.
39. Negron-Muntaner, F. "Feeling Pretty: West Side Story and Puerto Rican Identity Discourses." Social Text 18.2 63 (2000): 83-106.
40. D'Emilio, J. Sexual Politics, Sexual Communities: The Making of a

Homosexual Minority in the United States, 1940-1970. Chicago: University of Chicago, 1983: 105.
41. Walzer, L. Gay Rights on Trial: A Reference Handbook. Santa Barbara, 2002: 48.
42. Rupp, Leila J. A Desired Past: A Short History of Same-sex Love in America. Chicago, 1999: 163.
43. D'Emilio, J. Sexual Politics, Sexual Communities: The Making of a Homosexual Minority in the United States, 1940-1970. Chicago: University of Chicago, 1983: 122.
44. Weiss, Jillian. "Http://www.bilerico.com/2010/07/transphobia_in_the_gay_community.php." Bilerico Project. N.p., 2010. Web. 06 Mar. 2014. <http://www.bilerico.com/2010/07/transphobia_in_the_gay_community.php>.
45. D'Emilio, J. Sexual Politics, Sexual Communities: The Making of a Homosexual Minority in the United States, 1940-1970. Chicago: University of Chicago, 1983: 122.
46. D'Emilio, J. Sexual Politics, Sexual Communities: The Making of a Homosexual Minority in the United States, 1940-1970. Chicago: University of Chicago, 1983: 65.
47. "The History of Coming Out." Human Rights Campaign. N.p., n.d. Web. 06 Mar. 2014. <http://www.hrc.org/resources/entry/the-history-of-coming-out>.
48. "The History of Coming Out." Human Rights Campaign. N.p., n.d. Web. 06 Mar. 2014. <http://www.hrc.org/resources/entry/the-history-of-coming-out>.
49. Rupp, Leila J. A Desired Past: A Short History of Same-sex Love in America. Chicago, 1999: 144.
50. Herbert, A. Summer Camp. New York, 1963: 49.

David Kay is a sophomore at Stanford University, majoring in the Political Science Research Honors Track. He is the founder/president of Stanford PAW (People for Animal Welfare), a research assistant at the Clayman Institute for Gender Research and an author in the magazine Film Score Monthly. Contact him at dkay19@stanford.edu.

natural sciences

A Musical Model of Organic Compounds

Sherman K. Leung
Stanford University

Organic chemistry is an important field in modern science that not only requires a deep understanding, but also imagination, creativity and intuition. The goal of this study is to define and illustrate a novel approach to organic chemistry through the application of a musical model that denotes organic compounds as musical phrases. The model revolves around the denotation of carbon as the musical pitch C and the foundation of a carbon baseline. Reflecting important concepts in organic chemistry, this model challenges the nature of other interdisciplinary studies between music and other fields by establishing an unprecedented approach to an unexplored link between music and organic chemistry. Using music as an auditory approach to the chemical structures of organic compounds, this model bridges the arts and sciences to apply an artistic and musical perspective to a highly creative science.

The applications of organic chemistry have grown exponentially in the last few decades, where the synthesis of organic compounds has become essential in the development of pharmaceutics, plastics, petroleum, cosmetics, and food additives [1]. Central to the quality of a high standard of living, organic chemistry provides the foundations on which the fields of biochemistry, biotechnology, and medicine are built [1]. Organic chemistry has become one of the most important and innovative pursuits in modern science.

Models to describe the structure of organic molecules exist in chemoinformatics. A well-known model, Simplified Molecular-Input Line-Entry System (SMILES), follows a set of encoding rules to describe molecules with line notation using short ASCII strings [2]. Developed for computational calculations, SMILES allows a user to compress a molecule's structural information into ASCII strings. Though it reduces computational complexity, this compression keeps chemical trends from the original representation from being understood.

Conversely, in attempts to model music, computer programs have been used to mimic creativity in compositions [3], and model musical styles from a variety of composers [4] Breaking down musical composition into programmable algorithms, programmers [5,6] have found ways to represent the creativity of a composer with computer algorithms.

Despite all the links established in these interdisciplinary fields, music has yet to be used as a notation model for the sciences. Indeed, mathematical proofs have been modeled in music using an algorithm [7] that translates well-formed-formulas in proofs to musical representation. However, the author admits that the proofs are "unpredictable" and that the musical properties of the proofs are questionable [7].

The purpose of this study is to establish a link between chemistry and music by defining a musical model of organic chemistry that builds a system of musical notation to describe the chemical structures of organic compounds. Based on relationships between organic chemistry and music theory, the model defined in this study allows for the translation of organic molecules into music. Acting as a system to encode and denote organic molecules, the model also reflects important chemical properties that show further links between the two fields.

The merits and novelty of this particular study lies in the model's comprehensive ability to use music as a tool to represent chemistry. Unlike the connections made in other interdisciplinary studies, this model challenges and abandons common approaches to the same subjects in favor of discovering new perspectives.

This study proposes that an auditory understanding of organic molecules leads to an entirely new way to think about organic chemistry. By approaching organic chemistry from a

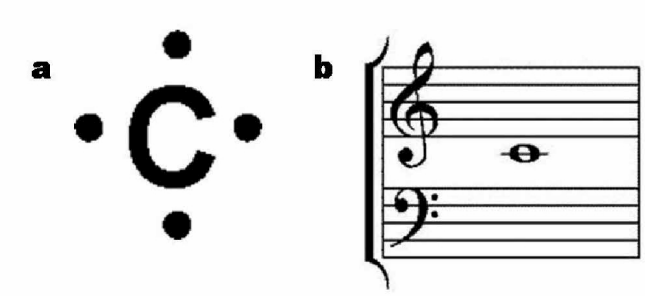


Figure 1. (a) Lewis structure of a carbon atom, **(b)** Middle C or C_4

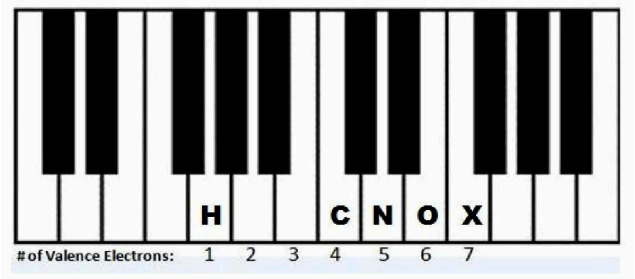


Figure 2. Common elements in organic chemistry are defined by the number of their valence electrons (Note: X represents a halogen)

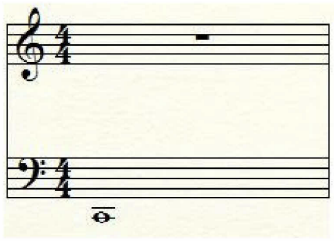


Figure 3. First carbon in a carbon chain defined as C_2

musical perspective, this model encourages its users to think creatively about organic molecules and appreciate the structures from a unique standpoint.

Fusing the fields of organic chemistry and music in an unprecedented manner, the successful application of this model may offer new insight and new connections between the fields of chemistry and music.

GUIDELINES FOR THE DENOTATION OF ORGANIC COMPOUNDS IN THE MUSICAL MODEL

Definition of Carbon

Organic chemistry is defined as the study of carbon-containing compounds [9]. As the building block of organic compounds and the central element in organic chemistry, carbon (Figure 1a) will be defined as the central note of music notation and the standard 88-keyboard – the pitch of C, a note that defines the treble and bass staffs with its position (Figure 1b).

Definition of H, N, O, and Halogens

Elements in organic chemistry will be defined by the number of their valence electrons. Each element will be assigned a note relative to carbon, which has four valence electrons (Figure 1a).

Thus, hydrogen, with one valence electron, should be three notes lower than C at the note G. In the same way, nitrogen, with five valence electrons, should be one note higher than C at the note D. Other common elements in organic chemistry will be denoted in the same fashion (Figure 2).

It should be noted that the noble gases are excluded from this model because of their absence in organic compounds.

Transposition of Carbon to C_2

To reflect the role of carbon chains as the backbone of organic chemistry, carbons in the main carbon chain of any organic molecule will be played in the bass clef. The first carbon of any carbon chain will be denoted as C_2 in scientific pitch notation in order to encapsulate the full range of an organic compound (Figure 3).

Bonding

The bonds between non-carbon elements and its corresponding carbon will be denoted by placing the non-carbon elements in the treble clef above the carbon baseline in the bass clef. Considering the simplest organic molecule, methane, each of the 4 hydrogens in a methane molecule is played as one quarter note on top of the one carbon of a methane molecule (Figure 4).

Consequently, the bonding between non-carbon elements and other non-carbon elements is also denoted in the same fashion. Elements that are more electronegative will be transposed above elements that are less electronegative. For example, in chloromethane, the chlorine atom is denoted as F_5, which is higher than the hydrogen at G_4 (Figure 5).

In representing two or more non-carbon elements that share a covalent bond (e.g. a hydroxide group), the elements will be denoted as two notes that share a beat. In the case of a hydroxide group (Figure 6), it is important to notice that instead of playing the oxygen above the hydrogen atom, the hydrogen is played on top of the oxygen to reflect the placement and order in which the atoms are bonded. Thus, hydrogen is played above the oxygen atom while sharing the same quarter note beat over carbon.

Subdivision of Carbon into a $^4/_4$ Time Signature

Considering that carbon can only have up to four bonds, each carbon in a carbon chain is designated one full measure in the common time signature of $^4/_4$. Each of carbon's four bonds will be given one quarter note in its measure. Thus, the model will indicate where non-carbon elements bond to carbon with the beat that they are in. With the exception of the terminal carbons, the order of the quarter note bonds for any particular carbon in a chain will be as follows:

The bond linking one carbon with the previous carbon atom will be defined as the first beat, and the bond linking the current carbon with the next carbon will be defined as the last beat. For the other two bonds, the second and third beat will denote the remaining bonds from left to right or top to bottom (Figure 7a).

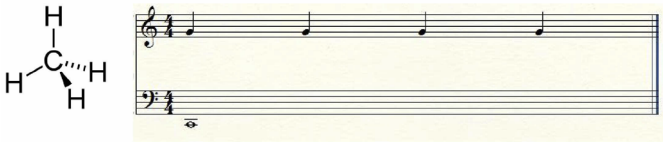


Figure 4. Methane, CH_4

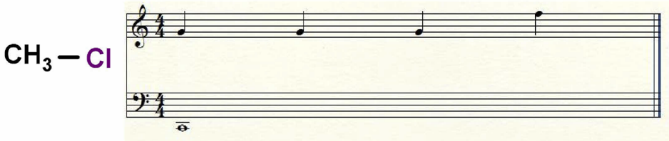


Figure 5. Chloromethane, CH_3Cl

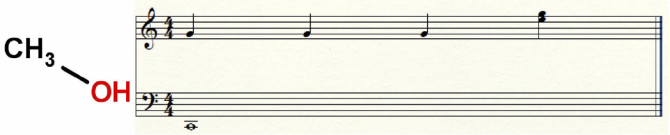


Figure 6. Methanol, CH_3OH

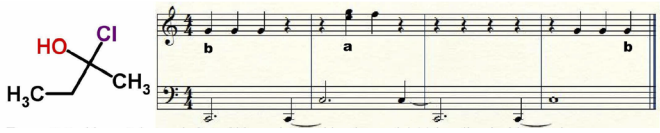


Figure 7. 2-chloro- 2-butanol, C_4H_9ClO, as depicted by the model. **(a)** bonding inside a carbon chain is specified from left to right, **(b)** terminal carbons

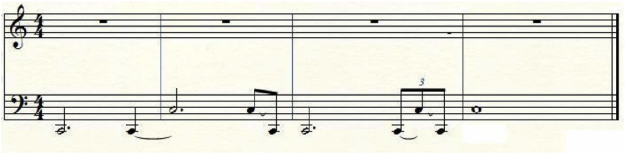


Figure 8. (a) single-bonded carbon, **(b)** double-bonded carbon, **(c)** triple-bonded carbon

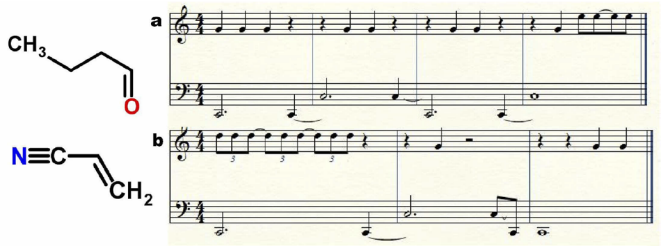


Figure 9. (a) butanal, C_4H_8O, **(b)** acrylonitrile, C_3H_3N

Regarding the terminal carbons, the first and last beats of the measure will be the leftmost and rightmost atoms bonded to carbon, respectively (Figure 7b).

It should be noted that the order of the second and third bonds of carbon changes depending on the orientation of the molecule.

Carbon Bonding

In order to establish a carbon baseline, subsequent carbons in a given chain of carbons will alternate between C_2 and C_3 in the bass clef; however, the first carbon in a carbon chain will always be C_2.

Bonding will be represented by slurs between two carbons, with the length of the slur corresponding to the length of the carbon bond between subsequent carbons in a carbon chain. Put more explicitly, carbon bonds will be slurred quarter, eighth or triplet notes to reflect single, double, and triple bonds respectively (Figure 8).

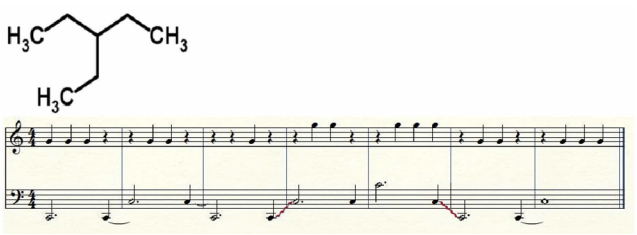


Figure 10. 3-ethylpentane, C_7H_{16}

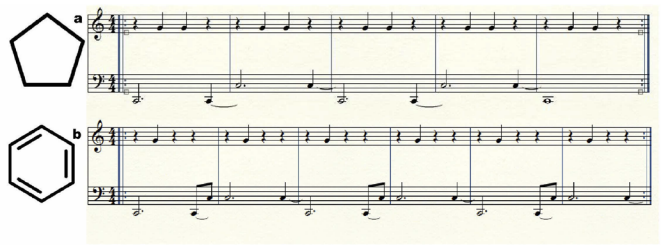


Figure 12. (a) cyclopentane, C_5H_{10}, **(b)** benzene, C_6H_6

Double and Triple Bonds Between Non-Carbon Elements and Carbon

The bonding of non-carbon elements to the carbon chain will follow the similar guidelines for the representation of carbon bonding. Elements that share a double or triple bond with a carbon will be played at the corresponding note length of either an eighth (Figure 9a) or a triplet (Figure 9b).

Side Chains

To denote side chains attached to a carbon chain, any side chain that branches off the main carbon chain will be played an octave higher in both clefs. To signify when a side chain breaks off from the main chain, a glissando in the bass clef will be played on the fourth beat of the measure where the side chain branches off the main chain. In denoting the side chain, all rules that apply to the main chain will also apply to the side chain.

When the end of the substituent is played, a second glissando returning to C_2 in the baseline will be played on the first beat of the measure after the end of the side chain. The return to the original range of the carbon baseline will signify the continuation of the original carbon chain (Figure 10).

It should be noted that the position of the glissando in the fourth beat is an exception to the rules defined by the subdivision of carbon. For this reason, the remaining non-carbon bond for the starting carbon in a side chain will be defined as the beat directly before the glissando.

In a case where two side chains break off at the same carbon, the second side chain will be denoted by playing the side chain in the octave below the original chain. Thus, in the last measure of the first side chain, the left hand will perform a glissando down to C_1 the entire side chain played below the original carbon chain. In order to define a consistent order in a compound with two side chains off of the main carbon chain, the first side chain will always be played an octave above and the second side chain will always be played an octave below (Figure 11).

Carbon Rings

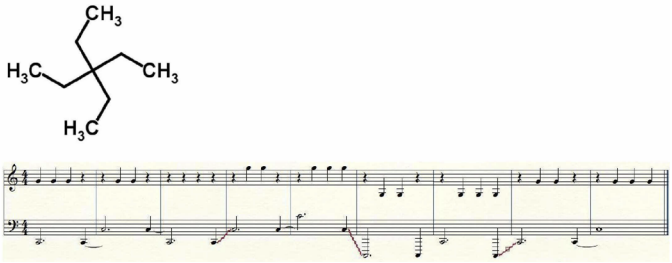


Figure 11. 3,3-diethylpentane, C_9H_{20}

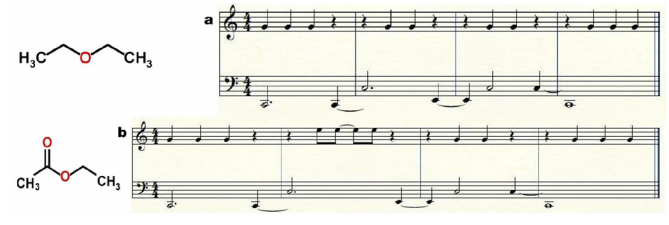


Figure 13. (a) diethyl ether, $C_4H_{10}O$, **(b)** ethyl acetate, $C_4H_8O_2$

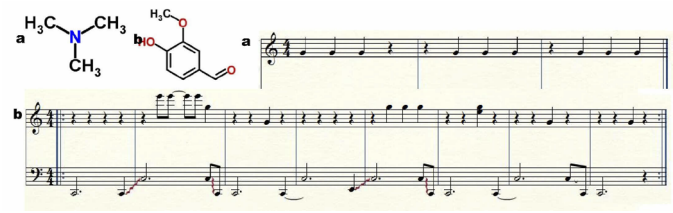


Figure 14. (a) trimethylamine, $N(CH_3)_3$, **(b)** vanillin, $C_8H_8O_3$

The cyclic nature of a carbon ring will be represented by the addition of repeat signs before and after one cycle is completed (Figure 12).

Non-Carbon Baselines

In structures where a non-carbon element is bound by carbon on both sides, the non-carbon atom becomes part of the baseline without its own measure.

In the example of ether, the oxygen atom will become part of the baseline to reflect the chemical structure. It should be noted that oxygen is not given its own measure because it does not share carbon's bonding characteristics. Instead, the oxygen is denoted in the octave range of the baseline as two tied quarter notes across two measures. As an exception to the alternating carbon baseline in other organic molecules, the baseline will not alternate after the denotation of the oxygen atom. Instead, the carbon baseline will remain on the same octave of C that was played before the oxygen atom. This is seen in the position of C_2 in the third measure of the denotation of ethyl acetate (Figure 13a). It should be noted that a non-carbon element in the baseline does not affect the order of the remaining beats in the measure (Figure 13b).

Side Chains Branching Off non-Carbon Elements

Side chains that branch off non-carbon elements will be depicted by glissandos made from non-carbon elements within the octave interval of the original baseline.

In trimethylamine (Figure 14), the molecule is modeled as if nitrogen is part of the baseline connecting two other carbons. The glissando that starts with D_2 (between the original octave of C_2 and C_3) shows the methyl group that branches off the nitrogen atom.

In vanillin, the same principle is applied to the methoxy group on the third carbon.

Organic Structures with Multiple Rings

When modeling organic compounds with multiple rings, three possibilities will arise: two rings connected by a single bond, two rings joined together by one side, or three rings connected together at one carbon.

In the musical denotation of the first possibility, the left repeat sign for the second ring will follow immediately after the right repeat sign for the first ring. A tied quarter note over the repeat signs will connect the two rings together.

In the second possibility, one ring will be denoted as a repeated sequence of measures within the ring. The ring in which the carbon numbering starts will begin and end the outer repeated sequence, while the inner repeated sequence will start

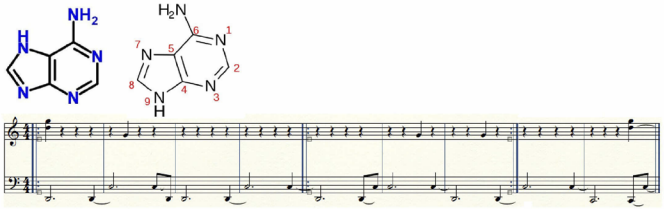


Figure 15. adenine, $C_5H_5N_5$

where the second ring attaches to the first. The placement of the smaller repeated sequence will be determined by where the bond links. The size of either ring will not affect which sequence is inside another.

Taking adenine as an example (Figure 15), the normal numeration of the molecule will be denoted by the model in the following order: [1,2,3,4 [9,8,7] 5,6], where the brackets around the numbers represent repeat signs.

In the third possibility, the order of the rings will simply follow the carbon numbering of the molecule while the same approach from the second possibility is used.

Carbon-like Behavior in non-Carbon Elements

When non-carbon elements are part of a ring structure, they will adopt carbon-like behavior in bonding (Figure 15). It should be noted that the perfect fourth in the first measure is a result of the tied denotation of the amine functional group on the last carbon.

Additional Notes

It should be noted that this model is best exhibited through the piano, where two hands can play both the bass and the treble clef at once.

Furthermore, though there are many ways to describe and name the same molecule, this model will adhere to the nomenclature standards set by the International Union of Pure and Applied Chemistry (IUPAC). Thus, the model will denote all of its musical phrases according to the IUPAC name of a molecule (e.g. using a chain or a ring as the base).

ANALYSIS

Nature of Carbon

Several points should be noted in regard to the model's ability to

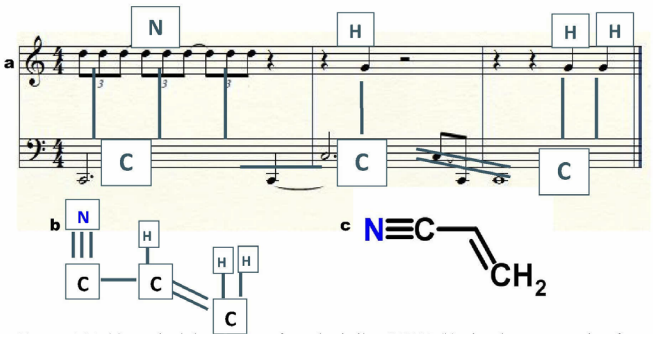


Figure 16. (a) musical denotation of acrylonitrile, C_3H_3N, **(b)** visual representation from score, **(c)** actual chemical structure

reflect principles in organic chemistry. First, the standard meter of $^4/_4$ is able to effectively embody the nature of carbon and its four bonds. Additionally, the carbon baseline in the model reflects the carbon backbone found in organic chemistry.

Visual Representation of Bonding

The model defines and reflects the nature of organic compounds in a manner that accurately represents the chemical structure of the compound. In addition to hearing the bonds in the musical representations of models, each bond in a molecule can be seen visually in the musical score.

Number of Elements

For any musical representation of a molecule, the number of measures containing a dotted half note or a whole note of C corresponds with the number of carbons. In the same way, the number of quarter notes corresponds to the number of atoms of its respective element. When counting quarter notes, it should be kept in mind that two tied eighth notes would count as one quarter note while two separate eighth notes would not. To keep consistent with the patterns found in the eighth notes, the two ties between three sets of triplets (e.g. the triple bond in Figure 17) will be approximated to count as one quarter note.

Electronegativity

Electronegativity is also modeled because the more electronegative elements are placed in the largest interval above any other element (i.e. hydrogen) that is attached to the same carbon. Thus, if the size of the interval between two non-carbon elements is taken as a representation of the magnitude of the dipole moment between the two elements, the model would reflect the electrodensity distributions of the dipole moments in compounds.

Hybridization

Consider a sp^3 hybridized carbon. Then, consider the depiction of the same carbon in the musical model and count the quarter notes in that particular measure. After adding any quarter notes tied to the carbon in the measure, the number of quarter notes corresponds with how many bonds a carbon atom shares with other elements, and thus – its hybridization. In a sp^2 hybridized carbon, the double bond of a carbon with another element would only take up one beat due to the tie between eighth notes. This concept is slightly complicated when considering triple bonds, but only because there is no way to tie three triplets in such a manner that they amount to one quarter note beat.

Bond Enthalpies of Perfect Intervals

From a musical perspective, the combination of C's and G's in models of hydrocarbons presents a listener with the tonic and the dominant of the C scale. The musical properties of these two notes in the scale present a stability of harmony that reflects the stability found in hydrocarbons. One can consider the bond enthalpies for C-C and C-H and observe how perfect intervals (i.e. fourths, fifths, and eighths) seem to result in higher bond enthalpies (Figure 16). Conversely, other intervals (e.g. the major second of C-N) seem to have lower bond enthalpies. There are exceptions to this connection, namely the bond enthalpies of carbon and the halogens; however, this is simply because the model does not distinguish between different halogens.

FUTURE APPLICATIONS

Applications in Teaching Chemistry

For interested chemistry students coming from different backgrounds in language, the model (i.e. the denotation of organic compounds) offers an approach that bridges barriers in teaching styles and language comprehension. For individuals with a background in musical notation, chemical concepts present in the model follow intuitively. Conversely, an individual from a chemistry background may develop a new appreciation for the applications of music after being exposed to this model.

For auditory learners, this model may prove to be helpful in supplementing the understanding of any basic knowledge of chemistry. The auditory context that underlies the understanding of this model works to effectively enhance and engage auditory learners.

Applications from ChemoInformatics

Applying this model to computer science, one could retrieve a molecule's structural information from PubChem and denote the molecule following the above model. Using this technology, it would be interesting to screen and compare a variety of denotations to find trends that may only be obvious audibly.

Applications to Biology

With the completion of the sequencing of the entire human genome and the capacity of the model to depict the nucleic acids (Figure 15), the possibility of denoting the entire human genome as music is conceivable with the use of computer applications. Taking this application one step further, a symphony of the human genome could be composed by designating each cell type as a different instrument in an orchestra.

This approach could be propagated to represent other interesting biological phenomena as well. If this model can be applied to appreciate chemical compounds and chemical reactions, it's possible to apply the same sort of musical understanding to biological systems using this model as a musical and chemical intermediate.

A Model of Organic Reactions through Chord Progressions

Despite its merits, there are several important components of organic chemistry that are not represented in the model. Most importantly, reaction mechanisms cannot be shown without the depiction of electrons through this model. Without the presence of electrons in the model, there is no way to show the movement and motion of electrons, and consequently, no way for organic reactions to be represented. Thus, a large practical part of organic chemistry is lost because of the inability to depict and denote the synthesis of new compounds.

Stemming from this limitation, a better way to model reactions through music may be found in the structure of a chord progression in a cadence.

Consider a cadence with several chords that resolve one chord into another. If each chord in a cadence is taken to be an intermediate of an organic reaction, the motion of electrons in an organic reaction (i.e. rearrangement, leaving groups, attacking, etc...) would be modeled by the movement of notes between chords. Suspensions, neighbor tones, passing tones, and appoggiaturas may all be used to denote different ways that electrons move around in a reaction.

New reactants may be represented by suspending the starting material with a sustained pedal and adding new reactants in a higher octave while the original starting material is sustained. Furthermore, resonance could be denoted by changing between inversions of the same chord.

Of course, this approach would require a way to simplify a molecule in order to allow a chord to represent an entire organic molecule, as opposed to the multiple bar-length measures that this model currently uses. However, the idea may offer a basis of a prospective model with the potential to supplement the denotation of organic molecules with the denotation of organic reactions.

CONCLUSION

This model provides an unconventional and novel approach to organic chemistry that may inspire new understanding of organic chemistry. The application of this model leads to a notation system of organic molecules that presents them in an entirely new dimension.

Unlike previous interdisciplinary studies, this model links the arts and the sciences by approaching science through an artistic standpoint. By establishing a new perspective to an unexplored link between two subjects, this model offers educators the ability to present chemistry in an auditory context.

In its atypical approach, this model brings a novel perspective that fosters appreciation of both fields - hinting that these two fields, chemistry and music, may be more bonded than we thought.

REFERENCES

1. American Chemical Society. Organic Chemistry. Division of Organic Chemistry, American Chemical Society 2012.
2. Weininger, D. SMILES, a Chemical Language and Information System. 1. Introduction to Methodology and Encoding Rules. Journal of Chemical Information and Modeling 1998.
3. Fauvel, J., Flood, R., & Wilson, R. J. Music and Mathematics: from Pythagoras to Fractals. Oxford University Press 2006.
4. Cope, D. Computer Models of Musical Creativity. MIT Press 2005.
5. Cope, D. Experiments in Musical Intelligence. A-R Editions. 1996.
6. Balaban, M., Ebcioglu, K., & Laske, O. E. Understanding Music with AI: Perspectives on Music Cognition. American Association for Artificial Intelligence 1995.
7. Nierhaus, G. Algorithmic composition: paradigms of automated music generation 2008.
8. Metamath Music. Retrieved from http://us.metamath.org/mpegif/mmmusic.html 2006.
9. Reingold, D. Organic Chemistry: An Introductory Text Emphasizing Biological Connections (Second ed.). Learning Solutions 2011.

Sherman is a sophomore at Stanford University pursuing a major in Computer Science and a minor in Chemistry. He has played classical piano at the state and national levels and began studying organic chemistry in high school. Inspired by an UChicago application essay prompt, he wrote a draft of this manuscript, which was nominated for the Davidson Fellowship "Outside the Box" category and won the Academic Paper Award from the National Society of High School Scholars. Sherman plans to finish a co-terminal degree in CS and may then pursue a medical degree.

What Accelerates Blue Carbon Sequestration?

Factors Influencing Variation in Carbon Content of Seagrass Meadow Sediments of Moreton Bay, Australia

John Getsy, Mark Bessen, Sophia Christel and Yari Greaney
Stanford University

In recent years, carbon markets have emerged as an economically driven method of combatting climate change [1]. Seagrass stores more carbon per hectare than terrestrial forests [2] and could hold up to $5.4 billion worth of stored carbon [3]. However, seagrass habitat is under threat and relatively little is known about the factors governing regional variations in its productivity. This experiment sought to provide insight into how species diversity, population density, canopy height, location relative to the shore, and exposure (i.e. intertidal or subtidal) might influence the amount of carbon stored in the sediment of the seagrass meadows of Moreton Bay (Queensland, Australia). Randomly placed quadrats were used to gather information about community properties and soil samples were analyzed for carbon content. Sediment carbon content was found to positively correlate with canopy height and meadow density. Additionally, subtidal soils, especially those dominated by the Syringodium isoetifolium had the highest carbon load. These results could prove useful for policymakers in finding areas of coastlines that are especially important to conserve because of their ability to store carbon. In addition to its growing monetary value, carbon (as CO_2) is a potent greenhouse gas. Understanding seagrass ecology is essential as we try to balance conservation and port development while combatting climate change.

The high concentration of atmospheric CO_2, which recently reached 393.66 PPM, [4] has been implicated in a myriad of human-induced environmental problems [5]. As the negative effects of ocean acidification and climate change become more apparent, it has become important for countries to preserve and expand natural areas with high rates of carbon sequestration (primary productivity). Seagrass plants tend to grow in sprawling meadows comprised of one (monoculture) or more (polyculture) species. Seagrass does not appear as substantial as terrestrial forests, but it is extremely effective at sequestering carbon. It has a high turnover rate, meaning that it grows and regrows quickly [6]. When seagrass plants die, they sink into oxygen-poor sediments; decomposition is slow, and the roots hold sediment in place. These factors contribute to the remarkably high net primary productivity of seagrass. Scientific estimates have placed the global productivity of seagrass between 27.4 Tg [7] and 112 [8] Tg of carbon per year, a range that surpasses that of terrestrial forests [9]. The variability in such estimates reflects a general lack of understanding of the global distribution of seagrass and factors that influence its productivity.

To compound this lack of knowledge about seagrass primary productivity, seagrass habitats are under threat. In Australia, proposed port developments and dredging threaten many of the seagrass meadows. Australia is endowed with bountiful seagrass meadows interspersed throughout the country's entire coastline, including the Torres and Bass Straits, encompassing between 51,000 and 92,500 km^2. The shallow waters off the east coast of Queensland, which include Moreton Bay, contain about 8.4% of Australia's seagrass [10, 11]. This wealth of seagrass could store as much as 136.1 Tg (0.1361 Gt) of carbon dioxide. Nelleman et al. (2009) dub the carbon stored in seagrass and other marine plants such as mangroves and the salt-tolerant plants comprising salt marshes "blue carbon" [12]. Blue carbon serves as a way to quantify coastal ecosystems' value in combating climate change, which incentivizes conservation and allows policymakers to make informed decisions about which seagrass-rich areas especially deserve protection. For those policymakers who do not see climate mitigation as a sufficient reason to preserve coastal ecosystems, the monetary value of blue carbon may provide a powerful incentive. Numerous scientists and economists have described the rise of carbon trading markets a way to provide an economic incentive to reduce emissions [13]. In 2005, the nations of the Kyoto Protocol met and discussed the potential of forest conservation and regeneration as a means by which to reach their emissions reductions quotas [14].

Metz et al. (2001) reported that individual countries had begun to research how the slowing of deforestation and the replanting of forests could contribute to net emissions reductions [15]. However, research in blue carbon sinks, especially seagrass, is a newer science. Numerous studies [16, 17,18] have suggested that seagrass is significantly more productive than terrestrial forests per hectare. A recent study from Edith Cowen University suggests that the carbon from Australia's seagrass could be worth as much as $5.4 billion based on the government's projection

Figure 1. Map of Study Sites (marked by numbered tacks) off North Stradbroke Island

that carbon will reach a value of $AUD 35 per ton by 2020 [19]. Productive, healthy seagrass meadows can also provide homes for numerous animal species, including commercially important prawns and crabs [20]. Seagrass meadows where these organisms are present can take on an additional value of $AUD 3800 per hectare and a seafood biomass comparable to that of a hectare of a tropical aquaculture system [21].

A variety of factors influence the productivity of seagrass, such as the coverage of the seabed, location with respect to the tide, latitude, type and variety of species present, and sediment size. Globally, the carbon content of seagrass sediment varies between 0% and 48.2% by dry mass [22]. Considering its exceptional ability to store carbon, scientists know relatively little about its global distribution and the factors that govern its productivity. Estimates for the global coverage of seagrass range from 177,000 km^2 to 600,000 km^2 [23]. Furthermore, the rate of import of carbon into (e.g. by sediment runoff) and export from (e.g. by grazers, and pieces of seagrass that die and drift away) seagrass meadows has not been extensively studied. It is also possible that the community composition of a meadow influences its productivity. Cardinale et al. (2007) maintain that the complementarity of species increases a meadow's ability to sequester carbon, and that previous studies underestimated the impact that other species had on the productivity of the most productive species in a meadow [24]. Complementarity refers to the process by which species exhibit niche partitioning that allows them to capture resources in ways that complement rather than compete with each other, thus enhancing the productivity of each species [25]. The purpose of this current research was to provide insight into the factors that govern the primary productivity of seagrass in Queensland, Australia's Moreton Bay.

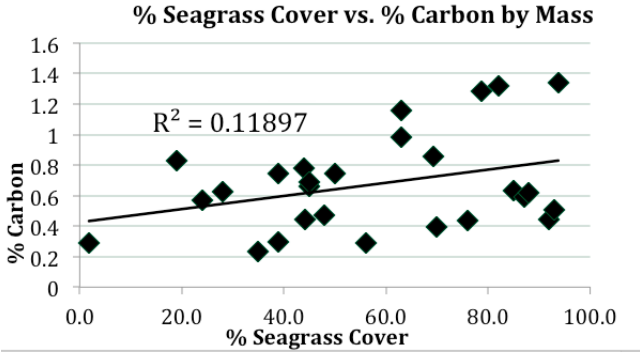


Figure 2. Soil's Seagrass Coverage vs. % Carbon by Mass, found by calculating difference in dry mass of soil before and after burning at 550°C

HYPOTHESIS

Based on the literature review, it was hypothesized that the percent seagrass coverage and canopy height in a meadow will positively correlate with the amount of organic carbon stored in the sediment. It was also hypothesized that the amount of organic carbon stored in sediments would be greater in intertidal sites with tidal recharge and in polyculture communities (i.e. ones with two or more species of seagrass). This is in keeping with the view of Cardinale et al [26].

MATERIALS AND METHODS

All samples were collected at sites in Moreton Bay, off the west coast of North Stradbroke Island, Queensland, Australia. Moreton Bay was ideal because of the availability of a research station, as well as its vast seagrass meadows. The bay contains a variety of different seagrass microhabitats all easily accessible by boat. Seven sites were used (Fig. 1). Three to five areas were randomly sampled at each site. At each of the sampled areas, 3-5 quadrats were placed (i.e. sites 1-7, sub-sites A-C or E each, and 3-5 quadrats per subdivision).

Seagrass Coverage Data

At each sub-site of each site, randomly placed $1m^2$ quadrats (with grids) were used to determine the percentage of the bottom covered by seagrass, the percentage of this seagrass's surface area covered by algae and the species composition of the area. The canopy height was determined by finding the mean of the three

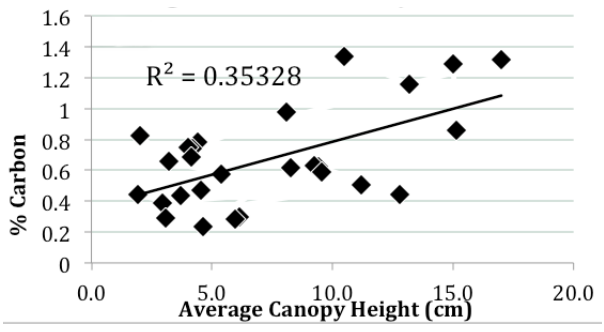


Figure 3. Canopy Height (defined as average of three tallest strands in the transect) vs. % Carbon by Mass in Sediment

[1] Multiplied by 0.46, a constant used when analyzing the carbon content of seagrass sediments (Samper-Villarreal et al., 2013).

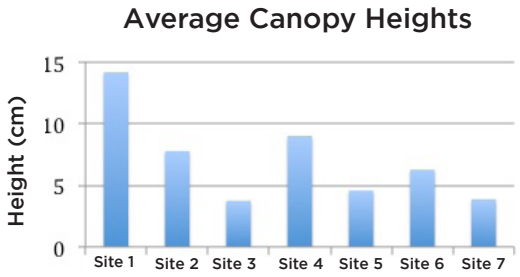


Figure 4. Average Canopy Heights by Site (average of all transects, about 15 per site)

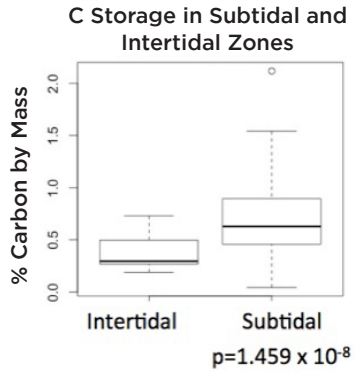


Figure 5. Carbon Storage in Subtidal and Intertidal Sediments

tallest pieces of seagrass in the quadrat.

Soil Data

A soil hand pump with a known sampling capacity was used to collect three sediment samples from each area in the site. These samples were stored in sealed bags. In the laboratory, the soil samples were incubated at 100°C for 24 hours. Once dehydrated, the samples were massed and homogenized using a mortar and pestle. Two grams of the pulverized mixture were taken from each of the samples and placed in aluminum crucibles. These crucibles were heated to 550°C for 6 hours to incinerate the organic matter in the soil. These samples were re-massed to determine how much mass they lost, as this difference[1] between the pre and post-incineration masses shows the percent of the sample's mass that was carbon.

RESULTS

The data demonstrated significant differences between some, but not all, of the study sites. Scatter plots were analyzed with a least squares regression analysis. For comparisons of two variables, a T-test (significant P value <0.05), and for comparisons of three or more variables, a basic ANOVA with Tukey honest significant difference test was used. The carbon content of seagrass meadow sediments correlated directly (r^2=0.12) with the density of the seagrass in the meadow (i.e. percent of the area covered; Fig. 2).

Similarly, carbon content of the sediment was directly proportional to the canopy height of the seagrass that covered it (r^2= 0.35; Fig. 3). Fig. 4 shows the average canopy heights at each site. The tallest plants tended to coincide with subtidal carbon-rich sediments. Based on the soil samples collected, it was concluded that subtidal sediments held significantly more carbon by dry mass than sediments of intertidal seagrass meadows (p=1.46 x 10^{-8}; Fig. 5).

Differences in measured soil carbon levels in and around seagrass meadows were not influenced by the presence of seagrass at the sediment core spot." (Fig. 6). Similarly, the carbon content of subtidal seagrass meadow sediment did not vary significantly based on whether or not it was near the shoreline (Fig. 7). In general, the amount of sequestered carbon in the sediments of monoculture and polyculture seagrass meadows did not vary significantly; nor did the amount of sequestered carbon vary among communities dominated by different species (Fig. 8) except for *Zostera capricorni* and *Halophila ovalis* (Fig. 9). Fig. 10 highlights the differences between the sequestration abilities of the species, with *Syringodium*-dominated meadows[2] having more carbon-rich sediments than *Zostera* and *Halophila*. Fig. 11 summarizes the variation across the seven study sites.

DISCUSSION

The average carbon by dry mass of the sediment samples analyzed varied between 0.168% and 1.337% (Fig. 11). While these

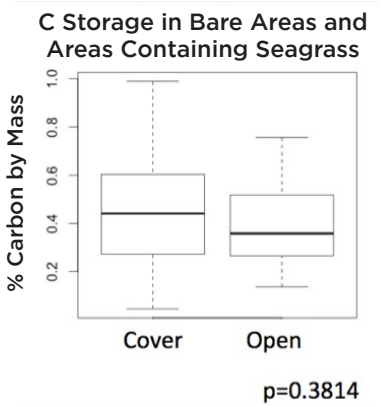


Figure 6. Carbon Storage in Bare and Covered Areas of Meadows

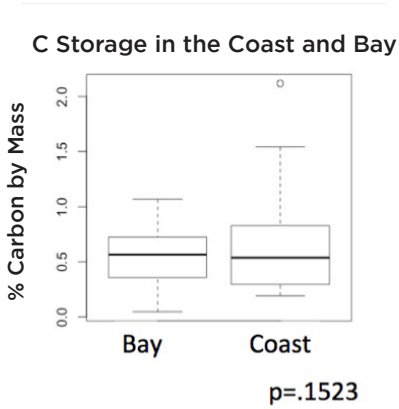


Figure 7. Carbon Content of Bay and Coastal Sediments

[2] The median amount of carbon by mass found in the sediments of the *Cymodocea*-dominated plots exceeds those medians of the *Zostera* and *Halophila*-dominated areas (Fig. 10), but more data is needed to demonstrate whether a statistically significant difference exists between them.

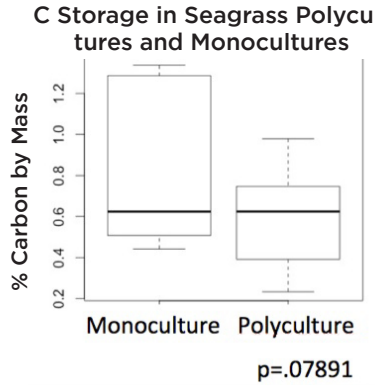


Figure 8. Carbon Content of Monoculture and Polyculture Seagrass Meadow Sediments

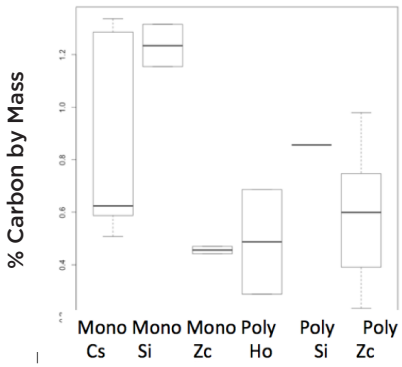


Figure 9. Sediment Carbon Content (% by dry mass) by type of community

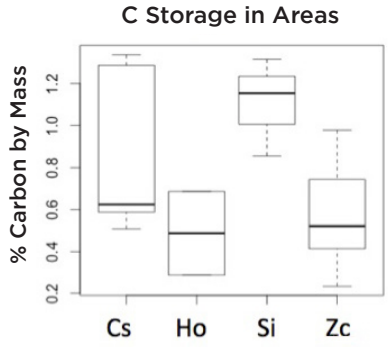


Figure 10. Carbon Storage capacity of Meadows Dominated by Different Species

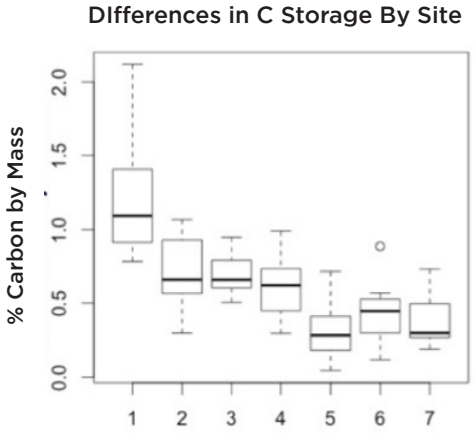


Figure 11. Summary of the Variation in Carbon Load in the Sediment Across Sites

figures are lower than the global average, they are still plausible figures for a seagrass meadow, and the seagrass beds of Moreton Bay could still be a significant carbon sink (especially considering that this experiment only examined a limited number of sites). A weak positive correlation (r^2=0.12) was found between the density of seagrass in a meadow and the soil's ability to hold carbon. Similarly, taller canopies also showed a weak positive correlation (r^2 = 0.35). These findings support the hypothesis that denser seagrass coverage and taller plants indicate a higher carbon load in the sediment. Since taller, more densely packed seagrass plants can catch drifting organic matter more easily than shorter, sparser meadows and have denser, more robust root systems, these results are relatively unsurprising.

However, the differences in stored carbon between intertidal and subtidal meadows (Fig. 5) contradict the related hypothesis. Analyzing the data with a T-test suggests that sediments of subtidal seagrass meadows store more carbon than those of intertidal ones ($p=1.5 \times 10^{-8}$). Multiple factors could explain this discrepancy. Intertidal seagrass tends to contain shorter plants than those found in subtidal seagrass meadows, and the data have suggested that height of seagrass plants correlates with the amount of carbon stored in its sediment. Another possibility involves the nature of the soil. It is possible that the intertidal sediments are more porous than the subtidal ones since they are disturbed more by waves. If so, they would have a diminished ability to maintain biomass. The sediment around North Stradbroke Island likely consists of medium-large silica grains (as it is a sand island); areas with larger grains would not be able to store carbon as well because oxygen could enter the sediment and biomass could escape.

One atypical site could also have caused this difference. Site 1 (of 7), known as Myora springs, receives runoff from the nearby mangrove. The salinity likely varies significantly with precipitation. The average canopy height at this site was the greatest of any site included in this study. Mangrove sediments tend to be anoxic and full of biomass (27), and taller seagrass plants will be able to catch this floating biomass, which will sink to the bottom to be incorporated into the sediment. Although the T-test points to statistically significant differences between subtidal and intertidal sediments' carbon storage, it does not show statistically significant differences between sediments in seagrass meadows closer to the coast and those farther out into the bay (Fig. 7). One especially surprising result was the lack of a statistically significant difference between bare and covered areas of seagrass meadows.

Based on the Tukey test used to analyze the data, the carbon content of sediments at site 2 differed significantly from those of sites 5, 6 and 7 (Fig. 11). Site 7 was located in the intertidal zone. It was originally hypothesized that intertidal sediments should store more carbon per square meter; however, other studies such as Lavery's [28] study of Australian estuarine seagrasses have found that the carbon content of subtidal sediments that support

Site Number	Intertidal or Subtidal?	Coastal or Bay?	Average % Coverage	Other Notes
1	Subtidal	Coastal	73.63	*Freshwater and sediment input from Mangrove runoff*
2	Subtidal	Bay	55.4	
3	Subtidal	Bay	34.4	
4	Subtidal	Coastal	86.67	
5	Sub-sites A-B: Subtidal; sub-site C: intertidal	Bay	48	*Sub-site C on tidal sandbar*
6	Subtidal	Bay	65.67	
7	Intertidal	Coastal	39.53	

Table 1. Average data from sites (n=15 per site) (see Figure 1 for Map)

Australian seagrasses exceeds that of comparable intertidal sediment. Additionally, the composite data of site 2 defy the trend that taller canopies and higher percent coverage correlate with more carbon-rich sediment (Fig. 4; Table 1). Many of the quadrats used in site 2 included monocultures of *Cymodocea*, which, like *Syringodium*, is especially effective at carbon sequestration (Fig. 10). Variations in shoot thickness and size could contribute, as these characteristics do vary between the species, as the seagrass at site 2 was taller than that found at sites 5 and 7 (Fig. 4).

Site 3 could have contained exceptionally carbon-rich sediments for similar reasons. This site mostly contained more of a balance between *Halophila* and *Zostera*, with sub-site E consisting of *Cymodocea*/*Halophila* polycultures. Sites 5, 6 and 7 were dominated by *Halophila* and *Zostera* (other than 6A's *Cymodocea* monocrop). Dugongs' (which were observed at several study sites) preference for *Halophila* could have contributed to the lower numbers recorded for stored carbon. Sections of site 5 were intertidal, as was all of site 7; the regular disturbance from wave action could impede carbon's ability to become buried in soil. Sites 2, 3, 5 and 6 were all close in proximity, which suggests that variables that were not accounted for contributed to the differences observed.

The carbon storage capacity of site 4 also differed significantly from sites 5 and 7. Similar to sites 2, 3, 5 and 7, there are likely unexplored variables contributing to the differences noted between sites 4, 5 and 7. Another possibility is that the data set is too small to portray the differences between these sites accurately. Nonetheless, the consistently high coverage of *Syringodium* (which has thick blades and robust roots), which was not found in sites 5 or 7, could contribute to this difference in sequestration.

The impact of species composition on carbon storage produced varied results. Not all species sequestered carbon in their sediments equally well. In fact, a Tukey test showed that monocultures of *Syringodium isoetifolium* had significantly more carbon-rich sediments than monoculture or polyculture meadows dominated by *Zostera capricorni* (Fig. 9). *Syringodium* has more robust shoots and root systems than *Zostera*, which could explain this variation. Another factor that could explain the variation between the species' capacity to sequester carbon could relate to the way that large herbivores graze them. Dugongs eat several species of seagrass, but they prefer *Halophila* to *Syringodium*. [29]. Conversely, green sea turtles (*Chelonia mydas*) prefer to graze on algae, but sometimes eat seagrass. When they eat seagrass, they prefer to forage in *Syringodium*-dominated meadows rather than *Halophila* dominated ones [30]. The differing preferences of these herbivores are significant because dugongs disturb the sediment considerably when they graze, and they tend to graze in groups in a relatively fixed location for a period of one month of more [31]. Sea turtles prefer algae to seagrass, even *Syringodium*, and do not graze in herds. Since dugongs cause more disturbances to the *Halophila*-littered sediments where they graze, they would release more biomass from the sediment than grazing sea turtles.

Although the findings are limited in geographic scale, they can be applied to other seagrass communities. Future research will test whether these factors have similar effects on seagrass's sequestration ability elsewhere. Additionally, localized studies like this one can be used to identify seagrass meadows that are most important in carbon storage. However, the fact that these results have implicated similar factors as previous studies is promising that the factors measured do correlate with seagrass productivity.

Despite the differences between *Halophila* and *Syringodium*-dominated communities, the Tukey test showed no other significant differences between polycultures and monocultures (Fig. 8), thereby supporting the null hypothesis rather than the view endorsed by Cardinale et al. (2007) and therefore the research hypothesis [32]. Similarly, seagrass meadows with different distributions of species (community composition) did not vary in their capacity to hold carbon in their sediments.

Although the findings are limited in geographic scale, they can be applied to other seagrass communities. Future research will test whether these factors have similar effects on seagrass's sequestration ability elsewhere. Additionally, localized studies like this one can be used to identify seagrass meadows that are most important in carbon storage. However, the fact that these results have implicated similar factors as previous studies is promising that the factors measured do correlate with seagrass productivity.

In summary, the data from this experiment alone does not provide conclusive evidence as to which factors are the most important in governing the ability of seagrass meadows in Moreton Bay to sequester carbon. However, the data does suggest that meadows with a significant amount of *Syringodium* tend to be more productive than those that do not. Additionally, subtidal meadows tend to have more carbon-rich sediments than intertidal ones. Numerous factors, such as sediment grain size, water salinity, water depth, flow rate and patterns of grazing could explain these variations. Further research will provide more answers as to why certain characteristics of seagrass habitats make them more conducive to high rates of carbon sequestration. In future studies, more data will be taken and the activity of tides and grazers should be noted. The more these factors are understood, the more informed scientists and policymakers can be when deciding which areas are especially deserving of conservation for carbon trading and conservation purposes.

LIMITATIONS AND SOURCES OF ERROR

Several factors impeded the execution of the methods outlined in this paper. Most notably, the data collection period was severely limited to a single week. Additionally, the time required to bake, pulverize, sort and incinerate the soil samples meant that all of the soil samples had to be collected early in the week. Collecting more data points would have provided a more accurate

representation of the variations in seagrass productivity across Moreton Bay. Sampling more sites would also have provided a more complete picture of the variations in the ability of the seagrass to sequester carbon throughout Moreton Bay.

Weather conditions, namely the high wave-action in the water during data collection, made soil sampling difficult. Additionally, the soil sampler often encountered bedrock before it was completely buried in the sediment. As a result, density measurements were not completely accurate. Furthermore, the ability to take samples from deeper depths could have revealed additional carbon stocks in Moreton Bay's sediment. Finally, due to constraints of time and available equipment, it was not possible to collect data on variables such as grain size, degree of tidal flow, water depth, salinity and patterns of disturbance and grazing. McLeod et al. suggest that all of these variables could influence the rate of carbon sequestration in the sediment of seagrass meadows, but that scientists do not know enough about the factors governing seagrass's capacity for sequestration [33]. Future studies that would include testing more sites and incorporate measurements of factors such as salinity, water depth, temperature, disturbance patterns (e.g. grazing and floods) and degree of tidal flow can address these shortcomings. Seagrass and other marine plants play vital roles in carbon sequestration, so as carbon markets grow, they will become especially valuable. Understanding the factors that make seagrass habitats better at storing carbon will prove important in the fight against climate change.

ACKNOWLEDGEMENTS

The author wishes to thank the following: Dr. Selina Ward and Professor Cath Lovelock, who organized the Stanford in Australia Targeted Research Program; Dr Olaf Meynecke and Matt Hayes for piloting our boats and navigating Moreton Bay; University of Queensland and Moreton Bay Research Station/staff for accommodating us as we collected data and for providing us with transportation and equipment; and Stanford University's Bing Overseas Studies Program, without which we would not have been able to travel to Moreton Bay to conduct our research.

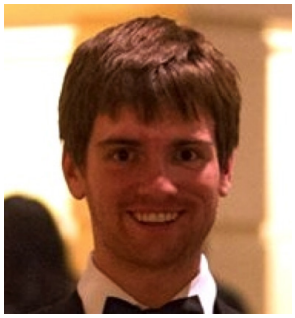
NOTES

1. Baldwin R & Cave M (2012) Understanding regulation theory, strategy, and practice (2nd ed.). Oxford: Oxford University Press.
2. Duarte C (1991) Allometric Scaling Of Seagrass Form And Productivity. Marine Ecology Progress Series, 77: 289-300.
3. Lavery P (2013). Variability in the Carbon Storage of Seagrass Habitats and Its Implications for Global Estimates of Blue Carbon Ecosystem Service. PLOS ONE, 8 (9): 1-12.
4. Trends in Carbon Dioxide. (n.d.) ESRL Co2 Trends RSS. Accessed November 21, 2013, from http://www.esrl.noaa.gov/gmd/ccgg/trends/
5. Solomon S et al. (2009) Irreversible Climate Change Due To Carbon Dioxide Emissions. Proceedings of the National Academy of Sciences, 106(6): 1704-1709.
6. Duarte, 289-300
7. Fourqurean J, Duarte C, Kennedy H et al. (2012). Seagrass ecosystems as a globally significant carbon stock. Nature, 5: 505-509. Accessed November 25, 2013, from http://www.nature.com/ngeo/journal/v5/n7/pdf/ngeo1477.pdf
8. Kennedy H et al. (2010) Seagrass sediments as a global carbon sink: Isotopic constraints, Global Biogeochem. Cycles, 24: GB4026, doi:10.1029/2010GB003848.
9. Fourqurean J. www.nature.com
10. Kirkman H (1997) Seagrasses of Australia, Australia: State of the Environment Technical Paper Series (Estuaries and the Sea), Department of the Environment, Canberra.
11. Lavery, pp 1-12
12. Nelleman C, & Corocan E (2009) Blue Carbon: The Role of Healthy Oceans in Binding Carbon: A Rapid Response. Birkeland Trykkeri AS, Norway: Birkeland Trykkeri AS
13. Baldwin
14. Tavoni M, Sohngen B, & Bosetti V. (2007) Forestry And The Carbon Market Response To Stabilize Climate. Energy Policy, 35(11): 5346-5353.
15. Metz B et al. (2001) "Climate Change 2001: Mitigation". Cambridge, UK: Cambridge University Press.
16. McLeod E et al. (2011) A blueprint for blue carbon: toward an improved understanding of the role of vegetated coastal habitats in sequestering CO2 Read More: http://www.esajournals.org/doi/abs/10.1890/110004. Frontiers in Ecology and Environment, 9(10). Accessed November 19, 2013, from http://www.esajournals.org/doi/abs/10.1890/110004
17. Duarte, 289-300
18. Lavery, 1-12
19. Lavery, 1-12
20. Kirkman
21. Di Carlo G, McKenzie LJ (2011) Seagrass training manual for resource managers. Conservation International, USA.
22. Campbell, JE et al. (2013) "Carbon storage in seagrass beds of the Arabian Gulf", unpublished paper, Smithsonian Marine Station, Ft. Pierce, FL USA 34949 (in press)
23. Green EP & Short FT (2003) World atlas of seagrasses. Berkeley, Calif.: University of California Press.
24. Cardinale, BJ et al. (2007) Impacts Of Plant Diversity On Biomass Production Increase Through Time Because Of Species Complementarity. Proceedings of the National Academy of Sciences, 104(46): 18123-18128.
25. Cardinale, 18123-28
26. Cardinale, 18123-28
27. Matsui, N (1998) Estimated stocks of organic carbon in mangrove roots and sediments in Hinchinbrook Channel, Australia. Mangroves and Salt Marshes, 2. Retrieved March 6, 2014, from http://link.springer.com/article/10.1023/A:1009959909208#page-1
28. Lavery, 1-12
29. Marsh, H (1982) et al. Analysis of stomach contents of dugongs from Queensland. Australian Wildlife Research, 9: 55-67. Accessed November 25, 2013, from http://geckodesign.biz/dugong/publications/JournalPapers/1982/Marsh%20et%20al%201982%20Wil%20Res%209.pdf
30. Taquet, C., Taquet, M. et al. (2006) Foraging Of The Green Sea Turtle Chelonia Mydas On Seagrass Beds At Mayotte Island (Indian Ocean), Determined By Acoustic Transmitters. Marine Ecology Progress Series, 306: 295-302.
31. Preen, A (1995) Impacts of dugong foraging on seagrass habitats: observational and experimental evidence for cultivation grazing. Marine Ecology, 124, 201-213. Accessed November 24, 2013, from the Google Scholar database.
32. Cardinale, 18123-18128
33. McLeod et al.

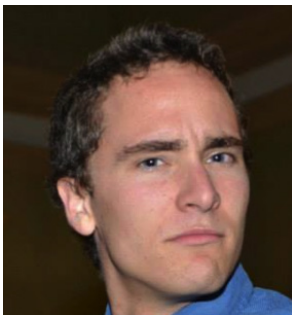
REFERENCES

1. Baldwin R & Cave M (2012) Understanding regulation theory, strategy, and practice (2nd ed.). Oxford: Oxford University Press.
2. Campbell, JE et al. (2013) "Carbon storage in seagrass beds of the Arabian Gulf", unpublished paper, Smithsonian Marine Station, Ft. Pierce, FL USA 34949 (in press)
3. Cardinale, BJ et al. (2007) Impacts Of Plant Diversity On Biomass Production Increase Through Time Because Of Species Complementarity. Proceedings of the National Academy of Sciences, 104(46): 18123-18128.
4. Di Carlo G, McKenzie LJ (2011) Seagrass training manual for resource managers. Conservation International, USA.
5. Duarte C (1991) Allometric Scaling Of Seagrass Form And Productivity . Marine Ecology Progress Series, 77: 289-300.
6. Fourqurean J, Duarte C, Kennedy H et al. (2012). Seagrass ecosystems as a globally significant carbon stock. Nature, 5: 505-509. Accessed November 25, 2013, from http://www.nature.com/ngeo/journal/v5/n7/pdf/ngeo1477.pdf
7. Green EP & Short FT (2003) World atlas of seagrasses. Berkeley, Calif.: University of California Press.
8. Kennedy H et al. (2010) Seagrass sediments as a global carbon sink: Isotopic constraints, Global Biogeochem. Cycles, 24: GB4026, doi:10.1029/2010GB003848.

9. Kirkman H (1997) Seagrasses of Australia, Australia: State of the Environment Technical Paper Series (Estuaries and the Sea), Department of the Environment, Canberra.
10. Lavery P (2013). Variability in the Carbon Storage of Seagrass Habitats and Its Implications for Global Estimates of Blue Carbon Ecosystem Service. PLOS ONE, 8(9): 1-12.
11. Marsh, H (1982) et al. Analysis of stomach contents of dugongs from Queensland. Australian Wildlife Research, 9: 55-67. Accessed November 25, 2013, from http://geckodesign.biz/dugong/publications/JournalPapers/1982/Marsh%20et%20al%201982%20Wil%20Res%209.pdf
12. Matsui, N (1998) Estimated stocks of organic carbon in mangrove roots and sediments in
13. Hinchinbrook Channel, Australia. Mangroves and Salt Marshes, 2. Retrieved March 6, 2014, from http://link.springer.com/article/10.1023/A:1009959909208#page-1
14. McLeod E et al. (2011) A blueprint for blue carbon: toward an improved understanding of the role of vegetated coastal habitats in sequestering CO2 Read More: http://www.esajournals.org/doi/abs/10.1890/110004. Frontiers in Ecology and Environment, 9(10). Accessed November 19, 2013, from http://www.esajournals.org/doi/abs/10.1890/110004
15. Metz B et al. (2001) "Climate Change 2001: Mitigation". Cambridge, UK: Cambridge University Press.
16. Nelleman C, & Corocan E (2009) Blue Carbon: The Role of Healthy Oceans in Binding Carbon: A Rapid Response. Birkeland Trykkeri AS, Norway: Birkeland Trykkeri AS.
17. Preen, A (1995) Impacts of dugong foraging on seagrass habitats: observational and experimental evidence for cultivation grazing. Marine Ecology, 124, 201-213. Accessed November 24, 2013, from the Google Scholar database.
18. Samper-Villarreal, J et al. (2013) "Carbon storage in seagrass meadows across gradients in water quality", paper presented at Australian Marine Science Association 2013 Golden Jubilee Conference, Jupiters Hotel, Gold Coast, 7-11 July
19. Solomon S et al. (2009) Irreversible Climate Change Due To CarbonDioxide Emissions. Proceedings of the National Academy of Sciences, 106(6): 1704-1709.
20. Tavoni M, Sohngen B, & Bosetti V. (2007) Forestry And The Carbon Market Response To Stabilize Climate. Energy Policy, 35(11): 5346-5353.
21. Taquet, C., Taquet, M. et al. (2006) Foraging Of The Green Sea Turtle Chelonia Mydas On Seagrass Beds At Mayotte Island (Indian Ocean), Determined By Acoustic Transmitters. Marine Ecology Progress Series, 306: 295-302.
22. Trends in Carbon Dioxide. (n.d.) ESRL Co2 Trends RSS. Accessed November 21, 2013, from http://www.esrl.noaa.gov/gmd/ccgg/trends/



John "Joe" Getsy is a Stanford University junior from Philadelphia, Pennsylvania majoring in Human Biology with a Notation in Science Communication. His main academic interests include public health, the environment, conservation and sustainable development. Outside of academics, Joe enjoys playing guitar and singing with his band, Air Bounce. He is also a member of the Leland Stanford Junior University Marching Band and the Delta Kappa Epsilon Fraternity. He is involved with health education research through the group HELP, led by Dr. TW Wiedmann. In the future, he intends to pursue an MD/PhD program.



Mark is a Geology and English major at Stanford.

Sophia is an Earth Systems major who has a particular interest in birds and conservation. Her interest in coastal ecosystems hearkens back to her childhood, spent roaming among the banks of pickleweed at the Palo Alto Baylands.

Yari is an Earth Systems major with a minor in Civil and Environmental Engineering at Stanford. She is interested in urban ecology, and how ecosystems can remain healthy within developed environments. Her research with the Bill Lane Center explores how various characteristics of green spaces within developed environments affect both human activity and biodiversity. She hopes to identify strategies that promote vibrant communities, both social and ecological. Yari is also interested in climate justice, dance, and architecture.

An Examination of the Fisheries Benefits of Ileyakl Beluu Marine Reserve

Laura Crews
Stanford University

No-take marine reserves are popular and effective tools for fisheries management in tropical and developing areas. Such reserves are often initiated at the local level without scientific basis for site selection or reserve design. At the request of Ngardmau State, Palau, this study was conducted to assess the impact of Ileyakl Beluu, a small, community-based reserve, on exploited reef fish to inform future management of the reserve. Fish size and abundance data collected within the reserve was compared to data collected at a nearby reference site to examine whether protection status influenced fisheries fecundity. Because this study neither found differences in fish biomass, abundance, or size for all fish, herbivores, or piscivores between the reserve and reference areas, nor did trends over time differentiate the two areas, results do not indicate that the reserve benefits fisheries. Possible reasons this study failed to observe a reserve effect were: (1) the reserve was too small to protect the mobile fish species targeted by surveys, (2) the reserve and reference areas were subject to similar fishing pressure, or (3) data was not powerful enough to detect slight fisheries benefits. Ngardmau should increase the size of Ileyakl Beluu and clarify the reserve's goals to maintain community support.

Fish are a major protein source for people in Pacific Island countries, but most of the world's reefs are overfished [1,2]. Because conventional, top-down management cannot combat unprecedented human impacts to reefs [3], community-initiated marine protected areas have become a popular fisheries and conservation management tool in coral reef ecosystems [4]. In fact, no-take reserves (reserves) are one of few viable management options in developing nations [5]. Reserves are the best way to protect species and habitats [7], and to restore fish assemblages and ecological processes [6]. As a result, advocates argue that the global reserve network should expand to combat reef decline [3].

Reserves have been shown to increase the biomass [8,9,10], size [9,10], abundance [6,10], and diversity [7,10] of fish species exploited outside the reserve. These enduring increases can begin soon after reserves are created, and are not a boom-and-bust phenomenon [7, 11]. Of particular importance to local fishermen, the benefits of reserves are not restricted to the area under protection; in a process known as spillover, migration increases the size and abundance of exploited fish near the reserve [7].

Though fewer large reserves are better than many small reserves at protecting ecosystem function through habitat continuity and preservation of species diversity [10,12], small reserves can still provide fisheries benefits [7]. In fact, increases in fish size, biomass, and abundance within the reserve are proportional to reserve size [10,11], and even small reserves have been shown to produce spillover effects [8]. This means that small reserves measure up to large reserves in fisheries benefits per unit area, but large reserves produce more benefits on an absolute scale. Though the magnitude of fisheries benefits is limited by reserve size, small reserves can be of further benefit by supplying larval reef fish to unprotected areas [13] and protecting fish spawning aggregations [4].

Reserves enhance fisheries and ecosystem health in many case studies, but success is not guaranteed. Management must include periodic monitoring and evaluation to guide decision-making [7,14]. This study quantified the efficacy of Ileyakl Beluu Reserve, Ngardmau State (population 166 in 2005), Palau. Ileyakl Beluu is a small (0.62 km_2), community-initiated no-take marine reserve protected since 2005. Surveys of fish size and abundance conducted in the reserve and at a nearby reference site between 2010 and 2012 were used to determine Ileyakl Beluu's effect on the marine environment. These results will guide management of the reserve to benefit fisheries in the future.

METHODS

Study Site

Located at 7° 38.83'N x 134° 32.90'E, Ileyakl Beluu is a patch reef bordered by channels, and is part of a large barrier reef system on the western side of the main island of Palau. Ileyakl Beluu was first protected by Ngardmau state law in 2005, and then inducted into the Ngardmau Conservation Area System (NCAS) in 2009 by NSPL 7-11. The current management plan, implemented in 2011, began with community meetings held in October 2009. These meetings identified overfishing, poaching by community members and outsiders, and illegal fishing as high-level threats to the marine environment. The community voiced that its ability to manage the reserve was limited by a lack of management plans, monitoring, public knowledge of regulations,

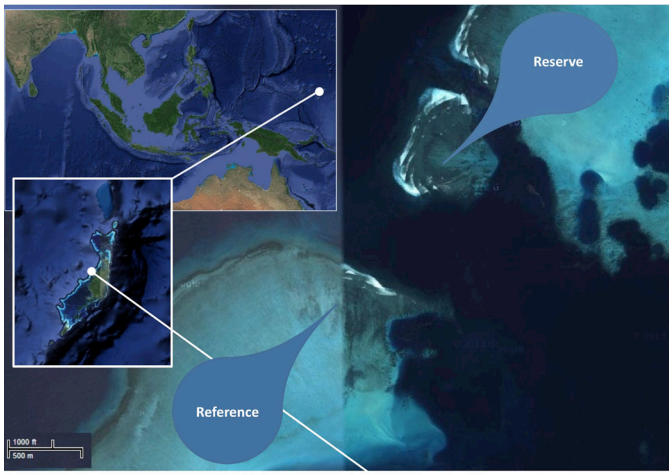


Figure 1. Ileyakl Beluu conservation area and reference area.

conservation officers, infrastructure, and boat fuel.

The 2011 management plan provided funding, personnel, and education to help the community manage Ileyakl Beluu. The plan stipulated that dredging, dumping, aquaculture, and harvesting of the mother-of-pearl snail Trochus spp. are prohibited in the reserve. Fishing for community purposes is allowed, but requires permission from the governor and the traditional chiefs. Entry for tourism requires a fee, but no diving activities currently take place inside the reserve (Board of Directors, NSCA, personal communication).

Formal goals were written into the management plan for the short (1 year), medium (2-3 year), and long (4-5 year) terms. In the first year, managers were to mark reserve boundaries, educate the public on the new laws, conduct a public awareness campaign about illegal fishing, and develop a buffer zone around the reserve. In the second and third years, managers were to encourage peer-to-peer education and enforcement, and secure the approval of fisheries closure from traditional chiefs. By 2015, managers hope to eliminate illegal fishing and ensure reefs are not damaged by dredging activities.

The reference site was an area of barrier reef (7° 38.37'N x 134° 32.57'E) located across Iwekako Channel approximately 1 km away from the reserve (see Figure 1). The reference site was selected because its depth and exposure resemble those of the reserve, but the site does not enjoy the same protections as its counterpart.

Field Techniques

Surveys were conducted on September 30, 2010; April 20, 2011; July 20, 2011; October 5, 2011; and April 3-4, 2012; at three randomly located sites inside the reserve and three randomly located sites in the reference area. At each site, a diver counted target fish in five, 5x50-meter transects aligned end to end and spaced one meter apart at 10m depth. Fish targeted for the study were those reef fish important for commercial and subsistence use (Table 1). Target fish were identified by species, and size was estimated. Fish biomass was calculated using parameters published in Fishbase [15].

Data analysis

Analysis of Similarity (ANOSIM) was used to examine fish community structure based on abundance for differences between Ileyakl Beluu and the reference area. Data was tested for normality using Shapiro-Wilk tests. Comparisons of biomass per unit area, density, and size, of all fish, herbivores, and piscivores between protected and unprotected areas were made using paired t tests if the data was normal and paired Wilcoxon tests if it was not. Linear regressions were used to test for trends over all survey periods in the aforementioned factors. t tests or Wilcoxon tests were used to look for differences in these factors between paired, one-year-apart survey periods to test for trends independent of seasonal variation. This study did not consider detritivores, omnivores, or invertivores as guilds because observations of these groups were infrequent, accounting for just 3% of total observations.

RESULTS

ANOSIM revealed that fish community structure at Ileyakl Beluu differed from that found at the reference area ($p>0.05$)(Figure 2). Despite this result, there were no significant differences between the protected and unprotected areas in biomass per unit area, density, or fish size for all fish, herbivores, or piscivores. Furthermore, there

Scientific Name	Palauan Name	Guild
Siganus lineatus	Kelsebuul	Herbivore
Siganus argenteus	Beduut	Herbivore
Naso unicornis	Chum	Herbivore
Naso lituratus	Cherangel	Herbivore
Cetoscarus spp.	Melemau	Herbivore
Hipposcarus longiceps	Ngeaoch	Herbivore
Siganus fuscescens	Meyas	Herbivore
Lethrinus olivaceus	Melangmud	Piscivore
Lethrinus obsoletus	Udech	Piscivore
Lethrinus xanthochilis	Mechur	Piscivore
Lutjanus bohar	Kedesau	Piscivore
Lutjanus gibbus	Keremlal	Piscivore
Caranx ignobilis	Erobk	Piscivore
Caranx melampygus	Oruidel	Piscivore
Plectropomus areolatus	Tiau	Piscivore
Plectropomus leopardus	Tiau	Piscivore
Plectropomus laevis	"Tiau, Katuu'tiau, Mokas"	Piscivore
Epinephelus fuscoguttatus	Meteungere'temekai	Piscivore
Epinephelus polyphekadion	Ksau'temekai	Piscivore
Valamugil seheli	Kelat	Detritivore

Table 1. Fish target species.

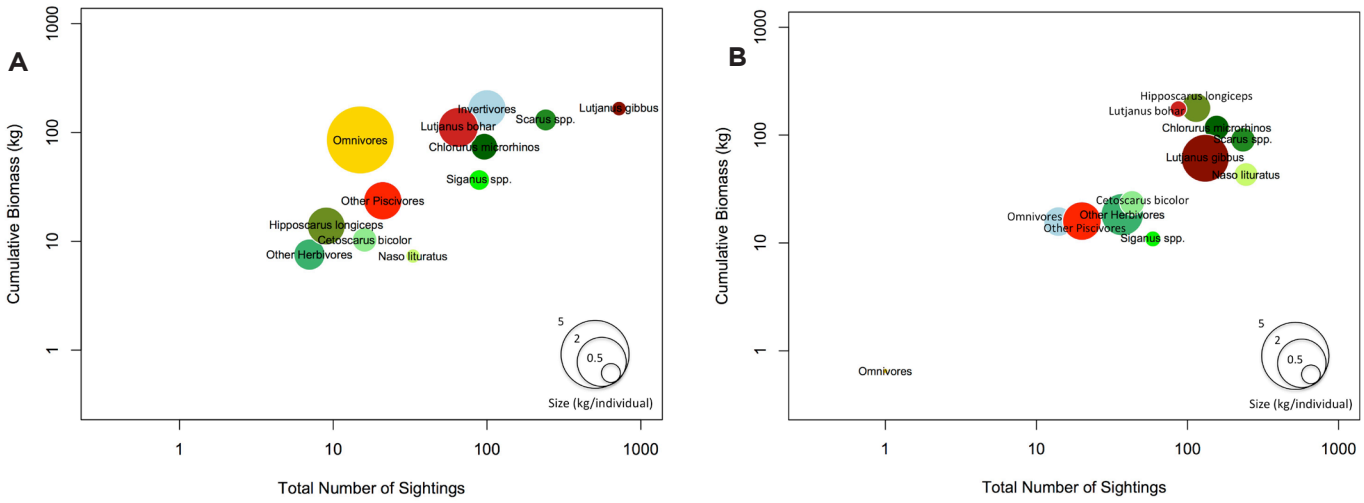


Figure 2. Cumulative biomass and frequency of observation of fish species in the reserve (a.) and reference (b.) areas. Circles are scaled to average fish size. Green circles are herbivores, red circles are piscivores, yellow circles are omnivores, and blue circles are invertivores. Note logarithmic scales.

were no differences in these factors at any of the five survey periods, excepting that herbivores were significantly larger in the reserve than in the reference area in April 2011 (t test $p<0.05$).

From September 30, 2010 to October 5, 2011 data revealed increases in reference fish abundance (Wilcoxon test $p<0.01$), reference fish size (t test $p<0.001$), and reserve and reference piscivore size (t test $p<0.05$ for both). There were no significant trends in total biomass per unit area over this time period.

Mixed trends in biomass, abundance, and size were observed from April 20, 2011 to April 3 and 4, 2012. This time period saw decreases in herbivore biomass (Wilcoxon test $p<0.05$) and herbivore density (Wilcoxon test $p<0.05$) in the protected area, and in fish density (t test $p<0.01$) and herbivore density (Wilcoxon test $p<0.05$) in the reference area. The average size of fish (Wilcoxon test $p<0.01$) and of herbivores (Wilcoxon test $p<0.01$) in the reference area increased over the same period, however, offsetting decreases in abundance and causing total biomass to remain constant.

There were no significant trends in biomass per unit area over all survey periods for all fish, herbivores, or piscivores (Figure 3a-b). The graph of total biomass per unit area versus time revealed that biomass inside and outside the protected area followed a similar pattern of variation.

Fish density did not vary predictably over all survey periods. The graph of fish density versus time showed that fish density was fairly constant, other than a spike in density in April 2011 in both the reserve and the reference areas (Figure 3c-d). The spikes in density in both the reserve and reference areas were caused by several large schools of fish. Because these high values were erratic, the differences in density of herbivores and of piscivores between the protected and unprotected areas were not significant.

The graph of fish size versus time was mixed, but protected and unprotected piscivores showed parallel, increasing trends (Figure 3e-f). Indeed, these trends were robust, with average reserve piscivore size increasing at a rate of 0.63 kg/year, and average reference piscivore size increasing at a rate of 0.54 kg/year ($R^2=0.80$, $F<0.05$ and $R^2=0.78$, $F<0.05$, respectively).

DISCUSSION

This study's results do not indicate Ileyakl Beluu benefits fisheries. There were no significant differences in biomass, abundance, or size for all fish, herbivores, or piscivores between the reserve and the reference areas. Examining trends over the entire survey period and over one year periods yielded jumbled results; there were no consistent trends toward increasing or decreasing biomass or abundance for any fish guild in the reserve or the reference. Rather, total biomass in both the reserve and the reference varied with uncanny similarity, suggesting it is controlled not by protection status but factors operating on a larger spatial scale (e.g. lunar or tidal cycle, turbidity). Piscivore size increased in both areas, though the parallel nature of these trends makes it unlikely this change is a result of protection.

Seven years after its creation, the reserve's benefits for fish populations, if they exist, should be evident. A review of over 100 studies found reserves exhibit significant increases in fish density and biomass after just 1-3 years [11]. Ileyakl Beluu has been protected for longer than three years, yet neither of these increases has occurred. Furthermore, it is unlikely new trends will emerge with time. Species' long-term responses will manifest in the first few years after protection [6], meaning future trends can be predicted soon after reserve establishment. The homogeneity of fish size, abundance, and biomass of the reserve and reference area is likely to continue in the future.

One explanation for the observed equality of the reserve and reference area is that the reserve was too small to protect the fish species targeted by surveys. Some studies conclude that small, community-based marine protected areas do not provide adequate protection for large, mobile species [4]. Reserves work best for species that are sedentary as adults [16], but the home range of reef fish targeted by fishermen can be several square kilometers [17]. Fish at Ileyakl Beluu likely traveled into and out of the protected area, at times exposing themselves to fishermen.

As an alternate explanation, fish biomass, abundance, and size between the reserve and reference area could have been similar because fishing pressure was uniform in these areas. Similar fishing pressure could have arisen because restrictions

were not adequately enforced. A global survey of reef reserves revealed that most lack adequate enforcement [17]. Illegal fishing may not be a pressing problem at Ileyakl Beluu anymore, however; since the implementation of the management plan in 2011, only one poacher has been caught in the reserve (Board of Directors, NSCA, personal communication). On the other hand, fishing pressure could be low both inside and outside the reserve. A reserve's apparent effectiveness depends on the intensity of exploitation in surrounding waters [10]; fish are functionally protected if they are not targeted in the reference area. Little to no fishing was reported near the reserve's boundaries (Board of Directors, NSCA, personal communication), so the reference area may not experience enough fishing pressure to quantify any potential benefits of the reserve.

It is possible that this study failed to observe an existing reserve effect because the data was not powerful enough to detect slight increases in abundance. Visual census data for fish can be too variable to detect reserve effects if survey effort is limited [18]. One study in the Mediterranean found 5-6 years of monitoring was needed to detect the effect of protection [18].

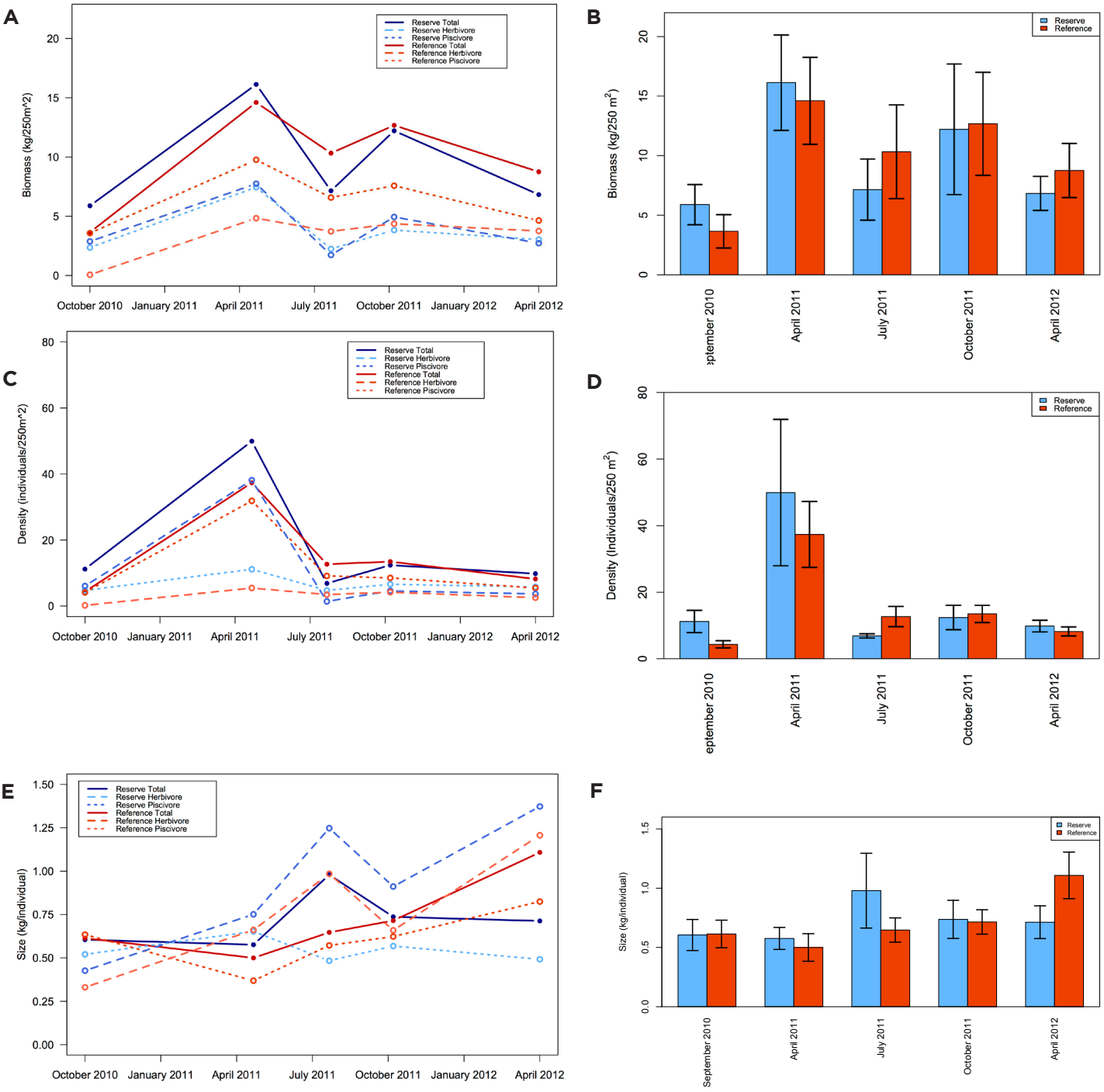


Figure 2. Cumulative biomass and frequency of observation of fish species in the reserve (a.) and reference (b.) areas. Circles are scaled to average fish size. Green circles are herbivores, red circles are piscivores, yellow circles are omnivores, and blue circles are invertivores. Note logarithmic scales.

Perhaps the statistical power gained from more surveys would reveal trends masked by the ecological noise in this study's data. The data's variability was confounded by the fact that increases in size and abundance of large fish species may still be small. Changes in slow-growing, late maturing species may take longer to detect than the time scales mentioned previously [11]. Furthermore, initial increases in biomass and abundance may be small. While piscivore abundance has been shown to increase the first ten years of protection, the effect was four to six times greater if the reserve had been protected for more than ten years [6]. This result agrees with Russ and Alcala's [5] finding that fish biomass within a newly protected reserve increases exponentially, meaning initial rates of change are small. In fact, full recovery of high trophic level fish populations requires decades [5,6]. Time and additional surveys could reveal fisheries benefits that were not detected by this study.

RECOMMENDATIONS

Future surveys should include sedentary target species to test if the observed similarity of the reserve and reference area was caused by similar fishing pressure (either because the reserve was fished illegally or because little fishing occurs in either the reserve or the reference) or because the reserve was too small to provide adequate protection for the mobile species targeted by this study. Because sedentary fish have small home ranges, their abundance should decrease more rapidly with distance from the reserve boundary than the abundance of mobile fish [8]. If surveys reveal sedentary fish are equally distributed between unprotected and protected areas, it is likely the two are similarly fished. If, however, sedentary fish are more abundant in the reserve, it is likely the reserve is too small to protect the mobile fish targeted by fishermen.

Even with insight from further surveys, Ngardmau should strongly consider expanding the size of Ileyakl Beluu. Although the results do not show that the small reserve benefited fisheries, it should still be expanded because the surest way to diagnose the reserve's failure to enhance fish stocks will be to alter its size and monitor the result. Reserves are subject to economies of scale, meaning small reserves are the most expensive to operate per unit area [19]. Increasing the reserve's size is cost effective, and could create currently unrealized fisheries benefits.

Expanding the diversity of fish considered by our surveys could reveal possible flaws in this study's design, and would be a step toward ecosystem-based management. Future surveys should include non-exploited species to test the assumed similarity of the reserve and reference areas; if the habitat of one is inherently better than the other, surveys should reveal higher biomass of non-targeted as well as targeted fish [9]. Furthermore, considering a broader fish population could reveal the reserve's effect on ecosystem function. Reef resilience depends on intact functional groups of fish [17], and some suggest future management should focus on ecosystem function (e.g. protecting grazers) [3]. More diverse baseline data could prove invaluable in assessing holistic changes in future reef health.

Finally, Ngardmau must engage the community in a goal-setting workshop to guide future management of Ileyakl Beluu or risk losing public acceptance of the reserve. Case studies show that, for marine protected areas to maintain community acceptance, the problem they are meant to solve must be well articulated [14]. Ileyakl Beluu's long-term goals are not clear from the management plan. Community meetings held in October 2009 established the goal of increasing fish abundance inside reserves by 20% by 2015, but this goal was not included in the management plant [21]. Lacking formal legislation, the reserve was confirmed only through conversation with community members to have been created to enhance fisheries. If intended benefits are indeed fisheries-related, people will be disappointed by the reserve. If goals are not fisheries-related, alternative expectations should be made clear so that the reserve is not seen as a failure. No-take reserves are too powerful a tool for conservation and fisheries management to be lost to suspicion seated in inaccurate expectations.

ACKNOWLEDGEMENTS

My work with the Palau International Coral Reef Center during the summer of 2013 was funded by the Stanford School of Earth Sciences Undergraduate Research Program and overseen by Rob Dunbar. PICRC researchers completed fieldwork for this study before I joined the project, though I helped with fieldwork (using the same procedures described in this report) for several other marine reserves in Palau. Victor Nestor of PICRC provided input on this paper's content and data analysis. Chris Doropoulos of the University of Queensland and David Koweek of Stanford provided comments on drafts of the paper.

REFERENCES

1. Pandolfi JM, Bradbury RH, Sala E et al. Global trajectories of the long-term decline of coral reef ecosystems. Science 2003; 301:955–958.
2. Jackson JBC, Kirby MX, Berger WH. Historical overfishing and the recent collapse of coastal ecosystems. Science 2001: 293(5530):629–637.
3. Bellwood DR, Hughes TP, Folke C et al. Confronting the coral reef crisis. Nature 2004; 429:827–833.
4. Hamilton RJ, Potuku T, Montambault JR. Community-based conservation results in the recovery of reef fish spawning aggregations in the Coral Triangle. Biological Conservation 2011; 144(6):1850–1858.
5. Russ GR, Alcala AC. Marine reserves: long-term protection is required for full recovery of predatory fish populations. Oecologia 2004; 138:622–627.
6. Micheli F, Halpern BS, Botsford LW et al. Trajectories and correlates of community change in no-take marine reserves. Ecological Applications 2004; 14(6):1709–1723.
7. Lubchenco J, Palumbi SR, Gaines SD et al. Plugging a hole in the ocean: The emerging science of marine reserves. Ecological Applications 2003; 13(1):S3–S7.
8. Abesamis RA, Russ GR, Alcala AC. Gradients of abundance of fish across no-take marine reserve boundaries: evidence from Philippine coral reefs. Aquatic Conservation: Marine and Freshwater Ecosystems 2006; 16:349–371.
9. Palumbi SR. Marine reserves and ocean neighborhoods: the spatial scale of marine populations and their management. Annual Review of Environment and Resources 2004; 29:31–68.
10. Halpern BS. The impact of marine reserves: do reserves work and does reserve size matter? Ecological Applications 2003; 13:S117–S137.
11. Halpern BS, Warner RR. Marine reserves have rapid and lasting effects. Ecology Letters 2002; 5:361–366.
12. Mills M, Weeks R, Pressey RL et al. A mismatch of scales: challenges in planning for implementation of marine protected areas in the Coral Triangle. Conservation Letters 2010; 3:291–303.
13. Harrison HB, Williamson DH, Evans RD et al. Larval export from marine reserves and the recruitment benefit for fish and fisheries. CB 2012; 22:1023–1028.
14. Agardy T, Bridgewater P, Crosby MP et al. Dangerous targets: Unresolved issues and ideological clashes around marine protected areas. Aquatic Conservation: Marine and Freshwater Ecosystems 2003; 13:353–367.
15. Froese R, Pauly D. Editors. FishBase 2013; World Wide Web electronic publication. www.fishbase.org version (06/2013).
16. Hastings A, Botsford LW. Comparing designs of marine reserves for fisheries and for biodiversity. Ecological Applications 2003; 13:S65–S70.
17. Mora C, Andrefouet S, Costello MJ et al. Coral reefs and the global network of marine protected areas. Science 2006; 312:1750–1751.
18. Ojeda-Martinez C, Bayle-Sempere JT, Sánchez-Jerez P et al. Detecting

conservation benefits in spatially protected fish populations with meta-analysis of long-term monitoring data. Marine Biology 2007; 151:1153–1161.
19. Balmford A, Gravestock P, Hockley N et al. The worldwide costs of marine protected areas. PNAS 2004; 101(26):9694-9697.
20. Pajaro MG, Mulrennan ME, Alder J et al. Developing MPA effectiveness indicators: Comparison within and across stakeholder groups and communities. Coastal Management 2010; 38:122-143.
21. Ngardmau Conservation Board with assistance from the Palau Conservation Society. Ongedechuul system of conservation areas ecosystem-based management plan 2011.

Laura Crews is an Earth Systems major from Girdwood, Alaska. She is interested in community development and small-scale sustainability projects. In the future, she hopes to work in the nexus of conservation and intelligent resource use in rural areas. She is fascinated by other cultures, and loved spending the summer learning from her Palauan co-workers. She is particularly proud of being brave enough to try fruit bat soup, a Palauan delicacy. On campus, she teaches outdoor leadership through the Outdoor Education Program and English as a Second Language through Stanford Habla.

Kinship Accuracy: Comparing Algorithms for Large Pedigrees

Danni Sun, Zhencheng Wang, Shuxin Yu

University of Illinois at Urbana-Champaign

Disease association studies to find alleles correlated with a given disease are commonly used in genetic epidemiology. However, calculating a kinship coefficient for large pedigrees with thousands of individuals remains computationally expensive. In this paper, we discuss new methods that could be used to enable disease association on large pedigrees with moderate accuracy and polynomial running time, with respect to the constant size of the sample. We choose the diploid Wright-Fisher Model to simulate the probability distribution of identity states through Monte Carlo Sampling. Since the kinship coefficient represents the expectation of the identity states probability, it can be an effective and efficient tool for analyzing the relatedness in a case-control association study.

A pedigree is a constrained graph designed to represent family relationships in genetic epidemiology. It treats individuals as nodes and uses edges to link parent and child where each individual has at most one male parent and at most one female parent. By tracing all alleles (variants on a gene) in the pedigree, one can determine how genotypes contribute to disease characteristics.

The two primary approaches for detecting disease associations are Linkage Analysis (LA) and genome-wide association studies (GWAS). LA provides strong evidence of genetic inheritance [1] but requires exponential running time based on the number of individuals in the pedigree. GWAS runs faster than LA [2] but is not accurate in the presence of population structure or cryptic relatedness [3] since the pedigree is unknown.

Here, we introduce two novel algorithms, the forward-in-time algorithm (FITA) and the backward-in-time algorithm (BITA) that achieve moderate accuracy with a polynomial running time in the size of the pedigree. The FITA and BITA estimate the kinship coefficients, which measure the relatedness between two individuals and are useful predictors of covariance and correlation between relatives in disease association studies. Our approach is conceptually divisible into two stages. First, we estimate the identity state distribution, a probability distribution of all possible relatedness between the alleles of two randomly selected individuals, using Monte Carlo sampling of inheritance in a pairwise model of relationships, the Pedigree Model. The Pedigree Model is leveraged in this paper to generate tests of association with greater accuracy than GWAS and greater speed than LA [4, 5, 6, 7]. Second, we derive the estimated kinship coefficients in linear time from the identity state distribution and test its accuracy through comparisons with actual kinship coefficients (see Figure 1). For large pedigrees, our algorithms obtain the kinship coefficients faster than traditional kinship calculations, which are quadratic in the number of individuals in the pedigree. For a pedigree with thousands of individuals, this change can make a critical difference by reducing running time from exponential to polynomial.

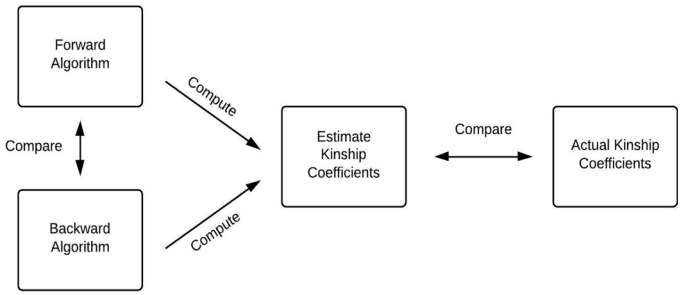


Figure 1. Experimental approach of the present study

BACKGROUND

To make the genetic relationships between individuals more precise, we introduce the term allele, which encompasses all types of genetic variants that are allowed at a particular site in a genome array. Our algorithms can be applied to all types of alleles but treat inheritance paths and single-site inheritance as Markov processes and do not consider genetic recombination.

Wright-Fisher Model

The Wright-Fisher (WF) Model is a haploid model of biological relationships that assumes random inheritance at each generation, non-overlap of generations, and no mutation [8,9]. Individuals choose parents from the previous generation uniformly at random, producing a binomial distribution on the number of identical alleles in the n^{th} generation G_n, except for the generation of founders G_1 [9]. We say two alleles are identical if they are copies of the same ancestral allele and are colored the same (see Figure 2a). The WF Model does not account for the diploid or gendered aspects of human genetics.

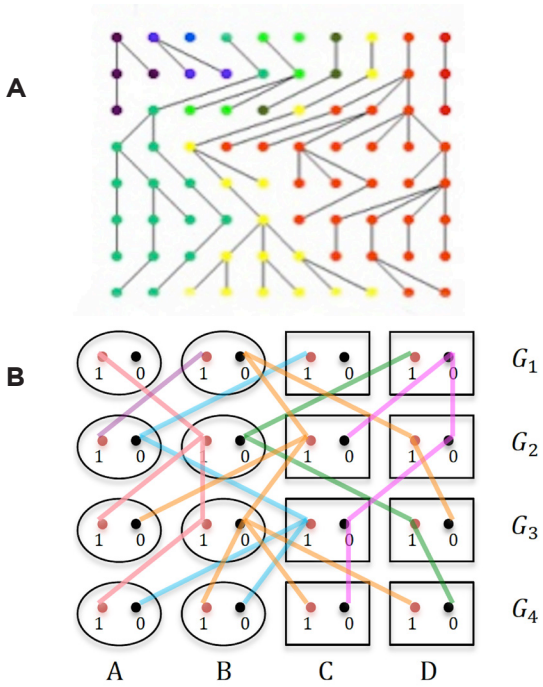


Figure 2. A.) Wright-Fisher Model, which does not account for gender. B.) Pedigree Model, which accounts for gender: Ovals are females and boxes are males. The number 1 represents maternal alleles, and 0, paternal alleles. Each colored line is an inheritance path.

Improved Wright-Fisher Model: Pedigree Model

Compared to the traditional Wright Fisher model, our new Pedigree Model accounts for gender, which makes the model more realistic: half of the *2N* diploid individuals in each generation are females, and the other half, males. Each individual has two alleles: one inherited from the mother, called the maternal allele, and the other from the father, called the paternal allele (see Figure 2b).

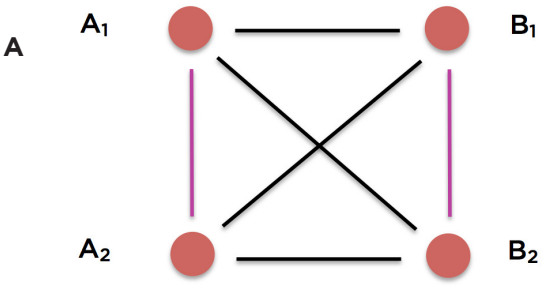
Inheritance Paths, Identity States, and PDIS

An **inheritance path** is a sequence of binary variables X_e, starting from one allele in the last generation to the ancestral allele in the first generation of founders. Each variable indicates which parent's allele was chosen: $X_e = 1$ for the maternal allele, $X_e = 0$ for the paternal allele. In addition, we represent the inheritance path based on the Markov property, a random process in which the future state depends only on the current state. Taking the maternal allele in individual B as an example (see Figure 2b), it has inheritance path 1010.

The **identity state** for two individuals is a graph with four nodes (see Figure 3a) where each individual has two nodes, one for each allele, and each edge indicates that the adjacent alleles are **identical by descent (IBD)**, i.e., they have the same ancestral origin [10].

We can categorize the edges into two types: *outbred edges* connecting alleles from different individuals indicate relatedness between two different individuals, and *inbred edges* connecting alleles within the same individual indicate relatedness between one individual's parents (see Figure 3a). The identity state can be obtained by tracking the inheritance path (but the inheritance path is not determined by the identity state since it is not a one-to-one mapping). Let $Y = \{A, B\}$ denote the set of two extant individuals, and let the inheritance graph induced by Y on the original inheritance path be R_y. Then the identity state for R_y is a graph with four nodes $V = \{A_1, A_2, B_1, B_2\}$ for the two alleles of individuals A and B, and the edges are (x, y) for elements $x \in V$ and $y \in V$ that are in the same connected component in R_y (Kirkpatrick, B. (2013, July). Lecture notes. Seminar, University of British Columbia. Vancouver, BC, Canada).

The **probability distribution of identity states (PDIS)** is constructed by considering all possible inheritance paths for the alleles of two individuals in a fixed pedigree. There are 2^{2n-2f} inheritance paths where n is the number of individuals and f is the number of founders (individuals without parents). The uniform distribution on inheritance paths induces a distribution (the PDIS) on the identity states through the mapping from the inheritance paths to the identity states, summarizing how often two individuals' alleles are identical by descent.



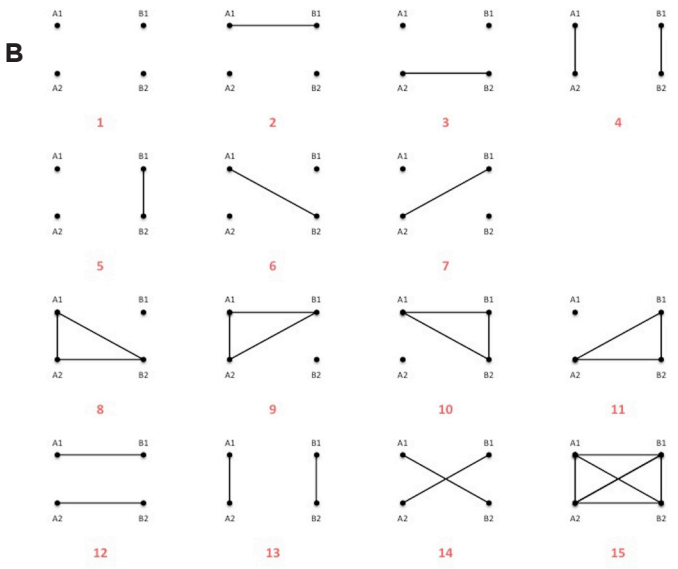


Figure 3. A.) The identity state for two individuals. Black lines mark the outbred edges and pink lines mark the inbred edges. B.) The 15 identity state graphs for four alleles from two individuals. A1, B1 are maternal alleles from individuals A and B and A2, B2are paternal alleles. Four alleles are ordered in a square and connected by an edge if they are inherited from the same ancestor. Therefore, each four alleles have 15 possible identity states that are indexed as i = 1,2,3...15.

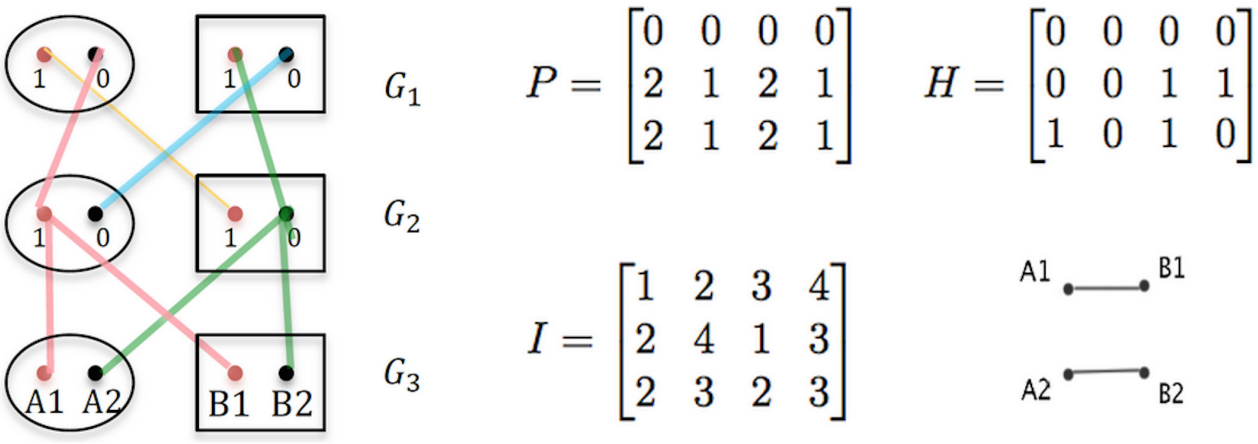


Figure 4. The founder generation is G_1 as usual. The matrix P encodes the parents' indexes for each individual. The matrix H which encodes the binary inheritance paths. Since the founders have no parents, their entries are set to zero. By tracking the allele relationships based on matrices P and H, we can mark each inheritance path with a unique number. In the matrix I, we index the alleles on the same inheritance path as 1 for yellow, 2 for pink, 3 for green, and 4 for blue. Since $A_1 = B_1 = 2$ (both colored pink), $A_2 = B_2 = 3$ (both colored green), we determine the corresponding identity state is 13.

METHODS

Here we introduce our two novel algorithms to compute PDIS: the Forward-In-Time Algorithm (FITA) and the Backward-In-Time Algorithm (BITA). The FITA is easy to implement and provides accurate results. However, it requires more time and computational space than the BITA, while still improving on the time and space requirements of the traditional method. For a pedigree on $2NG$ individuals, it requires time and space $O(NG)$. By contrast, the BITA will save running time and space because it only considers the ancestors of two selected individuals and checks the index of identity state while tracking inheritance paths. The BITA requires only time and space $O(G)$.

Forward-In-Time Algorithm

From a Pedigree Model with G generations, N males and N females per generation, we choose two individuals named A and B in the last generation uniformly without replacement. We outline the FITA first, then offer a simple example.

1. To begin, we create a $G \times 4N$ matrix P which stores the index of each individual's parent, where the mother is indexed by $m \in \{N+1, N+2, ..., 2N\}$ and the father by $f \in \{1, 2, ..., N\}$. In order to store inheritance paths, we create another $G \times 4N$ binary matrix H where the columns with odd indexes represent maternal alleles of the individuals in P and columns with even indexes represent their paternal alleles. Each binary number in $H[n,m]$ indicates which allele of the parents (in G_n with index m) was inherited. (See Example 1 below and the matrices P and H.)

2. Next, we create a new matrix called I with the same size as H to mark each inheritance path clearly by labeling all the alleles with the same value that were inherited from the same ancestral allele in a founder. Initially, all the alleles among the founders have unique values in I. Then we fill the matrix I by copying allele values from parent generation to child generation using information in P and H. (See Example 1 below.)

3. Finally, by checking the values in I $[G,m]$ (here m is the index corresponding to target alleles A_1, A_2, B_1, and B_2) and connecting identical alleles by edges, we get the index of the identity state. Since this algorithm simply fills the matrix I, whose size is bounded by $O(NG)$, the algorithm is $O(NG)$ in space and time.

Example 1: We consider $G=3, N=1$, meaning three generations and two individuals (one of each gender) per generation (see Figure 3b, which uses the same notation as Figure 2b).

Backwards-In-Time Algorithm

Unlike the FITA that generates the whole pedigree and set of inheritance paths for $2NG$ individuals, the BITA only focuses on the people who are related to the selected two individuals in the last generation, saving running time and space. Similar to the way we explain FITA, here we first outline the BITA algorithm, then give an example.

1. To begin, we generate a family graph for two selected individuals A and B (see Figure 5a) and also initialize four disjoint sets D_i, $i \in \{0,1,2,3\}$ where D_0 indicates allele A_1, D_1 indicates allele A_2, D_2 indicates allele B_1, and D_3 indicates allele B_2 (see Figure 5b).

2. Next, we flip a fair coin X_e to indicate which parent the allele was inherited from as mentioned in section 2.3. We join two disjoint sets D_i and D_j, $i,j \in \{0,1,2,3\}$ $i \neq j$, and set D_i as the root if i<=j, incident on edges e and e' in the inheritance path graph if and only if the two extant individuals have the same parent and $X_e = X_{e'}$ (see Figure 5 c & d).

3. Repeat step 2 for each generation until reaching the founders.

4. Finally, we check how the disjoint sets union with each other to get the index of identity state. For example, if D_0 and D_2 are merged into one set (see Figure 5d), i.e., A_1 and B_1 are inherited from the same ancestral allele, then the index of identity state is 2 (see the index of identity states in Figure 3b).

Example 2: We consider $G = 3$, $N = 3$ (see Figure 5). Unlike the FITA, which needs to analyze all individuals (18 in this case), we only analyze the individuals who are related to the two selected people (10 in this case).

All the notations are the same as in Figure 2b, except the black lines in Figure 5a, which are used to connect children and

their parents. Since the four alleles are unrelated to each other initially, the disjoint sets are only four roots (see Figure 5b). In the first iteration, we flip a fair coin X_e to decide whether the current allele inherits the maternal allele or paternal allele from its parent, giving the pink inheritance path and showing that A_1 and B_1 are IBD, inherited from the same allele (see Figure 5c). Also we form the union of the disjoint sets D_0 and D_2 and set D_0 as the root since 0<2. In the second iteration, we ignore B_1 and we only check whether A_1, A_2, and B_2 are IBD or not, since they are the roots of the remaining disjoint sets. In this example, A_2 and B_2 are inherited from the same ancestor but A_1 is not. Thus, we form the union of the disjoint sets D_1 and D_3 (see Figure 5f).

Computation of Actual Kinship and Estimated Kinship

After getting the PDIS, we use it to compute the estimated kinship coefficient for a pair of extant individuals, defined as the probability that two randomly chosen alleles from them are identical. We use a vector $n^{(s)}=(n_1^{(s)},n_2^{(s)},n_3^{(s)})$ on the alleles of two individuals *i,j* to represent the number of each type of edges in the identity state graph, where s indicates the identity state, $n_1^{(s)}$ is the number of outbred edges, $n_2^{(s)}$ is the number of inbred edges for individual *i*, and $n_3^{(s)}$ is the number of inbred edges for individual *j*. We use the probabilities of identity states $\hat{\mathbf{P}}$ to compute estimated outbred kinship $\hat{\Phi}_{ij}$ between two individuals *i,j* and estimated inbred kinship $\hat{\Phi}_{ii}$ and $\hat{\Phi}_{jj}$ for individual *i* and *j* respectively. The equations are defined as follows:

Equation 1.
$$\hat{\Phi}_{ij} = \sum_s \frac{n_1^{(s)}}{4}\hat{P}(s),$$

Equation 2.
$$\hat{\Phi}_{ii} = \frac{1+\sum_s \frac{n_2^{(s)}}{4}\hat{P}(s)}{2},$$

Equation 3.
$$\hat{\Phi}_{jj} = \frac{1+\sum_s \frac{n_2^{(s)}}{4}\hat{P}(s)}{2}.$$

The above computation, however, is rarely used since it is not easy to get the PDIS. In practice, a dynamic programming recursion to simulate the actual kinship coefficient is used. The recursive equations for computing the exact kinship is developed in detail in [5] and [6].

We have developed an algorithm that calculates the actual kinship coefficient using the data structure we created in BITA and FITA. We note that the traditional approach of calculating kinship coefficients in [5] and [6] depends on knowing if two people are lineal relatives, which can be computationally difficult. Therefore, we transform the formula in the following way to avoid this problem; if i and j are different individuals in the same generation, then:

Equation 4.
$$\Phi_{ij} = \frac{\Phi_{m(i)j}+\Phi_{p(i)j}}{2}.$$

Since $\Phi_{m(i)j} = \Phi_{jm(i)}$, $\Phi_{p(i)j} = \Phi_{jp(i)}$, we have

Equation 5.
$$\Phi_{jm(i)} = \frac{\Phi_{m(j)m(i)}+\Phi_{p(j)m(i)}}{2}.$$

Equation 6.
$$\Phi_{jp(i)} = \frac{\Phi_{m(j)p(i)}+\Phi_{p(j)p(i)}}{2}.$$

Thus, we have:

Equation 7.
$$\Phi_{ij} = \frac{\Phi_{m(j)m(i)}+\Phi_{p(j)m(i)}+\Phi_{m(j)p(i)}+\Phi_{p(j)p(i)}}{4}.$$

This result is important both practically and theoretically. Practically, by transforming the formula in this way, we are able to expand the kinship coefficient recursive equation in a level-order manner. Theoretically, the Pedigree Model is an improved WF Model (see section 2.1.2), because it retains the Markov property. In this case, the kinship coefficient of each generation is only related to its parent generation but does not further depend on any of its parent's ancestors.

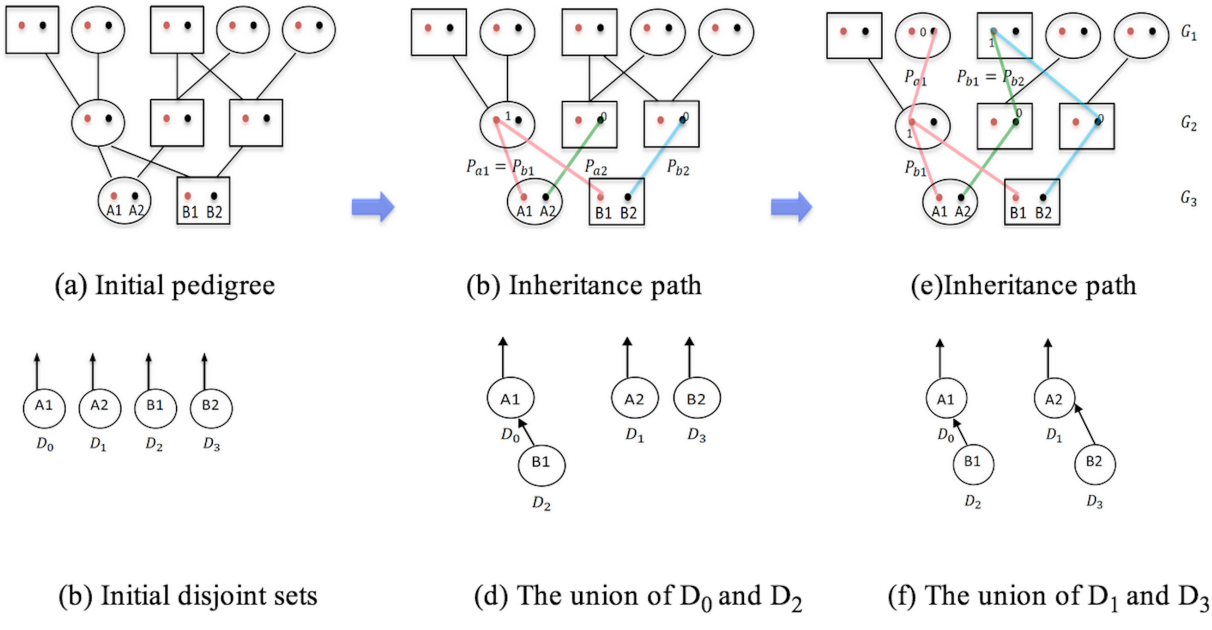


Figure 5. Generating inheritance paths on a fixed pedigree.

In the next section, we leverage the equivalence between estimated and actual kinship coefficients to verify the correctness of the Monte Carlo algorithm and help understand the error introduced by sampling.

RESULTS

If our algorithm estimates the PDIS accurately, we would expect coincidence of the PDIS generated by FITA and BITA and limited error between actual kinship and estimated kinship.

Coincidence of the PDIS generated by FITA and BITA

To get Figure 7, we average over the PDIS generated by 300 random pedigrees (N = 10, G = 10) and 5000 random inheritance paths for each pedigree. The red line indicates the PDIS generated by FITA and the black line indicates the PDIS generated by BITA. As shown in Figure 7, the two lines largely coincide, showing the correctness of both algorithms. The decreasing trend of the PDIS is reasonable since we ordered identity states by increasing number of edges (see Figure 3b), which makes related alleles less likely; alleles with same gender are more likely to be related than ones with different genders.

Comparison of actual kinship and estimated kinship

Based on one pedigree (N = 10,G = 10), we generate various sets of inheritance paths in ascending order (200, 1200, 3200, 6200, 10200, 15200, 21200, 28200, 36200) for each algorithm. For each set of inheritance paths, we compute the total estimated kinship $\hat{\Phi} = \hat{\Phi}_{ij} + \hat{\Phi}_{ii} + \hat{\Phi}_{jj}$ and the total actual kinship $\Phi = \Phi_{ij} + \Phi_{ii} + \Phi_{jj}$. Then we compute the distance between these two kinships, $\Delta\Phi = |\hat{\Phi} - \Phi|$, and the relative error $\Delta\Phi / \Phi$.

The relative errors for the two methods are both very small as a function of the number of sets of inheritance paths for each algorithm (see Figure 8, in which FITA is the black line and BITA the red). Even if there are only 200 sets of inheritance paths, the error is still less than 1.5%, which means that both algorithms for computing actual kinship work relatively well. The decreasing trend

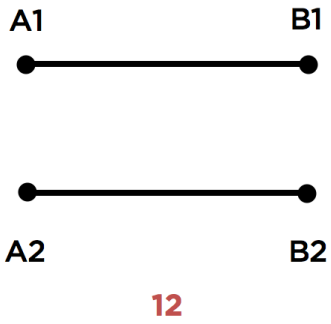


Figure 6. The two individuals in the third generation of Example 2 are related as follows: their identity state is number 12 (see Figure 3b).

of the relative errors means that the more inheritance paths we generate, the smaller the error is between the actual kinship and the estimated kinship. This conclusion is reasonable because for a fixed pedigree, there are only a certain number of different inheritance paths, 2^{2n}, where n is the number of non-founders. When number of inheritance paths generated is over this threshold, the simulations would cover all the cases, which causes the PDIS and estimated kinship to converge. We leave the mathematical properties of this convergence for future study.

DISCUSSION

First, we have demonstrated that using FITA and BITA to estimate kinship coefficient based on the Pedigree Model is accurate. As suggested in [5], actual kinship coefficients can be accurately estimated by Monte Carlo methods. The results we get from our implementation of both forward and backward algorithms support this argument well (both relative errors are within 1.5%, with N=10, G=10 and 200 sets of inheritance paths). This justifies the correctness of our implementation of FITA and BITA in both theoretical and practical terms.

Moreover, our algorithm BITA is a state-of-the-art method for calculating kinship coefficients running in linear time *O(G)*; while the traditional kinship coefficient calculation method computes the

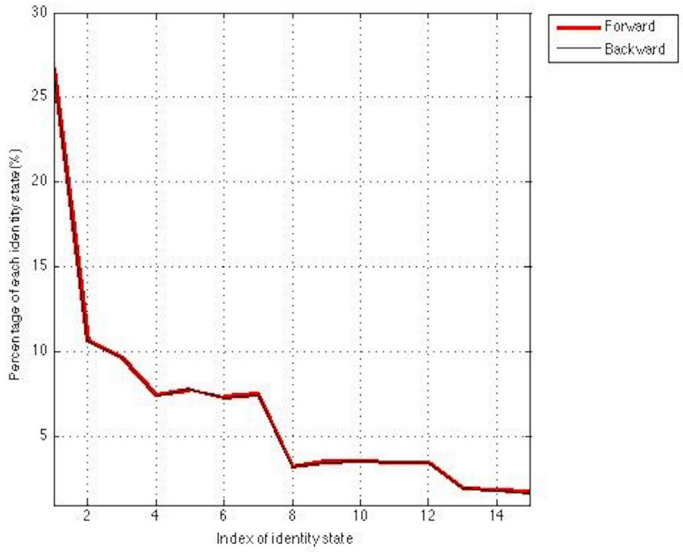


Figure 7. The simulated probability distribution of identity states (PDIS): see Figure 5 for the graphical index of identity states.

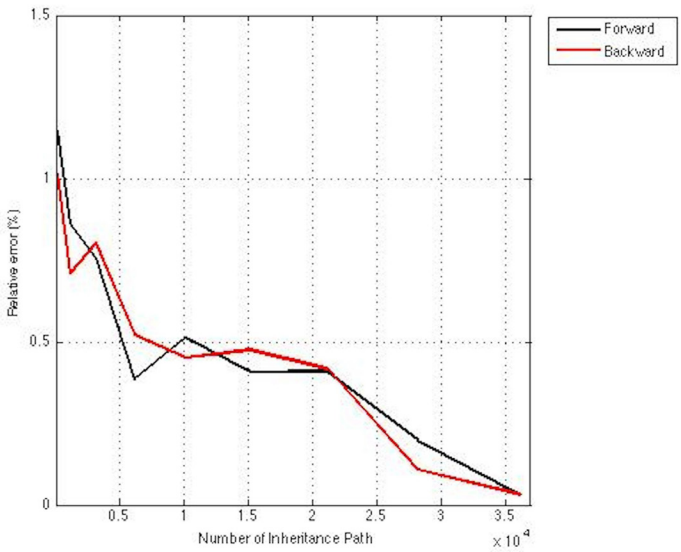


Figure 8. The relative error corresponding to the number of sets of inheritance paths for each algorithm, which decreases as the number of sets of inheritance paths increases.

actual coefficients in quadratic time *O(NG)*. This is a significant improvement that allows current methods of disease association to be applied to large pedigrees of thousands of individuals (large N) [4]. In addition, this performance improvement is achieved without loss of accuracy, as we still preserve the advantage of accounting for known relationships, where Thornton's method is preferable over GWAS. But unlike LA, our algorithm is faster, which makes disease association for large pedigrees possible. We can reach a relative error as low as 0.05% by tuning the number of sets of inheritance paths K (3.5×10^4 here) appropriately (see Figure 8). Notice that K is independent of G and N in our algorithm.

In addition, it is important that BITA does better than FITA in running time and memory consumption. In our implementation, the differences are *O(G)* in the BITA versus *O(NG)* in the FITA (see section 3). We experimented with relatively small datasets, but the gap for time and space between BITA and FITA was already large enough to be noticeable. Therefore, BITA will be even more preferable when enormous genetic data is involved, as in GWAS, and the uncontrollable scale of running time and memory becomes a big concern.

Finally, there is a decreasing trend in the error between the actual kinship coefficients and estimated kinship coefficients when the number of inheritance path increases (see Figure 8). More generally, stable probability distributions of identity states are helpful to improve the performance of both BITA and FITA estimates of kinship coefficients.

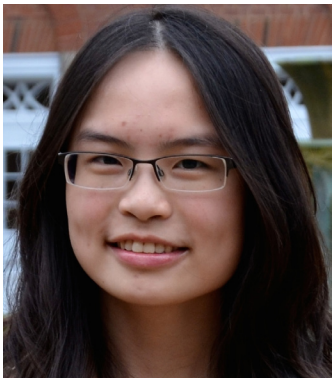
To further develop this work, we now hope to apply our estimates of the kinship coefficients in a statistical method that makes associations (called MQLS), which we hypothesize will produce more accurate association studies on larger pedigrees than can be achieved with existing kinship estimates [4].

ACKNOWLEDGEMENTS

We would like to thank Professors Kay Kirkpatrick and Bonnie Kirkpatrick for directing our work and for providing support, and Professor Bruce Reznick for suggestions. This research is supported by NSF Grant DMS-1106770.

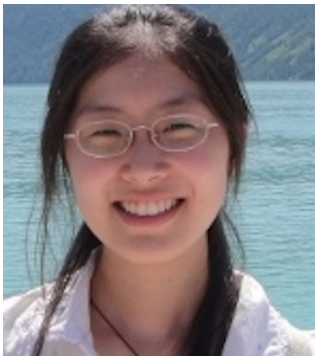
REFERENCES

1. Lander ES, Green P. Construction of multilocus genetic linkage maps in humans. Proc Natl Acad Sci USA 1987; 84:2363–2376.
2. Risch N, Merikangas K. The Future of Genetic Studies of Complex Human Diseases. Science 1996; 273:1516–1517. Genet 1981; 45:299–305.
3. Astle W, Balding DJ. Population Structure and Cryptic Relatedness in Genetic Association Studies. Statist Sci 2009; 24:451-471.
4. Thornton T, McPeek MS. Case-control association testing with related individuals: A more powerful quasi-likelihood score test. Am J Hum Genet 2007; 81:321–337.
5. Karigl G. A recursive algorithm for the calculation of identity coefficients. Ann Hum 1981; 45: 299-305.
6. Thompson EA. Pedigree Analysis in Human Genetics. Baltimore: Johns Hopkins University Press, 1985.
7. Jacquard A. Genetic information given by a relative. Biometrics 1972; 28:1101–1114.
8. Anderson E. An Example from Population Genetics: The Wright-Fisher Model. May 3, 1999.
9. Tavaré S. Ancestral Inference in Population Genetics. In: Picard J, ed. Lectures on Probability Theory and Statistics, Lecture Notes in Mathematics Book 1837. Berlin: Springer. 2004:3--180.
10. Donnelly KP. The probability that related individuals share some section of the genome identical by descent. Theor Pop Biol 1983; 23:34–63.



Danni Sun is a junior majoring in Mathematics at the University of Illinois at Urbana- Champaign. Her research interests lie in applied mathematics. Following the year, she is studying abroad in London with an internship on marketing research.

Zhencheng Wang is a senior student majoring in Computer Science at UIUC. His research interests include Computer Vision and Computer Graphics. He plants to further study these fields in graduate school.



Shuxin Yu is a senior student majoring in Computer Science at UIUC. Her research interest is Computer Vision, especially in medical image analysis. Her future plan is to achieve Master and Ph.D. degrees in her interest field.

Ants as Bioindicators of Ecosystem Health After Fire Regimes

Lisa Jackson, Hailey Juszczak, Nicolas Simon, and Aunika Swenson
Stanford University

Using ants as bioindicators of ecosystem health after fire has the potential to improve existing land management practices. However, few studies have focused on the efficacy of using ants as bioindicators in terrestrial ecosystems. This experiment strives to examine the suitability of ants as accurate bioindicators for ecosystem health after fire by employing comparisons of diversity across functional groups. Eighteen species of ants from nine genera were classified based on food and niche requirements, competitiveness, and reactions to environmental stress. Both ant species richness and the relative abundance of each functional group were examined for changes across four sites differing by times since last burn. The results indicate that ants can be used to effectively gauge post-disturbance ecosystem health using ant community composition rather than fluctuations in species richness. These conclusions have broader and important implications for land monitoring on North Stradbroke Island and in other ecosystems affected by fire or regular habitat disturbance.

Historically, land management researchers have focused on measuring species richness of vascular plants and charismatic vertebrates in terrestrial areas to determine and monitor ecosystem health [1]. Although invertebrates have been recognized as useful instruments of environmental management owing to their large abundance and diversity, ease of sampling, sensitivity to disturbance, and functional significance [1], their use as biological indicators has not been prominent in land management techniques. In fact, the use of invertebrates in terrestrial biological monitoring is only a relatively recent phenomenon. The method dates back to 1983, when ants were used as biological indicators – or 'bioindicators' – for assessing the success of restored mine sites in Australia [3].

Bioindicators are species, groups of species, or other taxonomic groups that reflect the biological health or state of an environment, the ecological impacts of change on an ecosystem, or the overall biodiversity within an area [4]–[5]. The aforementioned use of ants as bioindicators in the assessment of restored mine sites was so successful that mining industries throughout Australia now consider ant monitoring one of the best land management tools [1]. The success of ants as bioindicators relies upon classifying and tracking ants in functional groups. A model has been created prior to this study that classifies ant taxa by food and niche requirements, competitiveness, and responses to environmental stress (see Appendix) [6]–[8]. Studies including this one have employed this functional group model to examine changes in ant community composition in response to land-use, including habitat restoration [9] and fire [6].

Using ants as bioindicators of ecosystem health after fire has significant potential for application in Australia, as fire plays an important role in Australian land management. Control burns are used for many purposes, the most important of which is conservation [6]. Fire stimulates the germination and survival of native species, regulates invasive plants, and removes woody shrubs [10]. The control fires on North Stradbroke Island in Queensland, Australia follow the fire management guidelines issued by the Queensland government. However, the head rangers of Naree Budjong Djara National Park revealed that there is little to no monitoring of this controlled burned land, and no monitoring of the invertebrates on these lands (Head Rangers (Naree Budjong Djara National Park), personal communication, November 13, 2013). To date, there have been no published studies focusing on North Stradbroke Island that investigate changes in ant communities after fires. This study therefore strives to determine whether ants can serve as bioindicators for ecosystem health after fires on North Stradbroke Island and prompt further research implementing such a technique for monitoring areas around the world where recurrent fire is used in land management.

In order to investigate this question, we need to examine how ant biodiversity relates to ecosystem health. Operating under the assumption that ecosystem health increases with time since last burn [14]–[15], it is hypothesized that (i) ant species richness will positively correlate with time since fire, and (ii) high relative abundances of the most competitive ant species will be evident directly after a burn and will decrease with time, while less competitive, subordinate species will have low relative abundances directly after a burn and will increase with time.

METHODS

Study Site

North Stradbroke Island (bounded by latitudes 27°20'–27°45'S and longitudes 153°20'–153°33'E) is a large sand island (38 km long, 11 km wide) located near Brisbane in southeast Queensland, Australia. The study was conducted at four sites on North Stradbroke Island along East Coast Road and Beehive Road, representing four different histories of fire regimes (Fig. 1). Interviews

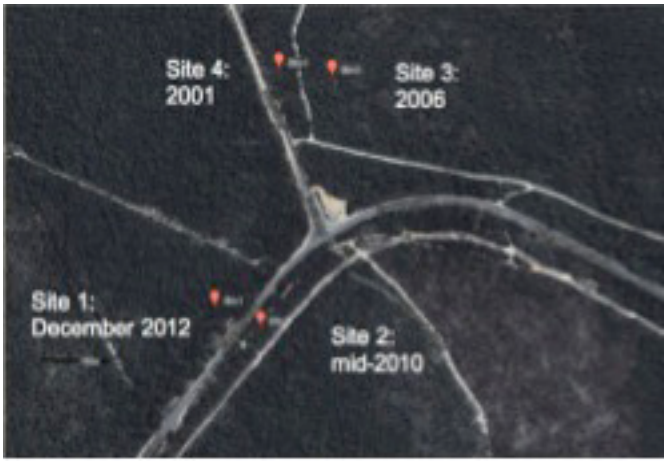


Figure 1. Study sites on North Stradbroke Island. Site 1 (burned in December 2012) and Site 2 (burned in mid-2010) were located south of the pictured intersection along each side of East Coast Road. Site 3 (burned in 2006) and Site 4 (burned in 2001) were located north of the intersection along the east side of Beehive Road.

were conducted with the North Stradbroke Island rangers to determine the known fire regimes for these sites. The four sites were last burned in December 2012, mid-2010, 2006 and 2001 (Head Rangers (Naree Budjong Djara National Park), personal communication, November 13, 2013). Each study site featured different vegetation composition and density (Fig. 2): the more recently burned Sites 1 and 2 are comprised of lower density understory and more open groundcover; Sites 3 and 4 contain dense heathland understory and groundcover.

Sampling

Ants were sampled using baits at two replicate transects in each of four locations on North Stradbroke Island in Queensland, Australia.

Two parallel 25-meter transect lines were randomly set at each study site, approximately 10 m apart. Ants were sampled using bait traps consisting of either honey, representing a "sugar" trap, or tuna, representing a "protein" trap, placed on a plastic specimen container lid, 4 cm in diameter. Each lid was positioned on an 8"x6" sheet of white waterproof paper, which was laid on a small plot of bare soil (i.e. vegetation and groundcover temporarily cleared). This setup comprised the specimen measurement area.

Each transect line had 12 bait traps spaced evenly apart: the traps were placed 2 m apart perpendicular to the transect line and 5 m apart parallel to the line. Six of each of these "sugar" and "protein" traps were made for each transect: six sugar traps were placed to the left of the transect line; six protein traps were placed to the right of the transect line running at 5 m intervals from 0 m to 25 m (Fig. 3).

Once the first bait trap was set down, a stopwatch was started. After 30 minutes, 60 minutes, and 90 minutes, each bait trap was checked for number of ant species present and their relative abundances. These observations were recorded for each bait trap. Individual ants were taken as samples for more accurate identification under a microscope at University of Queensland's Moreton Bay Research Station.

Also observed were the flora species present at each study site. Two 32-meter transect lines were set 1 m apart at each site. The number of different species and their relative abundances were recorded within the 1x1 m area, the 2x1 m area, the 4x1 m area, and the 8x1 m area. In the 16x1 m and 32x1 m areas, only novel species were recorded; no abundances or prior species observed were recorded in these areas. These measurements determined species area curves for plants at each study site.

Data Analysis

The diversity within a system is called alpha (α) diversity, which involves both species richness and abundance. To determine the effects of fire regimes on the α-diversity of ant species, a linear correlation analysis was used to compare time since a study site's last burn with diversity and richness of ants.

The α-diversity of flora species at each study site was determined by area curves and expressed by the Simpson's index:

$$\textit{Simpson's Index} = 1-\left(\frac{\sum n_i\,(n_i-1)}{N(N-1)}\right)$$

Figure 2. Photographs of study sites burned in December 2012 (1), mid-2010 (2), 2006 (3), and 2001 (4). Density of plant community groundcover and understory vary greatly depending on time since last burn.

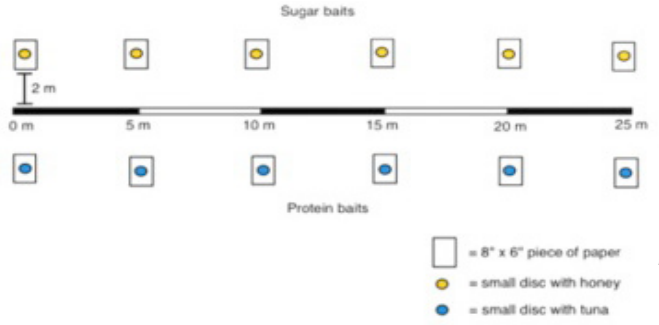


Figure 3. Sampling design used at each study site to assess ant communities after four different fire regimes.

where n_i = the number of individuals of species "i" in the system and N = the total number of individuals from all species within the system.

RESULTS

Flora Diversity

There were key differences in plant community structure at each site (Fig. 4). Based on flora α-diversity measured by the Simpson's index, which takes both species richness and abundance into account, plant diversity increased markedly, exhibiting a strong, positive correlation with time since last burn (R2 = 0.866).

Ant Abundance and Species Richness

An interesting trend occurred in the overall abundance of ants at each site (Fig. 5). Ant abundance increased substantially until Site 3, or approximately 7 years since last burn, and then noticeably decreased at Site 4. Species richness, or the number of distinct species present at each site, remained reasonably constant at around 10 species at the first three sites and then increased to 15 species at Site 4 (Fig. 6).

Community Composition according to Functional Groups

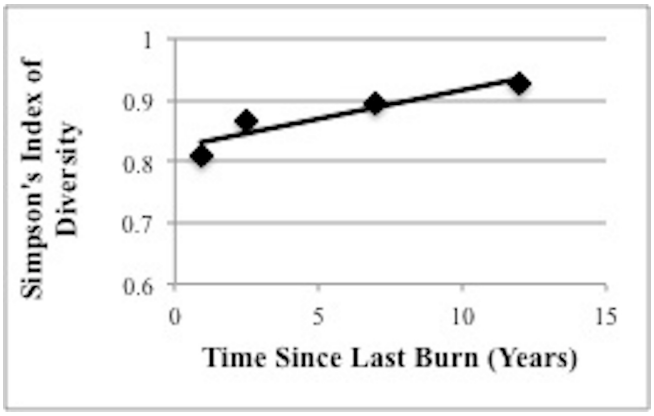
Ant community composition for the four sites according to the functional group model is displayed in Fig. 7. The dominant Dolichoderinae (DD) functional group dominated in the two most recently burned areas, and then DD abundance greatly declined as time since last burn increased in the third and fourth sites. The opposite trend was observed for the generalized Myrmicinae (GM) and subordinate Camponotini (SC) functional groups. Their relative abundances were lowest in the two most recently burned sites and they grew more dominant in the third and fourth sites. Opportunists (O) were most abundant in the most recently burned site and then largely absent as time since last burn increased.

DISCUSSION

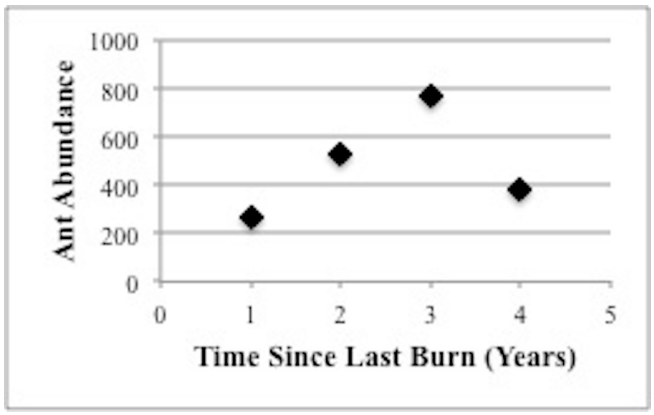
Ant Abundance and Species Richness

While ant abundance followed a non-linear trend, supporting our hypothesis, ant species richness surprisingly did not increase linearly with time since last burn. Our results are consistent with exis ting literature, which predicted that ant abundance would increase initially and then decrease at some point as time

A



B



C

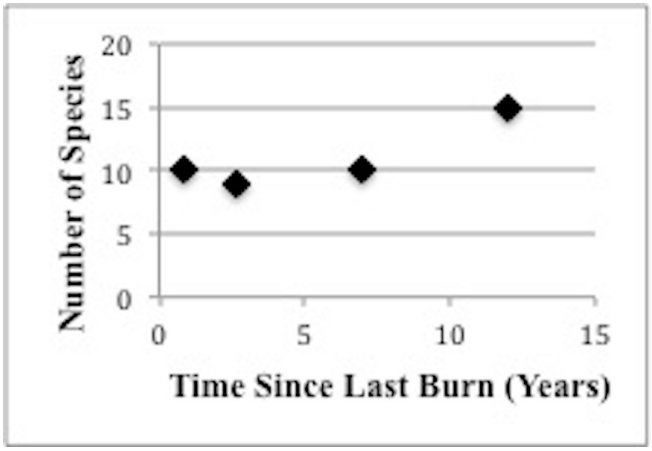


Figure 4. A) Effects of fire regimes (based on time since last burn) on floral diversity (calculated by Simpson's index) in four habitats of North Stradbroke Island. R2 = 0.866. B) Effects of fire regimes (based on time since last burn) on ant abundance in four habitats of North Stradbroke Island. C) Effects of fire regimes (based on time since last burn) on species richness (number of species) in four habitats of North Stradbroke Island.

since last burn increased. This phenomenon is attributed to plant community structure immediately after fire and then during restoration.

Like in other Australian environments, heath species on North Stradbroke Island tend to enter and dominate ecosystems that have not been disturbed for several years [7]. Thus, it was hypothesized that plant diversity would increase at each successive site. Our data support this conjecture (Fig. 4). As heath cover and plant diversity increase in the habitats that have had more time to recover, groundcover such as leaf litter and shrubbery also increases. This results in more difficult terrain for ants to travel across. Furthermore, increased heath cover decreases the amount of sunlight that reaches the ground [7]. Since ants are ectotherms, they rely on sunlight for energy and will have less energy in heathland environments. Thus, not only do ants have more difficult terrain to cover in heath-dominated ecosystems, they have less energy to do so. The observed decrease in ant abundance between the third and fourth sites may be a result of increased heath cover and plant diversity creating conditions more challenging for ant survival.

We expected ant species richness to increase linearly with ecosystem health. However, there was no increase in ant species richness in the first three sites; there was actually a slight decrease between Site 1 and Site 2. The predicted increase was only observed between sites 3 and 4, where species richness increased by 50% between the two sites.

Long-term studies of invertebrates conclude that changes in species richness depend on a variety of factors [19], including vegetation structure changes following disturbance [20]–[21]. Changes in species richness have been shown to differ between studies, either staying relatively unaffected [22] or declining [21]. In other words, there does not seem to be a general trend in the scientific literature regarding the effects of disturbance on species richness.

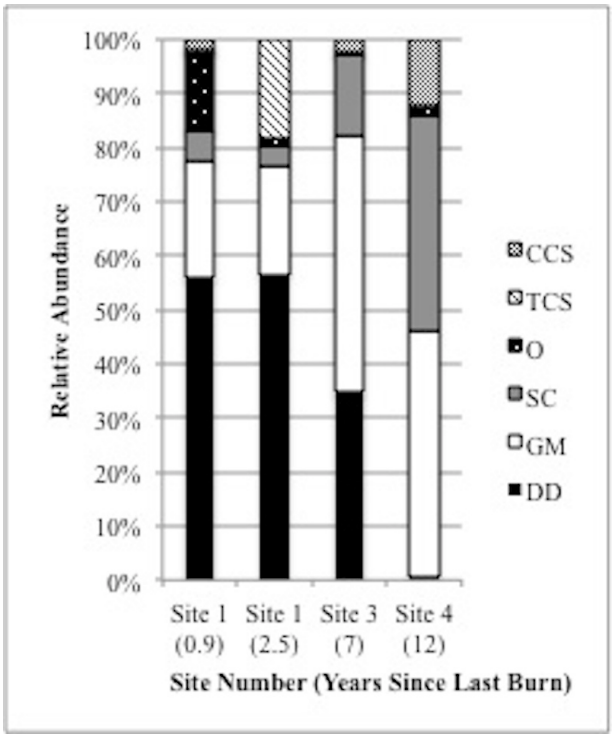


Figure 5. Effects of fire regimes (based on time since last burn) on relative abundance of ant functional groups in four habitats of North Stradbroke Island. The functional groups are: DD – dominant Dolichoderinae; GM – generalized Myrmicinae; SC – subordinate Componotini; O – opportunists; TCS – tropical climate specialists; CCS – cool climate specialists.

Ultimately, a species richness analysis does not provide a complete explanation for the clear inconsistency between the floral and faunal diversity present in our data. Our first hypothesis – that species richness would increase as ecosystem health improved – remains incomplete. Species richness, therefore, is insufficient and inadequate for assessing ecosystem health after fires. This finding is consistent with past literature, which suggests that the predictive power of species-level assays of ecosystem health is relatively low [23]. A more comprehensive explanation for the changes in the data can be derived from an analysis of ant community structure.

Community Composition According to Functional Groups

When considering ant functional groups at the fours study sites, it was hypothesized that the most competitive, aggressive ant species (e.g. Iridomyrmex reberrus) would dominate in the more recently burned areas, but that their relative abundance would decrease with time [6], [10]. The opposite trend was anticipated for less competitive, more subordinate ant species (e.g. Camponotus species or Notoncus species): a low relative abundance at more recently burned sites and a higher prevalence as time since last burn increased [24]. Andersen [6] attributes these trends to the indirect effects that fire has on modifying habitat, food, and competition. Recently burned areas are characterized by open habitats with high insolation and sparse groundcover [6], enabling Iridomyrmex to rapidly move and actively forage [7]. As ecosystems are left undisturbed, however, conditions become less favorable for dominant Dolichoderinae due to lower sunlight exposure caused by denser canopy cover, and more difficulty moving around as groundcover builds up [7]. These stressful conditions cause DD abundance to decrease and, as a result, the abundance of more subordinate groups that are better equipped to deal with the less favorable conditions will increase.

The data support this conjecture: DD were predominant in Site 1 and Site 2. The more open habitats of more recently burned areas offer more insolation (more energy for ectotherms) and allow these fast-moving ants to actively forage and recruit in large numbers. DD have a major competitive influence on other ant species and prevent other species from gaining access to food sources when present in an ecosystem [25].

As time since disturbance increases, however, canopy density and groundcover increase, which create more stressful conditions for the more aggressive ant species. DD have a more difficult time moving through increased leaf litter and heathland, and

have less energy due to decreased sunlight reaching the ground. They cannot recruit as effectively, and drop in abundance. Meanwhile, with less competitive influence from the dominant species and more niches to fill, the more subordinate functional groups (e.g. GM, SC, TCS and CCS) can enter these habitats.

These results support previous studies that show a correlation between time since rehabilitation and clear successional patterns in ant functional groups [26]. Past literature on invertebrate responses to fire come to a similar conclusion: using functional groups to interpret ecosystem health after fires helps simplify complex ecological structures [24]. Analyzing ant species richness alone does not offer a comprehensive analysis of ecosystem health. Instead, determining the relative abundances of specific functional groups within an ecosystem allows for a more complete understanding of how the ecosystem has recovered from disturbance.

Ants provide a promising tool to accurately assess ecosystem health after fires. Their ubiquitous nature, diversity, and relatively predictable functions make them ideal bioindicators of ecosystem health following disturbances. Although the generalizability of the results of this study is unknown, further research could examine the variety of ecosystems the functional group pattern applies to. With proper instruction our methods can be repeated and implemented on North Stradbroke Island and in additional ecosystems around the world.

ACKNOWLEDGEMENTS

We thank Dr. Claire Baker of the University of Queensland for her endless support and direction throughout the project, Dr. Selina Ward for organizing the Targeted Research Project program, the head rangers of Naree Budjong Djara National Park Base for sharing their information on the current fire regimes of North Stradbroke Island, and the Bing family and Stanford in Australia for making this research possible.

APPENDIX

Functional groups are based on schemes used in previous work (Andersen 1991, Anderson 1995, King et al. 1998). Species observed in the field belonging to each functional group are listed in Table 1.

REFERENCES

1. Anderson AN, Fisher A, Hoffman BD, Read JL, Richards R (2004) The use of terrestrial invertebrates for biodiversity monitoring in Australian rangelands, with reference to ants. Austral Ecol. 29: 87–92.
2. Hawkins CP, Norris RH, Hogue JN, Feminella JW (2000) Development and evaluation predictive models for measuring the biological integrity of streams. Ecol. App. 10: 1456–1477.
3. Majer JD (1983) Ants: bio-indicators of minesite rehabilitation, land-use, and land conservation. Env. Manag. 7: 375–383.
4. Majer JD, Orabi J, Bisevac L (2007) Ants (Hymenoptera: Formicidae) pass the bioindicator scorecard. Myrmecol. News 10: 69–76.
5. McGeoch MA (1998) The selection, testing and application of terrestrial insects as bioindicators. Biol. Rev. 73: 181–201.
6. Andersen AN (1991) Responses of ground-foraging ant communities to three experimental fire regimes in a savanna forest of tropical Australia. Biotropica 23: 575–85.
7. Andersen AN (1995) A classification of Australian ant communities, based on functional groups which parallel plant life-forms in relation to stress and disturbance. J. Biogeogr. 22: 15–29.
8. King JR, Andersen AN, Cutter AD (1998) Ants as bioindicators of habitat disturbance: validation of the functional group model for Australia's humid tropics. Biodivers. Conserv. 7: 1627-1638.
9. Andersen AN (1997a) Ants as indicators of ecosystem restoration following mining: a functional group approach. In Finlayson CM, Hale P & Lamb D (Eds.) Centre for Conserv. Biol., Univ. Queensland, Brisbane: 319–325.
10. Underwood EC, Fisher BL (2006) The role of ants in conservation monitoring: If, when, and how. Biol. Conserv. 132: 166–182.
11. Bowman DMJS (1998) The impact of Aboriginal landscape burning on the

Functional Group	Species Observed	Characteristics
Dominant Dolichoderinae (DD)	Iridomyrmex reburrus	Highly abundant, active, and aggressive ants belonging to the genus Iridomyrmex that have a major competitive influence on other ant species. They are particularly abundant and recruit well in open habitats with high levels of insolation enabling rapid movement and foraging activity, and often absent from highly shaded areas.
Generalized Mrymicinae (GM)	Pheidole megacephala; Pheidole sp. 2; Pheidole sp. 3; Crematogaster consobrinus	Unspecialized but highly competitive ants with generalized nesting habits and dietary requirements. They are very competitive at rich food sources due to ability to recruit rapidly and defend food sources. Individuals are not highly active or aggressive so rely on mass mobilization for success.
Subordinate Camponotini (SC)	Polyrhachis ammon; Polyrhachis hookeri; Camponotus consobrinus; Camponotus sp. 2; Camponotus sp. 3	Unspecialized but highly competitive ants with generalized nesting habits and dietary requirements. They are very competitive at rich food sources due to ability to recruit rapidly and defend food sources. Individuals are not highly active or aggressive so rely on mass mobilization for success.
Opportunist (O)	Rhytidoponera metallica; Rhytidoponera sp. 2; Technomyrmex sophiae	Unspecialized but highly competitive ants with generalized nesting habits and dietary requirements. They are very competitive at rich food sources due to ability to recruit rapidly and defend food sources. Individuals are not highly active or aggressive so rely on mass mobilization for success.
Tropical Climate Specialist (TCS)	Leptomyrmex rufipes; Leptomyrmex sp. 2	Generally found in the humid tropics in areas where dominant Dolichoderines are not abundant.
Cool Climate Specialist (CCS)	Notonocus capitatus; Notoncus sp. 2; Notoncus sp. 3	Generally found in cool-temperate zones where dominant Dolichoderines are not abundant.

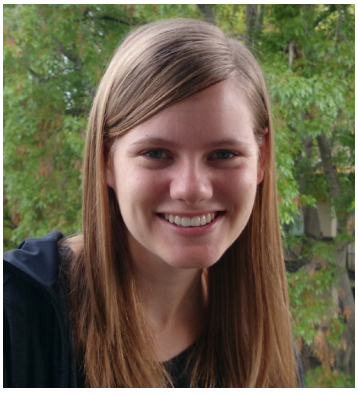
Table 1.

Australian biota. New Phytol. 140, 385–410.
12. Ross A, Prangnell J, Coghill B (2010) Archaeology, cultural landscapes, and indigenous knowledge in Australian cultural heritage management legislation and practice. Heritage Management 3: 73–97.
13. Bird RB, Bird DW, Codding BF, Parker CH, Jones JH (2008) The "fire stick farming" hypothesis: Australian Aboriginal foraging strategies, biodiversity, and anthropogenic fire mosaics. Proc. Natl. Acad. Sci. USA 105: 14796–14801.
14. Andersen AN (1996) Fire ecology and management. In Finlayson C. M. & von Oertzen I. (Eds.) Landscape and Vegetation Ecology of the Kakadu Region, Northern Australia: 179–195.
15. Bond WJ, Keeley JE (2005) Fire as a global 'herbivore': the ecology and evolution of flammable ecosystems. Trends Ecol. Evol. 20: 387–394.
16. Andersen AN (1988) Immediate and longer-term effects of fire on seed predation by ants in sclerophyllous vegetation in south-eastern Australia. Aust. J. Ecol. 13: 285–293.
17. Andersen AN, Yen AL (1985) Immediate effects of fire on ants in the semi-arid mallee region of northwestern Victoria. Aust. J. Ecol. 10: 25–30.
18. Andersen AN, Müller WJ (2000) Arthropod responses to experimental fire regimes in an Australian tropical savannah: ordinal-level analysis. Austral Ecol. 25: 199–209.
19. Morris MG (1975) Preliminary observations on the effects of burning on the Hemiptera (Homoptera and Auchenorhyncha) of limestone grassland. Biol. Conserv. 7: 312–319.
20. Brian MV, Mountford MD, Abbot A, Vincent S (1976) The changes in ant species distribution during ten years postfire regeneration of a heath. J. Anim. Ecol. 45: 115–133.d
21. York A (1994) The long-term effects of fire on forest ant communities: management implications for the conservation of biodiversity. Memoirs of the Queensland Museum 36: 231–239.
22. Merrett P (1976) Changes in the ground-living spider fauna after heathland fires in Dorset. Bull. Br. Arachnol. Soc. 3: 214–221.
23. Andersen AN (1997b) Using ants as bioindicators: multiscale issues in ant community ecology. Cons. Ecol. 1.
24. York A (1999) Long-term effects of repeated prescribed burning on forest invertebrates: Management implications for the conservation of biodiversity. In: Australia's Biodiversity–Responses to Fire: Plants, birds and invertebrates: 181–266. Biodiversity Technical Paper No. 1. Department of Environment and Heritage, Canberra.
25. Andersen AN (2000) A global ecology of rain forest ants: functional groups

Lisa Jackson is a junior at Stanford pursuing a degree in Human Biology with a concentration in Adolescent Development and Behavior. Lisa is interested in observing and understanding what drives human behavior, with a particular focus on social functioning. Her research interests include understanding the biological mechanisms underlying complex social behaviors, and she is currently a research assistant in the Parker Lab in the Department of Psychiatry and Behavioral Sciences. Lisa is starting an independent research project to investigate the neurobiology of autism.

Hailey Juszczak is a junior majoring in Human Biology at Stanford University. Her area of concentration is the neurophysiology of human behavior and disease. She is currently studying the interoceptive abilities of children suffering from PTSD and Prader-Willi syndrome as a research assistant at the Center for Interdisciplinary Brain Science Research. Outside of academics, Hailey is an Executive Director of Stanford's chapter of Relay for Life and loves to travel, having spent the majority of the previous year studying abroad in Madagascar and Australia and gallivanting through Mexico and Iceland.

Nicolas Simon is a junior at Stanford University majoring in Human Biology with a concentration in Infectious Diseases and Community Health. His academic interests include studying the interaction between ecological change and emergence of infectious diseases, and how this impacts health in developing nations. Beyond the classroom, Nicolas loves working with kids as a counselor for Camp Kesem Stanford, traveling, surfing, exploring nature, and reading.



Aunika Swenson is junior at Stanford University. As an Earth Systems major she studies the interactions between human activities and the environment, with a particular focus on how natural environments impact human health. She is currently a research assistant for PhD candidate Gregory Bratman in the Stanford Psychophysiology Lab, and their research investigates the ways in which nature experiences impact emotion and cognitive functioning. Aunika is also a student intern at the Pediatric Bipolar Disorders Program, and volunteers in the geropsychiatric ward at the Menlo Park VA Hospital. In her free time she enjoys traveling, hiking, and playing ice hockey and ultimate Frisbee.