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A Letter from the Editor:

The Journal of Undergraduate Research and Scholarly Excellence has grown into so much more than a journal for undergraduates by undergraduates. While our flagship journal maintains the mission to share the most prominent undergraduate work across all disciplines, our organization has expanded into the JUR Press which now publishes a new line of discipline-specific journals and books. Our first disciplinary journal will be released in 2013 as a collaboration with the Rocky Mountain Sustainability and Science Network in the form of a journal of sustainability.

As our scope of services has expanded, so too has our staff. In addition to our core team of editors, operations and management personnel at our headquarters on the campus of Colorado State University, we also have staff working in countries around the world such as Mexico, India, and the United Kingdom. Most exciting, we have established JUR Editorial Offices on the campuses of the Autonomous University of the Yucatan (Mexico), the University of New Haven (Connecticut), and Schreiner University (Texas). This spring, JUR hosted our first annual Editors' Training Conference at our headquarters on the campus of Colorado State University. This annual event will allow us to expand our editorial board to the campuses of over twenty-two domestic and international institutions over the course of the next five years!

This May, I will be graduating with a degree in Biology from Colorado State University. My next endeavor includes medical school. Thus, in keeping with the policies of JUR, I will be stepping down as the Editor in Chief to make room for the next generation of JUR's leadership. I would like to thank all those who have helped facilitate our expansion during my tenure at the Journal of Undergraduate Research and Scholarly Excellence. I extend my special thanks for the amazing support we have received from our donors, Colorado State University, and the surrounding community. My most sincere thanks go to our Faculty Advisory Board and our Faculty Supervisor, Dr. Mark Brown. Several years ago, Dr. Brown had the vision of establishing a journal for undergraduates, by undergraduates. We know we have made him proud. I would also like to thank our hundreds of referees for helping us select the most professional and high impact pieces for our publication and I thank our authors for having the courage to submit their amazing work.

I would like to personally thank the amazing staff with whom I have had the pleasure of working for the past fourteen months. Getting to know each one of you on a personal and professional level has been a life changing experience for me. Our staff exhibits the strongest work ethic and nothing that this organization has accomplished could have been done without the personal commitment made by each of you. I wish you all the best.

Finally, I am excited to hand the reins over to our new Editor in Chief, Deanna Cox. Deanna is completing her second year as a Natural Resource Management major in the University Honors Program at Colorado State University. As an Associate Editor for Natural Resources, Deanna has taken great strides and exhibited unequaled initiative and leadership potential. As Editor in Chief, I know she will take the journal to new heights. I invite our readership, referees, and staff to join me in welcoming Deanna.

Sincerely,
Pasha Lookian
Editor in Chief
Journal of Undergraduate Research and Scholarly Excellence

Table of Contents

Table of Contents

Staff

Height variations in football shoes (cleats) for running backs and receivers may not alter ankle spatting effects in football field drills

By Peyton P. Faganel, Ty C. Drake, Angela R. Dahl-Miller, ATC and David S. Senchina, PhD
Drake University

Abstract:

Spatting is one option for increasing ankle support for football players. It is unknown how the effects of spatting may be influenced by the height of the football shoe (cleat). Nine young adult males (21.6 ± 1.8 yr., 180 ± 1.7 cm, 72.6 ± 2.4 kg) performed two football drills (60 yd. sprint and 25 yd. cutting drill) with their ankles taped and spatted, once in low-top and once in mid-top football cleats. Heart rate, performance time, and measures of foot comfort and stability were recorded for each drill. Four ankle range-of-motion (ROM) measurements (dorsiflexion, plantarflexion, talar eversion, talar inversion) were recorded for both conditions, plus additional control conditions. Subjects rated their feet as significantly more comfortable in the low-top compared to the mid-top ($p=0.015$). Although there were no significant differences in perceptions of stability, the average stability rating was higher for the mid-top. Expectedly, ankle ROM decreased in a statistically significant manner (all $p \leq 0.06$) with increasing layers on the ankle: barefoot > taped only > taped + shod > taped + shod + spatted. However, there were no significant differences in ROM by shoe height when ankles were either taped + shod + spatted or taped + shod. Heart rate and performance outcomes did not differ based on cleat. Despite the fact that the mid-top cleat covers the ankle malleolus, whereas the low-top cleat does not, it appears that shoe height influences perception but not performance or ROM when ankles are taped or taped and spatted.

Introduction

Football players take care in selecting their footwear, and different cleat models are marketed for different positions. In this paper, the word "cleat" will be used in the common or vernacular sense to refer to the entire shoe and not just the studs on the sole, which are technically also called "cleats" or "studs" or "spikes". Running backs and receivers usually choose a low- or mid-cut cleat, whereas linemen or linebackers usually choose a mid-cut (¾-cut) cleat. Lower-cut cleats are lighter weight, whereas higher-cut cleats provide more support.

The main reason why an athlete chooses a more supportive, higher-top shoe is to reduce the risk of or to stabilize pre-existing ankle sprains by limiting ankle range-of-motion (ROM). Early work suggested that athletes who wore high-top shoes did not experience lower ankle sprain rates compared to athletes wearing low-top shoes.[3,4] Subsequent work suggested that shoes with taller uppers may reduce sprain rates depending on loading forces and foot angle.[5,9,13] Most recent reviews conclude high-top shoes reduce ankle ROM and can reduce ankle sprain rates.[19,20] Most of these studies occurred within the context of basketball.

In addition to the cleat, football players often wear other ankle support measures such as tape jobs, spat jobs (additional tape jobs over the cleat and sock), or ankle braces. Standard taping and bracing restrict ROM and are effective at reducing the rates of or stabilizing pre-existing ankle sprains.[1,19] Also, a recent comprehensive review concluded that standard tape jobs, braces, and high-tops do not impact performance.[15] Much less is known about spatting. In terms of ROM, spatting or spatting + taping is more restrictive than taping alone, though there may be a loosening effect during exercise.[10,12,16,18] In terms of performance, spatting + taping has not been associated with declines in performance when compared to taping, but one study reported spatting + bracing induced larger performance losses than bracing alone.[2,12,16] In terms of athletes' perceptions, conditions for tape alone or brace alone were perceived as more comfortable than conditions involving spatting; for "stability," athletes either perceived spatted conditions were more stable or equivocal to other conditions.[12,18] It is not known whether the height of the cleat modifies the effects of spatting.

The purpose of this investigation was to determine if the height of football cleats typically used by running backs or receivers changed how athletes performed in football drills when their ankles were taped and spatted. Nine young adult males had their ankles taped and spatted in both low-top and mid-top football cleats in a balanced randomized crossover design. Subjects performed both a sprint and cutting drill in both conditions where performance time, footwork errors, heart rate, and perceptions of ankle comfort and stability were recorded.

Materials & Methods

Subject Characteristics

All procedures were approved by the Drake University Institutional Review Board (Drake IRB ID 2009-10031) prior to the commencement of the experiment, and all subjects gave written informed consent prior to participation. Subjects were included if they were male, between the ages of 18-35, and could safely and competently exercise in a size 11 football shoe. Subjects were excluded if they presented any physical conditions that precluded safe exercise, or if they had sustained an ankle injury within two years of the study. Nine young adult males participated. Subject characteristics were as follows, expressed as means ± standard error: age = 21.6 ± 1.8 yr., height = 180 ± 1.7 cm, mass = 72.6 ± 2.4 kg, body fat

Figure 1: Subject wearing low-top cleat (top) and mid-top cleat (bottom). The white tape mark indicates the ankle lateral malleolus.

percentage = 12.3 ± 0.9%, resting heart rate = 66.4 ± 4.1 beats per minute (bpm). For comparison to the football shoes used in the study, subjects were asked to bring their normal training shoes. The average mass of the subjects' training shoes was 364.6 ± 9.9 g. ROM was determined using a standard 12" goniometer (HPMS Inc.) with all measurements taken at rest and on the right ankle. The four measurements taken were dorsiflexion, plantarflexion, talar eversion, and talar inversion.

Shoes and Taping Regimens

The football shoe used in this study was a size 11 Nike Land Shark molded cleat. All shoes were brand new when the study began. Masses of the low-top and mid-top cleats were 439 g and 447 g, respectively. The low-top shoe does not cover the ankle lateral malleolus, whereas the mid-top shoe does (Fig. 1). All subjects completed the experiment in the same two pairs of shoes and were given identical socks to wear (BodyGlove Inc.) that extended midway up the leg. Ankle taping was performed directly on the ankle itself, whereas the spatting occurred over the ankle taping, sock, and cleat (Fig. 2). The same researcher (Faganel) did all the taping. For the ankle taping, both of the subjects' ankles were sprayed with an adhesive (Tuf-Skin Tape Adherent; Cramer Products, Inc.) over which were placed two heel-and-lace pads (3×3-in. cushioned pad and Skin-Lube lubricating ointment, both Cramer Products Inc.). A single layer of underwrap was applied (2 ¾" prewrap; Mueller Sports Medicine Inc.) Standard ½" athletic tape (Johnson & Johnson, Inc.) was used with half-width overlap between adjacent tape strips. The taping procedure is described in detail elsewhere, but consisted of anchor strips, stirrup strips, alternating medial and lateral heel-locks, figure-8's, and finishing strips.[12] Spatting methodology was identical to ankle taping except the following: no underwrap/spray adhesive was used because the tape was applied directly over the cleat/sock, tape strips were adjusted when going under the cleat so that the tips of the studs were left uncovered, and an extra volume of tape was necessary given the added size of all coverings atop the ankle.

Procedures

Initial anthropometric measurements were taken in the laboratory. Height and weight were determined by a physician's scale (Health-o-Meter Professional), and body composition was determined via bioelectrical impedance analysis, or BIA (BodyStat Inc.). Subjects were fitted with a heart rate monitor worn directly against the chest (F6 model; Polar Electro Oy). Football drills were performed on artificial turf ("Field Turf" 2nd generation; Tarkett Sports Co.) at the Drake University outdoor football stadium.

Prior to exercise, subjects were allowed up to five minutes to warm up on the turf in their normal training shoes. Subjects were asked to remove their shoes and socks. Goniometry was performed barefoot, and again after both of their ankles had been taped. The same researcher (Drake) performed all the goniometry. Subjects were given a pair of socks and either the low-top or the mid-top football cleat to wear (order randomized). Goniometry was repeated, both of the subjects' ankles were spatted, and goniometry was again performed. Subjects completed both football drills (described below) then the spatting and cleat were removed. Subjects were then given the other football shoe, goniometry was performed, both of the subjects' ankles were spatted a second time, then goniometry was repeated. Thus, goniometry was performed on six different ankle conditions: (1) barefoot, (2) tape-only, (3) tape + low-top, (4) tape + low-top + spat, (5) tape + mid-top, and (6) tape + mid-top + spat. However, exercise was performed in only two conditions: tape + low-top + spat and tape + mid-top + spat. Only two conditions were tested during exercise to minimize both physical and psychological fatigue.

The sprint drill was always conducted before the cutting drill. For the sprint drill, a 60-yd. distance was measured and subjects ran the distance as fast as possible. For the cutting drill, ten disc cones (Adidas Inc.) were arranged on the ground in two columns of five cones each such that they alternated. Subjects were asked to run ("cut") from the first cone in column A, to the first cone in column B, to the second cone in column A, and so forth, to the end of the series. Cones within a column were spaced 5-yd. from each other. The distance between the two columns was also 5-yd., but cones within the two columns were staggered such that the "forward distance" from a cone in one column to the cone on the other column was 2.5-yd. The total "forward distance" of the cutting drill was 25-yd., even though the subject ran over twice that distance within the drill.

After completing each drill, time-to-completion and heart rate were recorded. Accuracy measures (number of correct contacts and number of misses) were also recorded for the cutting drill. Subjects were presented with two separate 10-cm visual analogue scales after each drill for perception of comfort and stability. Each scale consisted of a 10-cm line anchored with two directives on either end. For the comfort scale, the anchor phrases were "least comfortable imaginable," [sic] and ""most comfortable imaginable," [sic] whereas for the stability

Figure 2: Ankle taping (top) and ankle spatting (bottom) techniques.

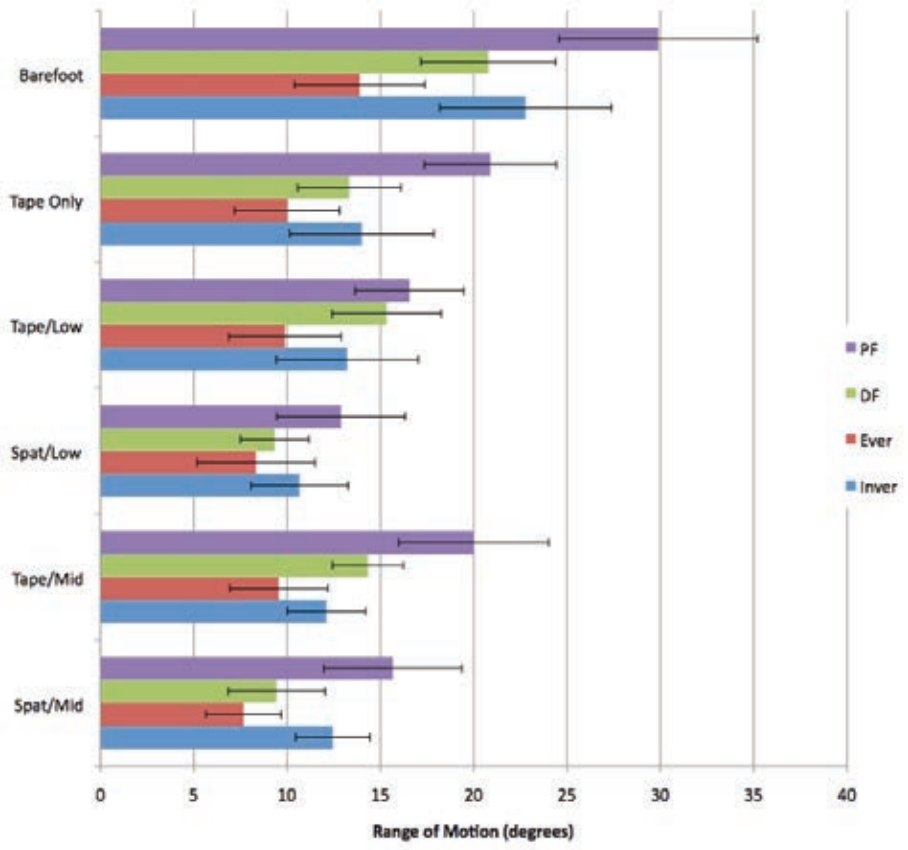

Figure 3: Ankle ROM (as determined by manual goniometry) for all conditions tested in this study. "Low" and "mid" indicate data from the low- and mid-top football cleats, respectively. "Tape" indicates the ankle was taped, "Spat" indicates the ankle was spatted in addition to being taped. For ROM measurements: PF = plantarflexion, DF = dorsiflexion, Ever = talar eversion, Inver = talar inversion.

scale, the anchor phrases are the same only the word "comfortable" was replaced with "stable." Subjects were asked to mark a vertical line along the continuum based on how their ankle felt after performing each drill in comparison to wearing an "ideal football shoe."

Statistics

Univariate ANOVAs were performed using PASW 19.0 (SPSS, Inc./IBM) with significance defined as $p<0.05$, and a trend defined as $0.05<p<0.1$. For ROM, independent factors included test performed (the four goniometry measurements) and ankle condition (the six ankle conditions), with degrees of motion being the dependent factor. For perception scales, independent factors included the drill (sprint or cutting) and the ankle condition (tape + low-top + spat or tape + mid-top + spat), with comfort or stability rating serving as the dependent variable. The ANOVAs for the performance parameters were structured similar to the perception scale ANOVAs, only a separate ANOVA was performed with each performance outcome serving as a distinct dependent variable. Whenever significant main effects were uncovered (such as a main effect of test or a main effect of ankle condition in the case of ROM), follow-up post-hoc pairwise comparisons (using LSD) were conducted.

Results

Range-of-Motion

Expectedly, there was a significant difference in absolute ROM for all four ROM tests (dorsiflexion, plantarflexion, talar eversion, talar inversion; Fig. 3) used in this study (main effect $p<0.001$). There was also a significant difference in ROM between the six ankle conditions (main effect $p<0.001$). Pairwise comparisons between the six ankle conditions revealed that the barefoot condition had much greater ROM when compared to all five other ankle conditions (all ≤ 0.001).

In terms of the effects of the football cleat itself on ROM, there were no significant differences between ROM in the tape-only condition compared to tape + low-top or tape + mid-top. Similarly, no significant differences in ROM were found when tape + low-top was compared to tape + mid-top, nor when tape + low-top + spat was compared to tape + mid-top + spat. Taken together, these results indicate that the height of the football shoe itself does not alter ROM in any comparisons, despite the fact that the low-top shoe does not cover the ankle malleolus, whereas the mid-top shoe does.

In terms of the effects of taping regimens, there was a significant difference when the tape-only condition was compared to tape + low-top + spat ($p=0.011$) and there was a trend towards a significant difference when tape-only was compared to tape + mid-top + spat ($p=0.057$). The tape-only condition was associated with greater ROM in both instances. There was significantly greater ROM for tape + low-top compared to tape + low-top + spat ($p=0.038$), and for tape + mid-top compared to tape + mid-top + spat ($p=0.026$). Taken together, these results suggest that both taping regimens restricted ROM over the barefoot condition, and that spatting on top of standard ankle taping restricted ROM more than standard ankle taping alone.

Comfort and Stability Perception

There was a significant difference in subjects' perception of comfort such that they perceived the low-top as being more comfortable than the mid-top ($p=0.015$; Fig. 4). However, there was not a significant difference in perception of stability between the two football shoes despite the fact that average stability scores were lower for the low-top versus the mid-top. There were no significant differences between the two drills in terms of subjects' perceptions of comfort and stability.

Performance Parameters

No significant differences were found between the low-top and mid-top shoes for any of the performance parameters measured (heart rate and time-to-completion in both drills, and measures of accuracy in the cutting drill) in either of the two drills.

Discussion

Our main findings were that the height of the football cleat did not change the effects of spatting on ankle ROM, despite the fact that the mid-top cleat covers the ankle malleolus, whereas the low-top cleat does not, and that subjects perceived the spatted low-top cleat as more comfortable

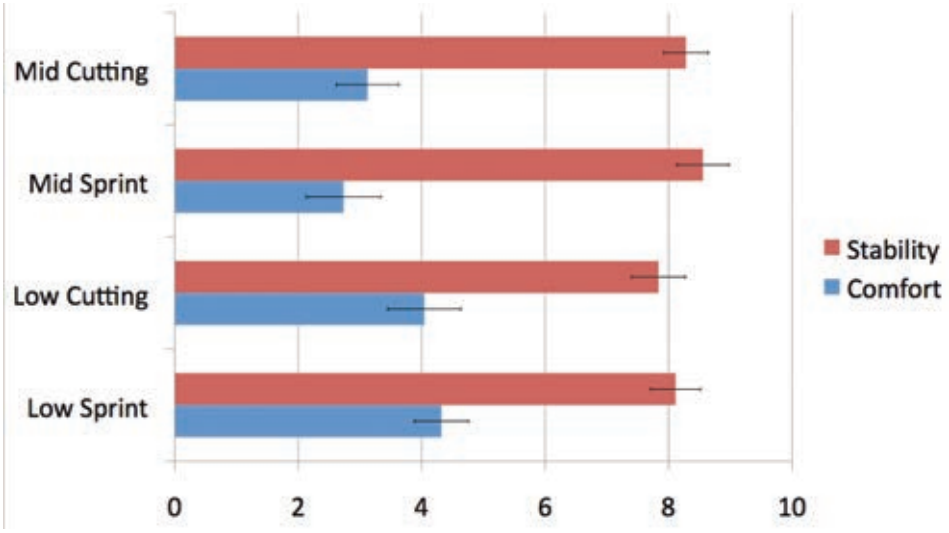

Figure 4: Athletes' perceived ankle comfort (blue) and ankle stability (red) assessed using a 10-cm visual analogue scale.

than the spatted mid-top cleat. Our data also hinted that subjects might have perceived the spatted mid-top cleat as more stable than the spatted low-top cleat, but our sample size was too small to achieve statistical significance.

These results are consistent with others in several ways, but also expand on current knowledge. Previous research has shown that ankle ROM decreased with increasing layers of tape or clothing on the ankle.[10,12,16,18] Our results (Fig. 3) echoed that, but also showed that football cleat height (at least among cleats like those commonly worn by running backs and receivers) does not influence ROM when ankles are taped and spatted. It is possible that differences would be found if a low-top cleat was compared to a high-top cleat because the difference in the height of the uppers would be greater, though brand new true high-top football cleats were not available at the time of the study. [Note: "¾ cleats" are the same as "mid-top cleats" in football footwear.] Some football players opt to wear soccer cleats because they are designed better for running on turf. Soccer cleats often have a lower cut than low-top football cleats, but it may be difficult to make comparisons between soccer cleats and football cleats due to differences in the uppers (materials, stitching patterns) or in studs and stud configurations. Future studies on football shoe height effects could explore soccer cleats or high-top cleats in comparison to the more widely used low- or mid-top football cleats as used in this study.

Regarding perception, we could not locate any studies that specifically investigated whether athletes found athletic shoes of varying heights to be more or less comfortable. The lack of research in this area has recently been discussed elsewhere.[7] One study investigating three different types of ankle braces reported that braces that elicited the higher ratings of perceived stability also elicited the lower ratings of perceived comfort, indicating that the two perceptions may be inversely related.[17] Both our team and another have shown that comfort expectedly decreases as layers of ankle restriction increase.[12,18] Athletes in our study rated the low-top cleat more comfortable than the high-top cleat when their ankles were taped and spatted, a finding congruent with the above studies, yet still novel. Though stability ratings were not significant in our study (likely due to small sampling size), they were lower for the mid-top cleat versus the high-top cleat, which would be consistent with the inverse relationship between comfort and stability noted earlier. Another interesting future experiment would be to have athletes wear shoes of differing heights during simulated games and then rate those shoes for comfort and stability as it appears there is no data on how the height of an athletic shoe influences its comfort or stability ratings. Such a study would be most feasible with basketball shoes or football cleats as manufacturers oftentimes produce a series of shoes with more or less identical soles but different uppers. Further research could also be done across different stud types such as blade, edge, flat, molded, pivot disk, or screw-in.

Performance outcomes did not differ based on ankle condition in our study, suggesting that there are no spatting-shoe height interactions among the cleats we tested. Previous work has indicated that spatting may or may not have performance effects.[2,12,16]

These findings may be helpful for athletes as well as coaches, athletic trainers, or other game personnel including sports medicine professionals. For athletes, the results suggest that low-top and mid-top cleats normally worn by running backs and receivers may provide similar benefits during practice drills and presumably during game scenarios, though we did not directly test the latter. In either practice or game scenarios, spatting may provide more of a placebo effect than actual benefit and the height of the cleat upper does not change the effects of spatting. For coaches and athletic trainers, these results suggest the additional height of mid-top cleats does not appear to have any additional benefits over low-top cleats, at least for players of builds similar to running backs and receivers, and at least for contexts such as those used in this study. Cleats selection, at least in terms of height, should be based on player preference. More importantly, the extra time, effort, and money required to spat ankles may not be justified from an injury prevention standpoint. All that said, the spatting of ankles may be an integral part of pre-game rituals for some teams, analogous to donning armor before battle, and as such may serve important social or psychological roles unrelated to athlete safety.

This work has some limitations. First, we tested only two ankle conditions in two football field drills. It is likely other results may have been obtained if other ankle conditions were used in other drills. The fact that our exercise bout lasted less than 10-min. does have an experimental design advantage though. Tape jobs are known to loosen as early as 15-min. post-application, and our study design eliminated that possible factor.[6,8,11] Second, we tested cleats most often used by running backs, receivers, and defensive backs, and our subjects were anthropometrically analogous to that population. It is likely that using different cleat models or athletes of different builds would produce different results. This means our results may not be applicable to all football players, as our athletes were on the smaller end of the football spectrum. Third, our sample size could have been increased to generate more statistical power, though given our specific results, the addition of more athletes may not have enhanced the findings too much. Fourth, our results were all based on acute effects and do not directly speak to injury prevention. Just because an ankle is more restricted does not mean it is more protected from ankle sprains.[19] One

study showed that National Football League athletes had similar ankle injury rates when spatted versus not spatted; although their sample size was low (12 or fewer athletes per group), that particular study may suggest the relation between spatting and injury prevention is poor.[14]

In conclusion, we demonstrated that there were perceptual differences, but not performance or ROM differences, when athletes' ankles were taped and spatted in football cleats of varying heights similar to what running backs or receivers use. Directions for future research are many and include investigating cleats that have greater differences in the heights of their uppers, recruiting athletes resembling a more diverse range of football player body shapes, investigating spatting performance in different types of drills, determining whether these effects are consistent on natural grass versus turf (as employed in this study), and determining whether similar results are found on actual game scenarios versus practice drills (as employed in this study). It is clear from the present data and literature review that much basic research still needs to be conducted on football cleats.

Acknowledgments

We would like to thank the nine participants who volunteered their time for our study and Matt Miller for coordinating our use of the football field with Athletics. Drake and Faganel collected all the data. Dahl consulted on ankle taping and study design procedures and Senchina was the research mentor. Drake, Faganel, and Senchina produced the graphics and wrote the paper.

References

[1]Abián-Vicén, J., Alegre, L.M., Fernández-Rodríguez, J.M. and Aguado, X. (2009) "Prophylactic ankle taping: elastic versus inelastic taping." *Foot and Ankle International* 30.3. Pg 218–225.

[2]Barlow, G,. DuBois, B., Kern, B. and Niedermeyer, R. (2010) "The effect of spatting on speed, agility, power, and balance." *Journal of Undergraduate Kinesiology Research* 6.1. Pg 55–62.

[3]Barrett, J. and Bilisko, T. (1995) "The role of shoes in the prevention of ankle sprains." *Sports Medicine* 20.4. Pg 277–280.

[4]Barrett, J.R., Tanji, J.L., Drake, C., et al. (1993) "High- versus low-top shoes for the prevention of ankle sprains in basketball players: A prospective randomized study." *American Journal of Sports Medicine* 21.4. Pg 582–585.

[5]Brizuela, G., Llana, S., Ferrandis, R. and García-Belenguer, A.C. (1997) "The influence of basketball shoes with increased ankle support on shock attenuation and performance in running and jumping." *Journal of Sports Science* 15.5. Pg 505–515.

[6]Cordova, M.L., Ingersoll, C.D. and LeBlanc, M.J. (2000) "Influence of ankle support on joint range of motion before and after exercise: a meta-analysis." *Journal of Orthopedic Sports Physical Therapy* 30.4. Pg 170–177.

[7]Iacovelli, J.N. (2011) *Effect of field condition and shoe type on lower extremity injuries in American football.* M. A. Thesis, Department of Industrial Engineering, University of Iowa.

[8]Meana, M., Alegre, L.M., Elvira, J.L.L. and Aguado, X. (2008) "Kinematics of ankle taping after a training session." *International Journal of Sports Medicine* 9.1. Pg 70–76.4.

[9]Ottaviani, R.A., Ashton-Miller, J.A., Kothari, S.U. and Wojtys, E.M. (1995) "Basketball shoe height and the maximal muscular resistance to applied ankle inversion and eversion moments." *The American Journal of Sports Medicine* 23.4. Pg 418–423.

[10]Pederson, T.S., Ricard, M.D., Merrill, G., Schulthies, S.S. and Allsen, P.E. (1997) "The effects of spatting and ankle taping on inversion before and after exercise." *Journal of Athletic Training* 32.1. Pg 29–33.

[11]Purcell, S.B., Schuckman, B.E., Docherty, C.L., Schrader, J. and Poppy, W. (2009) "Differences in ankle range of motion before and after exercise in 2 tape conditions." *American Journal of Sports Medicine* 37.2. Pg 383–389.

[12]Reuter, G.D., Dahl, A.R. and Senchina, D.S. (2011) "Ankle spatting compared to bracing or taping during maximal-effort sprint drills." *International Journal of Exercise Science* 4.1. Pg 49–64.

[13]Ricard, M.D., Schulties, S.S. and Saret, J.J. (2000) "Effects of high-top and low-top shoes on ankle inversion." *Journal of Athletic Training* 35.1. Pg 38–43.

[14]Scranton, P.E. Jr., Whitesel, J.P., Powell, J.W., et al. (1997) "A review of selected noncontact anterior cruciate ligament injuries in the National Football League." *Foot and Ankle International* 18.12. Pg 772–776.

[15]Thacker, S.B., Stroup, D.F., Branche, C.M., et al. (1999) "The prevention of ankle sprains in sports. A systematic review of the literature." *American Journal of Sports Medicine* 27.6. Pg 753–760.

[16]Trower, B., Buxton, B., German, T., et al. (1997) "The effects of prophylactic taping on ankle joint motion and performance." *Journal of Athletic Training* 42(Suppl 2). Pg S–14.

[17]Ubell, M.L., Boylan, J.P., Ashton-Miller, J.A. and Wojtys, E.M. (2003) "The effect of ankle braces on the prevention of dynamic forced ankle inversion." *American Journal of Sports Medicine* 31.6. Pg 935–940.

[18]Udermann, B., Miller, K., Doberstein, S., et al. (2009) "Spatting restricts ankle motion more effectively than taping during exercise." *International Journal of Exercise Science* 2.2. Pg 72–82.

[19]Verhagen, E.A., Van der Beek, A.J. and Van Mechelen, W. (2001) "The effect of tape, braces and shoes on ankle range of motion." *Sports Medicine* 31.9. Pg 667–677.

[20]Verhagen, E.A.L.M. and Bay, K. (2010) "Optimising ankle sprain prevention: a critical review and practical appraisal of the literature." *British Journal of Sports Medicine* 44.15. Pg 1082–1088.

Beware Thin Air: Altitude's Influence on NBA Game Outcomes

By Shane Moore and Jonathan Scott
Colorado State University

Introduction

Few can dispute the effects altitude has in sports. When one examines the statistics released from Major League Baseball, it is easy to see why Denver, Colorado's Coors Field is a sought-after destination for offensive production. At an elevation of 5,280 feet, a baseball hit in the mile-high city will travel an additional 9-10% further than it would in sea level Yankee Stadium.[1] The cause of this dramatic effect is found in the less-dense air of the high altitude environment when compared to the density of air particles at sea level—deemed the "Coors Field Effect."[1]

Aside from the physics of altitude, there is much evidence that demonstrates the influence that the density of air has on athletes' performance as well. Observations at the 1968 Mexico City (elev. 7350) Summer Olympics showed those athletes from Kenya, who traditionally train in the city of Eldoret (elev. 7,000-9,000 feet), dominated in medium and long-distance events.[2] The performance of soccer teams at high altitudes caused FIFA, a governing body in the professional soccer community, to ban international matches played above 8,000 feet.[3] The same research that led to this ban showed high altitude teams perform much better in lower altitude games, to the tune of an average increase of half a goal per 1,000m descended.[3]

The NBA, the premier league for professional basketball, has two teams that hold home games above 4,000 feet, three between 1,000 and 1,200 feet, and the remaining 25 teams play at a home court below 1,000 feet above sea level.[4,5] Professional basketball players are regarded as highly conditioned athletes with programs emphasizing both endurance (aerobic, oxygen-dependent exercise) and quick bursts of speed (anaerobic, oxygen-independent exercise). Of the two types of exercise, it has been found that in a game, basketball is around 80% anaerobic and 20% aerobic.[6] However, different positions require different "attitudes" on the court, some of which will require a consistently fast player while others will require a man to fight for a dominant position.[6] With the staggering evidence pointing to the effect altitude has on athletic performance, high-altitude teams like the Denver Nuggets (Pepsi Center elevation: 5197 ft) and the Utah Jazz (EnergySolutions Arena elevation: 4268 ft) may hold a significant advantage over their sea-level opponents.[4] With the basic understanding that lower air density, and thus less oxygen per cubic centimeter, grants a performance advantage to high-altitude teams, our hypothesis is the point differential for a team in a given game is directly affected by the altitude of the game site with respect to each team's home altitude. In short, we believe high-altitude teams hold an advantage both home and away.

Using regression analysis of data from the 2010-2011 NBA season, we aim to discern, *ceteris parabis*, the significance of the advantage high-altitude teams gain from descending, and conversely, the disadvantage low-altitude teams face when ascending. NBA statistician Dean Oliver has written that the four primary factors that contribute to winning basketball games are:

1) Shooting, measured by effective field goal percentage

(Effective Field Goal % = (Field Goals Made + 0.5*Three-point Field Goals Made)/Field Goals Attempted)

EFG% = (FGM + 0.5*FG3M)/FGA

2) Turnover percentage

(Turnover % = Possessions/Turnovers = (Field Goals Attempted – Offensive Rebounds + Turnovers + 0.4 * Free Throws Attempted)/Turnovers)

TO% = Poss./TO = (FGA - OR + TO + 0.4 * FTA)/TO

3) Offensive rebounds per 100 rebounding opportunities

(Offensive Rebounding % = [Offensive Rebounds / (Offensive Rebounds + Opponents Defensive Rebounds)] * 100)

ORB% = [OR / (OR + Opponents Def Reb)] * 100)

4) Success at the foul line

(Free Throws Made/Field Goals Attempted)
FTM/FGA

(Oliver, 2004).

We will include both home and away values for each of these measures as well, to capture the effects of both defensive production and opponent skill on point differential. We will define the final independent variable and subject of our analysis, altitude difference (*DALT*), as: home team arena altitude (HA) – away team arena altitude (AA). The dependent variable we will use, as a team's measure of success on the basketball court, is margin of victory (*MOV*), defined as: points for (PF) – points allowed (PA).

Research on this topic has several potential uses. Investors considering potential locations for new sports teams or existing teams seeking new homes can use the information our research will provide to determine how location will contribute to their team's ability to succeed in highly competitive professional sports leagues. Furthermore, teams considering trades or free agents should also take into account how a player who is conditioned to play in a high-altitude environment might receive performance benefits with relocation to a low-altitude city, and vice-versa. However, when such transactions take place, players moving from high-altitude cities may lose the competitive advantage they held over the rest of the league during their high-altitude tenure. Relocations like this would correct the potential statistical abnormality that high-altitude training gives to their performance, thus revealing what was previously perceived as superior talent as mere conditioning (if one assumes that conditioning will reflect location and decrease after some time spent at a lower altitude).

Economic Analysis

The relevant economic model for this analysis is a production function. A production function is any combination of inputs for which a producer produces the

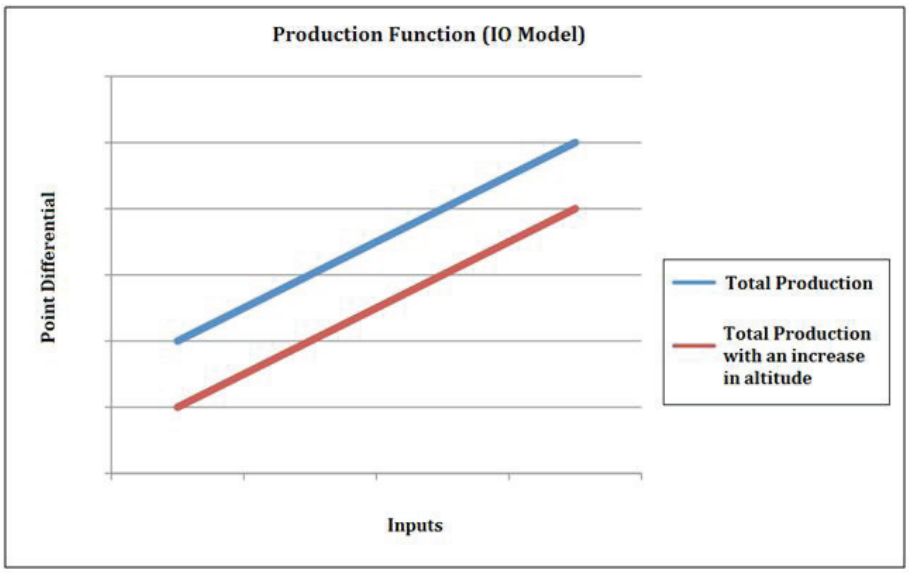

Figure 1: Relationship between game altitude and point differential in NBA basketball games

highest amount of output.[7] In a typical equation, the quantity produced is based on the function of capital and labor.[7] A general production function equation takes the following form:

$$Q = f(X_1 + X_2 \ldots + Xn)$$

where Q, quantity, is a function of in variable and fixed inputs (X).[7] The contribution an addition unit of input makes to the overall product is measured by the marginal productivity as is shown in the equation:

$$\text{Marginal product of an input} = \Delta q/\Delta x \quad [7]$$

Measuring success in NBA basketball games is quite straightforward; the team that scores more points wins. Teams employ differing strategies for achieving this objective, but all have essentially the same inputs. In the equation proposed in the hypothesis, the output of a given team's production in a game, points, is a function of inputs that most importantly include field goal percentage, turnover percentage, offensive and defensive rebounding, free throw shooting, steals and blocks. These inputs, as in a production function, can be put together in any combination to produce the output, points.[7] That is to say, a team can emphasize outside shooting and have roster dominated by shooters, or a team constructed of more tall players to emphasize rebounding and second chance points.

However, it is the aim of this study to determine whether altitude, measured by the difference between game site elevation and the away team's home elevation, is a significant determinant of outcomes in the production function. Holding all inputs constant, adding altitude as a new technology with an effect on the production of points will constitute a substantial factor in a team's production function if it significantly affects output, points (both scored and allowed). We will not consider altitude change as an input, but rather as a technology change because it affects the efficiency of all inputs in the function. As the hypothesis states and is demonstrated in Figure 1, we believe there is an inverse relationship between altitude change and point differential; as a team ascends their point differential (points for - points against) will decrease, and vice-versa. As Figure 1 shows, we expect a change in altitude to shift the production function down. In other words, holding all else equal, a change in the away team's altitude difference will shift the away team's *MOV* function. The regression analysis of the inputs in this production function will aim to discern the marginal productivity of a change in altitude. For clarity we will interpret altitude change in feet/1000, because the marginal effect of a one-foot change is likely quite small.

Previous Research

Using data from the Seoul (1988), Barcelona (1992) and Atlanta (1996) Olympic Games, Tcha and Pershin studied determinants of performance by country and sport.[8] The authors used econometric modeling to determine the impact of various geographic, social, economic and demographic factors on revealed comparative advantage (RCA).[8] Tcha and Pershin divided the whole of the Olympic Games categorically into swimming, athletics, weights, ball games, gymnastics and other, running regressions for each separately.[8] They also compared variables that included relative altitude, coast length, temperature, population, gross domestic product, GDP per capita, and dummy variables for former/current socialist countries and Asian and African countries.[8] From the results, the authors then compared sport specialization with RCA and economic variables such as GDP per capita. The results from the research showed high-income countries did not specialize in sports but rather they diversified their medals showing they were able to be competitive in several sports, a pattern, they claim, to be analogous to a developed economy's behavior in production.

In regards to medals awarded and relative altitude, the regression analysis showed the relative altitude variable was only statistically significant in the athletic (coefficient = 0.8279) and weight events (coefficient = -0.9662). For ball sports, which include basketball, relative altitude showed a statistically insignificant coefficient (-0.2696). According to their research, altitude is not a significant determinant in the outcome of ball sport events. However, lumped together under the ball sports category were disparate sports such as table tennis and volleyball. These sports differ from basketball in type of conditioning demanded, so one would assume that endurance does not play a relatively significant role in determining match outcomes. Were Tcha and Pershin to disaggregate their categories further, running separate regressions for each ball sport, their results would be more conclusive and pertinent to our research. Nonetheless, their analysis showed that relative altitude plays a significant role in determining a country's success in aerobic events (the athletics category), giving credence to our hypothesis.

Research of the effects of altitude in forecasting sports performance includes analysis of FIFA World Cup Qualifying matches in South America.[9] Following a 2007 FIFA ruling that no World Cup Qualifying matches be played at elevations above 8,200 feet, Rómulo A. Chumacero analyzed the effects of various factors on outcomes in soccer matches. Included are the quality of the teams playing, socioeconomic characteristics of the countries playing, crowd effects, humidity, temperature, and altitude.[9] Found significant were team rankings prior to the game, humidity and temperature; among the insignificant variables were the socioeconomic factors, the crowd effects,

and, most notably, altitude.[9]

Chumacero's results may initially be discouraging for our hypothesis. However, he notes that, because of FIFA's belief that altitude is a significant determinant of match outcomes, there is a mandatory acclimatization period of one week for matches played above 9000 feet, and two weeks for matches played above 9800 feet.[9] Also, Chumacero's two regressions resulted in R^2 values of 0.208 and 0.209, respectively, so it is apparent that his estimated equations are not effective in forecasting match outcomes and require further research to conclusively say that altitude is insignificant as an independent variable.[9]

Previous literature includes inquiry in modeling success in basketball games as well. Estimating technical efficiency in basketball games, Rimler *et al.* modeled factors for success in 296 games played in the 2005-2006 season of the men's NCAA Atlantic 10 Conference.[10] Independent variables included in the authors' proposed production function were two-point field goal percentage, three-point field goal percentage, free throw shooting percentage, offensive rebounds, defensive rebounds, personal fouls, assists, turnovers, blocked shots, and steals.[10] Rimler *et al.* found that shooting percentages and offensive rebounding positively impacted point production and turnovers negatively impacted point production, while the effects of defensive variables (e.g. blocks, steals, defensive rebounds) were negligible.[10] Assists appeared to be significant determinants of point production, but Rimler *et al.* deem that assists are merely correlated with shooting percentage and affect point production insofar as they help the offense generate easier shots.[10] The authors conclude that high efficiency contributes to success on the basketball court, but employment of resources (the independent variables suggested by Rimler *et al.*, e.g. shooting percentage and offensive rebounding) is far more important.[10]

The variables that Rimler *et al.* found statistically significant in their analysis in NCAA basketball games are, though formulated slightly differently, the same as proposed in our hypothesis. Shooting percentage (both two- and three-point as well as free throw), turnovers and offensive rebounding were concluded to be significant determinants of point production by the authors, reinforcing our proposal to use these variables in our regression analysis.

Data and Methodology

For this analysis we will use OLS regression, adjusted for robust standard errors to correct for heteroskedasticity, performed with Gretl 1.9.0. Game data was obtained from Basketball-reference.com, and altitude data is from Google Earth.[5,4] We begin with the following comprehensive estimated equation, to include all potentially influential inputs:

$$MOV = \beta_1 + \beta_2 EFG\% + \beta^3 TOV\% + \beta_4 ORB\% + \beta_5 (FT/FGA) + \beta_6 DRB + \beta_7 STL + \beta_8 BLK + \beta_9 AEFG\% + \beta_{10} ATOV\% + \beta_{11} AORB\% + \beta_{12} A(FT/FGA) + \beta_{13} ADRB + \beta_{14} ASTL + \beta_{15} ABLK + \beta_{16} DALT + \alpha_1 HTL + \alpha_2 LTH + \varepsilon$$

In this equation the dependent variable, margin of victory (*MOV*) is calculated by: (points for – points allowed) for each team in each game of the 2010-11 NBA season. The first independent variable, effective field goal percentage (*EFG*% = (FGM + 0.5*FG3M)/FGA) is a measure of shooting percentage with differential weight on three-point shooting.[11] Next is turnover percentage (*TOV*% = Poss./TO = (FGA - OR + TO + 0.4 * *FTA*)/*TO*), a measure of possessions per turnover, signifying the likelihood that a team will turn the ball over on a given possession.[11] We also expect offensive rebounding percentage (*ORB*% = [OR / (OR + Opponents Def Reb)] * 100) to be significant as well, because more offensive rebounds lead to more opportunities to score. We include the next independent variable, success at the foul line (*FTM/FGA*) because it takes into account the relative frequency that a team gets to the free throw line as well as the ability to make the free throws attempted. Defensive rebounding (*DRB*) is likely important as well because it prevents the opponent from getting offensive rebounds, thus limiting second chance points. Steals (*STL*) are potentially significant determinants of success in basketball games because they transfer possessions from the offense to defense, leading to opportunities to score. The next variable included in the regression is blocked shots (*BLK*), which have promise for influence on *MOV* because they deny field goal attempts.

The independent variable under scrutiny in this study is altitude, which we have formulated in several ways. The first version of the altitude variable is altitude difference (*DALT* = home team arena altitude – away team arena altitude). Alternately, we will also try two dummy variables, one for low-altitude teams playing at high altitude (*LTH*) and one for high-altitude teams playing at

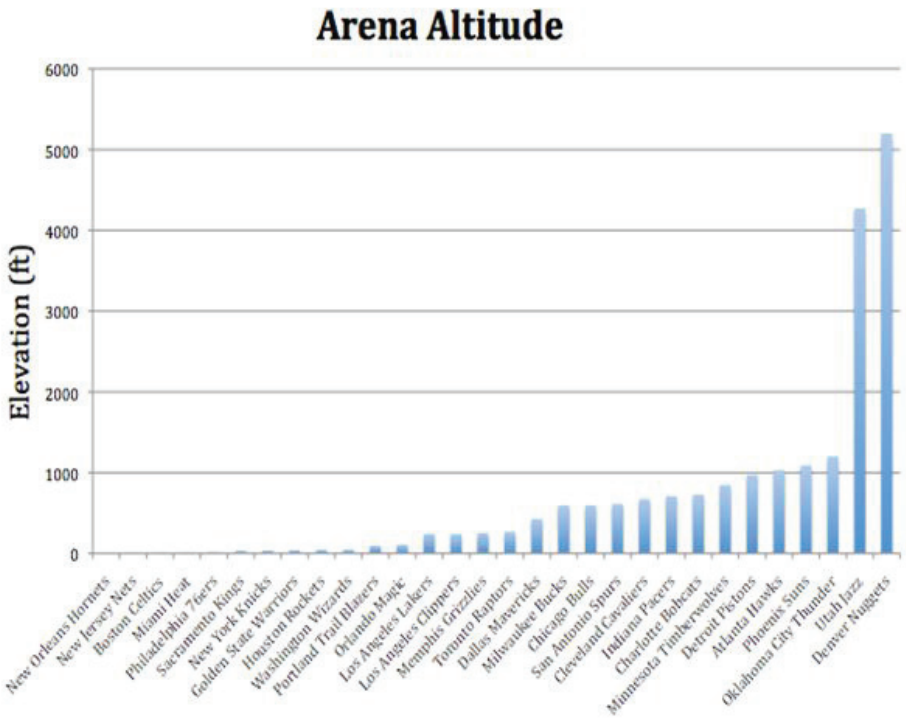

Figure 2: Elevations of all NBA team arenas

Table 1: Summary statistics

Variable	Mean	Min	Max	N
PTS	101.134	59	144	1230
APTS	97.967	56	137	1230
MOV	3.163	-41	55	1230
ALT	675.890	2	5197	1230
DALT	3.644	-5195	5195	1230
HTL	0.063	0	1	1230
LTH	0.063	0	1	1230
FG	37.778	23	56	1230
FGA	81.192	59	120	1230
FG%	0.466	0.291	0.658	1230
TP	6.561	0	21	1230
TPA	18.128	4	38	1230
TP%	0.356	0	0.833	1230
FT	19.017	3	45	1230
FTA	24.888	4	59	1230
FT%	0.764	0.375	1	1230
ORB	10.962	2	27	1230
DRB	30.820	14	50	1230
TRB	41.781	21	66	1230
AST	22.233	8	40	1230
STL	7.421	0	22	1230
BLK	5.270	0	15	1230
TOV	13.335	3	28	1230
PF	20.282	7	34	1230
EFG%	0.510	0.316	0.736	1230
TOV%	7.636	3.415	29.067	1230
ORB%	26.406	4.7	51.2	1230
FT/FGA	0.238	0.033	0.672	1230
AFG	36.713	21	53	1230
AFGA	81.239	59	111	1230
AFG%	0.453	0.296	0.629	1230
ATP	6.354	0	22	1230
ATPA	17.898	3	41	1230
ATP%	0.351	0	0.700	1230
AFT	18.186	4	43	1230
AFTA	23.841	4	52	1230
AFT%	0.761	0.357	1	1230
AORB	10.863	2	26	1230
ADRB	30.133	15	47	1230

(Continued)

Variable	Mean	Min	Max	N
ATRB	40.996	24	60	1230
AAST	20.764	4	37	1230
ASTL	7.232	0	18	1230
ABLK	4.459	0	14	1230
ATOV	13.845	4	28	1230
APF	21.143	8	37	1230
AEFG%	0.492	0.305	0.703	1230
ATOV%	7.316	3.333	20.700	1230
AORB%	25.838	0.222	50	1230
AFT/FGA	0.227	0.045	0.566	1230

Source: Data retrieved from Sports Reference LLC (http://www.basketball-reference.com/)

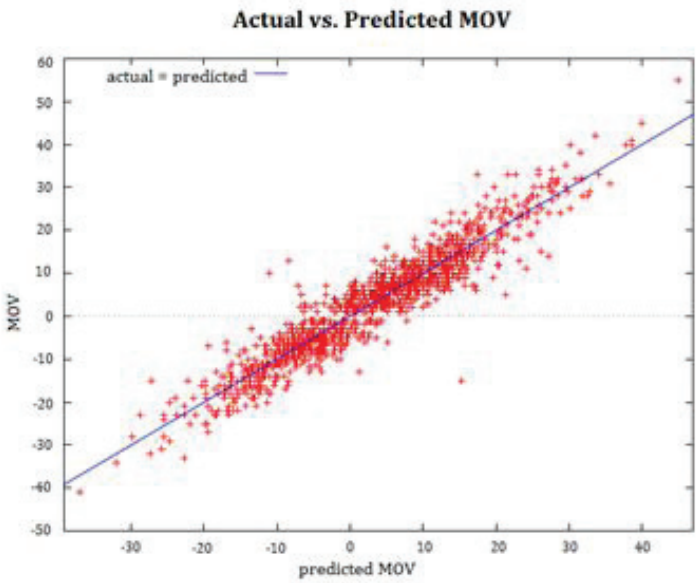

Figure 3: Actual vs. Predicted Margins of Victory (demonstrating goodness of fit) for Model (4)

low altitude (*HTL*). If the altitude difference is greater than or equal to 3069 the dummy *LTH* gets a 1, if not, a 0. This is because all other teams play at least 3069 feet below Utah (EnergySolutions Arena, home of the Utah Jazz, has an elevation of 4268 feet, minus the altitude of the next highest arena, Chesapeake Energy Arena, home of the Oklahoma City Thunder, with an elevation of 1199 feet). This captures the effect of a low-altitude team playing in either Denver or Utah. If the altitude difference is less than or equal to -3069, the dummy *HTL* gets a 1, if not, a 0. This is to capture the effect of a high-altitude team (Denver or Utah) playing in a low-altitude arena. Our sample size is 1230 games, representing all 82 games in the 2010-11 season for each of the 30 NBA teams. Margin of victory minimum (-55) and maximum (55) values represent the January 11 game in which the Los Angeles Lakers beat the Cleveland Cavaliers by 55 points.[5] As shown in Figure 2, there is not much variation in arena elevation in the NBA. Aside from the Utah Jazz and the Denver Nuggets, who both play above 4200 feet, the rest of the league plays below 1200 feet. The minimum altitude (2 ft) is New Orleans Arena, and the maximum altitude (5197 ft) is Denver's Pepsi Arena; the altitude difference minimum and maximum values seen in Table 1 represents matchups between the New Orleans Hornets and the Denver Nuggets. Notably, the mean altitude is 675.89 feet, significantly below the NBA's two high-altitude teams in Denver and Utah. As one might assume, the game statistics show considerable variation, demonstrating a wide variety of team performances throughout the season.

Results

Various potential formulations of OLS regressions are shown in Table 2. Margin of victory is measured for the home team, so the coefficients found for each variable signify the effect that a one-unit change has on the home team's *MOV*. For example, in Model (4), the model with the highest R^2 value (a measure of a model's goodness of fit), the coefficient on *EFG*% is 135.307. This means that if a team's *EFG*% increased by one unit, their *MOV* would have a resulting increase of about 135 points, holding all else equal. This, however, is entirely unrealistic, so by dividing the coefficient by 100 we get the effect a one-percentage point change in *EFG*% has on the home team's *MOV*: 1.35 points. As shown in Table 1, *TOV*% and *ORB*% are recorded differently than *EFG*%, they are recorded in whole numbers rather than as a decimal. As such, one needn't divide these coefficients by 100 to see their marginal effects on *MOV*. Model (4)'s coefficient for *TOV*%, 0.930, therefore means that a one-percent increase in *TOV*% (Possessions/Turnovers) results in a 0.93-point increase in the home team's *MOV*. Similarly, the coefficient on *ORB*% (Offensive rebounds per 100 opportunities) in Model (4) is 0.471, meaning that a one-percent increase in *ORB*% results in a 0.471-point increase in the home team's *MOV*. The variable for success at the foul line, *FT/FGA*, has an estimated coefficient of 25.393 in Model (4). This means that, all else equal, if a team were to make one free throw per field goal attempted (*FT/FGA* = 1), their *MOV* would increase by over 25 points, as compared to a game in which that team made zero free throws per field goal attempted (*FT/FGA* = 0). The variable for defensive rebounds (*DRB*) has a coefficient of -0.145 in Model (4), which is rather counterintuitive. One would think that, *ceteris parabis*, one additional rebound for the home team would increase their *MOV*, not decrease it. Each defensive rebound prevents one's opponent from getting an offensive rebound, therefore increasing the home team's possessions and limiting the opponent's possessions. However, as seen in Table 2, the coefficient is approximately -0.14 in each of the specified models, meaning that an additional defensive rebound for the home team results in a 0.14-point decrease in that team's *MOV*. One potential explanation for this coefficient is an increased amount of defensive rebounds could signify an increased amount of shots taken by the opposing team, and with more shots taken the opponent has more opportunities to score points. Further study must be devoted to resolve this uncertainty. The coefficient for blocks (*BLK*) has a similarly counterintuitive coefficient (-0.208). This means for each block the home team achieves their *MOV* decreases by 0.208 points. One possible explanation is that blocked shots do not lead to turnovers, but rather are likely to be rebounded by the offense and result in more high-efficiency shots. The coefficient for steals (*STL*) is a positive 0.292, denoting the positive effect that forcing turnovers has on scoring opportunities and *MOV*. The away variables (denoted by the prefix A) have the opposite effect of the variables explained above on determining home team margin of victory. In Model (4), all of the aforementioned variables are statistically significant at the 1 percent level aside from *ABLK*, which is statistically significant at the 10 percent level. Our work confirms previous research on the determinants of basketball game outcomes, which includes shooting percentages, offensive rebounding, and turnovers, but unlike Rimler *et al.* we found defensive rebounds, steals and blocks to be significant.[10,11]

In Models (1) & (3) we included the dummy variables for low altitude team playing at high altitude (*LTH*) and for high altitude team playing at low altitude (*HTL*) in an attempt to capture the effects of playing at an altitude significantly different than their home arena altitude. However, the inclusion of these variables had an adverse effect on the explanatory power of the variable *DALT* because multicollinearity issues arose when the three altitude variables were included. In order capture effects of altitude differences, *DALT* was determined to have more explanatory power and was substituted for *LTH* and *HTL* variables.

In the models where only *DALT* was used to measure the effects of altitude (Models (2) and (4)), the coefficients were nearly identical, both rounding to 0.0002. *DALT* was found to be statistically significant in both Models (2) and (4): at the 1 percent level in Model (2) and at the 5 percent level in Model (4). This consistency across various model specifications is demonstrative of robust results, showing that altitude is indeed a significant determinant of *MOV*. The coefficient of 0.0002 shows the effect a 1-foot increase in altitude has on the margin of victory (+0.0002 points/foot). However, when viewed in terms of 1,000-foot increments the effect on margin of victory is interpreted as (+0.2 points/1,000 ft). This result is unlike the research evaluated above (e.g. Tcha and Pershin, 2003; Chumacero, 2009), insofar as where we find altitude to be a statistically significant determinant of game outcomes, they did not.

Team dummy variables were included in Model (4) to capture any home court advantage effects (aside from altitude). It is noteworthy that *DALT* remained significant in Model (4), even after accounting for any home court effects. This shows that the advantage that high-altitude teams have does not come from any intangible arena effects, but from altitude. Included in Model (4) are dummies for all teams except the Washington Wizards; all coefficients are relative to this omitted team. The only teams that were seen to have any statistically significant coefficients were the Golden State Warriors (10 percent level of significance) and Portland Trail Blazers (5 percent level of significance). Explanations for these coefficients are beyond the scope of this research but may be attributed to arena atmosphere, noise, alcohol sales, etc.

Among the models specified, the R^2

Table 2: Regressions

Variable	(1)	(2)	(3)	(4)
const	3.205 (0.379)	3.663 (0.308)	3.461 (0.335)	4.005 (0.355)
EFG%	135.871*** (8.16e-214)	135.555*** (1.40e-213)	134.835*** (5.84e-221)	135.307*** (4.54e-210)
TOV%	0.985*** (2.48e-22)	0.987*** (1.72 e-22)	0.993*** (1.67 e-22)	0.930*** (1.15 e-20)
ORB%	0.479*** (1.68 e-81)	0.479*** (4.56 e-81)	0.484*** (4.85e-84)	0.471*** (5.29 e-78)
FT/FGA	26.341*** (1.60e-45)	26.075*** (1.39 e-44)	26.219*** (7.41e-45)	25.393*** (7.05 e-39)
DRB	-0.144*** (0.007)	-0.144*** (0.007)	-0.137** (0.011)	-0.145*** (0.008)
STL	0.320*** (2.08 e-08)	0.323*** (1.89 e-08)	0.321*** (2.44e-08)	0.292*** (1.09 e-06)
BLK	-0.202*** (9.02 e-05)	-0.205*** (8.33 e-05)	-0.198*** (0.001)	-0.208*** (0.001)
AEFG%	-136.580*** (1.19 e-209)	-136.777*** (1.41 e-210)	-136.118*** (3.35e-209)	-136.367*** (3.95 e-199)
ATOV%	-1.354*** (1.45e-45)	-1.354*** (1.81 e-45)	-1.360*** (5.02e-46)	-1.335*** (1.86 e-43)
AORB%	-0.435*** (1.28 e-56)	-0.435*** (2.75 e-56)	-0.433*** (1.09e-55)	-0.438*** (5.59 e-53)
AFT/FGA	-25.590*** (1.39 e-52)	-25.578*** (8.70 e-54)	-25.447*** (3.65e-52)	-25.154*** (2.04 e-49)
ADRB	0.145*** (0.007)	0.142*** (0.009)	0.145*** (0.007)	0.146*** (0.007)
ASTL	-0.386*** (2.67 e-09)	-0.390*** (1.76 e-09)	-0.382*** (4.46e-09)	-0.394*** (2.05 e-09)
ABLK	0.093 (0.104)	0.085 (0.127)	—	0.107* (0.090)
DALT	-9.082e-5 (0.683)	0.0002*** (0.010)	—	0.0002** (0.015)
HTL	-1.578 (0.146)	—	-1.182*** (0.009)	—
LTH	1.122 (0.277)	—	0.805* (0.098)	—
HOME_ ATL	—	—	—	0.224 (0.829)
HOME_BOS	—	—	—	-0.093 (0.925)
HOME_ CHA	—	—	—	-0.203 (0.828)
HOME_CHI	—	—	—	0.793 (0.409)
HOME_ CLE	—	—	—	-0.901 (0.336)
HOME_ DAL	—	—	—	-0.476 (0.691)
HOME_ DEN	—	—	—	0.399 (0.724)
HOME_ DET	—	—	—	0.082 (0.932)

(Continued)

Variable	(1)	(2)	(3)	(4)
HOME_ GSW	—	—	—	1.765* (0.057)
HOME_ HOU	—	—	—	1.238 (0.171)
HOME_ IND	—	—	—	0.822 (0.364)
HOME_ LAC	—	—	—	-0.569 (0.546)
HOME_ LAL	—	—	—	1.327 (0.177)
HOME_ MEM	—	—	—	0.426 (0.667)
HOME_ MIA	—	—	—	0.500 (0.604)
HOME_MIL	—	—	—	0.424 (0.657)
HOME_ MIN	—	—	—	-0.756 (0.399)
HOME_ NJN	—	—	—	-0.565 (0.582)
HOME_ NOH	—	—	—	0.326 (0.747)
HOME_ NYK	—	—	—	1.552 (0.140)
HOME_ OKC	—	—	—	1.102 (0.230)
HOME_ ORL	—	—	—	-0.055 (0.958)
HOME_PHI	—	—	—	0.127 (0.893)
HOME_ PHX	—	—	—	-0.732 (0.446)
HOME_ POR	—	—	—	2.231** (0.014)
HOME_ SAC	—	—	—	-0.448 (0.620)
HOME_SAS	—	—	—	0.102 (0.914)
HOME_ TOR	—	—	—	-0.179 (0.863)
HOME_ UTA	—	—	—	-0.175 (0.863)
Adjusted R2	0.891	0.891	0.891	0.892
Model S.E.	4.161	4.161	4.163	4.140
Observations	1230	1230	1230	1230

Source: Data retrieved from Sports Reference LLC (http://www.basketball-reference.com/)
Notes: Alternative model formulations using OLS; *** = significant at the 1 percent level; ** = significant at the 5 percent level; * = significant at the 10 percent level; p-values shown in parenthesis underneath each coefficient

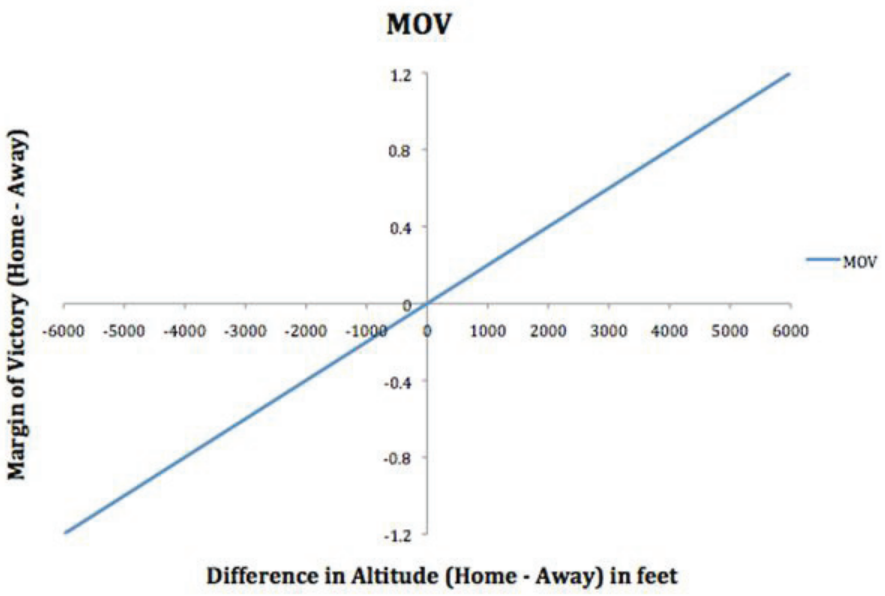

Figure 4: Difference in altitude's effect on margin of victory

values are all very similar (0.891) with Model (4) only slightly higher at 0.892. This signifies a marginal improvement in the goodness of fit, and all four versions of the model have good explanatory value in determining the margin of victory for a home team. We believe that Model (4) is the specification that best forecasts home team *MOV*. This model includes no insignificant variables, incorporates home team dummy variables to account for any intangible home arena effects and has the highest R^2 value of the variations specified. Model (4)'s R^2 of 0.892 means that over 89% of the variation in *MOV* is explained by the regression's variables. As Figure 3 demonstrates, Model (4) fits the data quite well and as such has substantial predictive power.

Conclusion

Based on results gathered from the regression analysis, we can prove a correlation between home and away altitude and NBA game performance. This means that high-altitude teams do hold an advantage over their low-altitude counterparts. To interpret the *DALT* coefficient, the difference in altitude (home minus away) must be considered. At 0.0002, the coefficient is significant in that a 1,000-foot increase in the altitude difference between home and away teams leads to a 0.2-point advantage to the home team. However small this may seem, the New Orleans Hornets (elevation 2 ft.) face a disadvantage of almost 1.04-points when playing the Denver Nuggets at 5,197 feet above sea level. The same holds true for the Nuggets traveling to New Orleans. The difference in altitude becomes -5,195, leading to about a 1.04-point disadvantage for the Hornets. These results show an obvious advantage for high-altitude teams in the NBA.

Our original hypothesis held to be true; there exists a positive correlation between the size of the altitude difference of home and away teams and margin of victory in the NBA. We can conclude that altitude, as seen as a new technology in the production function, affects point production for any team in the NBA. The results do not agree with previous research conducted by Chumacero and Tcha & Pershin, whose literature showed altitude as an insignificant input in the outcomes of soccer and ball sports, respectively. The results did show certain defensive inputs, blocks, steals and defensive rebounding, to be significant, contrary to the findings of Rimler *et al.*

Nonetheless, certain changes could be made in order to better predict margin of victory, and therefore determine with more certainty to what extent altitude is an important technology in the production function of NBA game performance. For example, including more seasons to have even more observations would prove helpful to reinforce the robust nature of our findings. Also, we would like to compare vertical distance traveled to horizontal distance traveled in order to discern travel affects on NBA performance. Inclusion of a dummy variable for back-to-back games could prove to be significant as well, especially if the second were played at a high altitude. All of these research variables would help to strengthen the predictive power of the regression and better predict margin of victory for a given NBA team.

References

[1]Heiny, E., & Schaffer, J. (2006) "Home Runs & Hole-in-one." *Scientific Daily*. ScienceDaily LLC. http://www.sciencedaily.com. (Accessed 03/01/2012).
[2]N/A. (2009) "Mexico 1968" *Olympic.org*. The International Olympic Committee. http://www.olympic.org/mexico-1968-summer-olympics. (Accessed 02/26/2012).
[3]Dickinson, E. (2007) "High Altitude Football Teams Have Significant Advantage Over Lowland Teams." *Eureka Alert*. http://www.eurekalert.org. (Accessed 03/01/2012).
[4]Google Earth (Version 6.0.3.2197). (2012). Google Inc. http://www.google.com/earth/index.html. (Accessed 03/01/2012).
[5]N/A. (2011) "2010-11 NBA Schedule and Results" *Basketball-Reference.com*. Sports Reference LLC. http://www.basketball-reference.com/leagues/NBA_2011_games.html. (Accessed 03/09/2012).
[6]Brittenham, G. (1996) *Complete conditioning for basketball*. Human Kinetics Publishers. Pg 4-6.
[7]Pindyck, R., & Rubinfeld, D. (2009) *Microeconomics*. Pearson Education, Inc. Pg 197-198.
[8]Tcha, M., & Pershin, V. (2003) "Reconsidering Performance at the Summer Olympics and Revealed Comparative Advantage." *Journal of Sports Economics*. 4.3. Pg 216-239.
[9]Chumacero, R. (2009) "Altitude or Hot Air?" *Journal of Sports Economics*. 10.6. Pg 619-638.
[10]Rimler, M., Seongho, S., & Yi, D. (2010) "Estimating Production Efficiency in Men's NCAA College Basketball: A Bayesian Approach." *Journal of Sports Economics*. 11.3. Pg 287-315.
[11]Oliver, D. (2004) "Roboscout and the Four Factors of Basketball Success." *Journal of Basketball Studies*. http://www.rawbw.com/~deano/. (Accessed 02/26/2012).

Inhibition of Human Glutaminase by 5-[3-bromo-4-(dimethylamino)phenyl]-2,3,5,6-tetrahydro-2,2-dimethylbenzo[a]phenanthridin-4(1H)-one

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Abstract:

The reliance of cancer cells on glutamine catabolism to provide biosynthetic precursors and sustain energy requirements has focused interest on glutaminase inhibition as a potential strategy for treating malignant tumors. "Compound 968", 5-[3-bromo-4-(dimethylamino)phenyl]-2,3,5,6-tetrahydro-2,2-dimethylbenzo[a] phenanthridin-4(1H)-one, was recently reported to selectively block the growth of human breast cancer and B lymphoma cells by inhibiting glutaminase. The present study was undertaken to characterize the kinetics of 968 inhibition of the GAC isoform of human glutaminase in order to determine its mechanism of action and to facilitate the design of more effective benzophenanthridinone derivatives. However, when assayed in the absence or presence of either low or high phosphate concentration, very little inhibition of recombinant GAC was observed with concentrations of 968 up to 50 μM. By contrast, the glutaminase activity in intact mitochondria was inhibited about 30% by 10 μM 968. Thus, the reported effects of this small molecule on the growth of transformed cells may involve a mechanism other than direct binding to glutaminase.

Introduction

Glutamine is the most abundant amino acid in the blood.[1] This distinction reflects the important role of glutamine in nitrogen transport as well as its ability to serve as an anaplerotic substrate for the citric acid cycle. The ability of glutamine to be catabolized to α-ketoglutarate is particularly important for cancer cells. Cancer cells are known to rapidly metabolize glucose, but favor an anaerobic process in which the pyruvate produced by glycolysis is reduced to lactic acid instead of entering the citric acid cycle as acetyl CoA.[2] To maintain the citric acid cycle, cancer cells depend upon the hydrolysis of glutamine to glutamate, which is subsequently oxidized to α-ketoglutarate.

Hydrolysis of glutamine is catalyzed by the mitochondrial enzyme glutaminase. Three glutaminase isoforms have been described in mammals: KGA and GAC, which are splice variants of the *GLS1* gene, and LGA, which is encoded by the *GLS2* gene. LGA is found only in the liver, while KGA occurs in a number of tissues, including kidney and brain, and GAC is most abundant in the pancreas and heart muscle.[3] Since it was first identified in 1999, GAC has attracted particular interest because it is the principal isoform of glutaminase in malignant cells such as human breast cancer cells. Its expression is also increased in human B lymphoma and in prostate cancer cells.[4] These observations suggest that selective inhibition of GAC could attenuate the growth of malignant cells.

A number of glutaminase inhibitors have been reported. Some, such as the KGA inhibitor L-2-amino-4-oxo-5-chloropentanoic acid (CK), are reactive analogues of glutamine that bind to a site that is specific for glutamate.[5] Another reactive analogue, 6-diazo-5-oxo-L-norleucine (DON), competes with glutamine for binding to the active site of KGA.[6] More recently, the compound bis-2-(5-phenylacetamido-1,2,4-thiadiazol-2-yl)ethyl sulfide (BPTES) was identified as a particularly potent uncompetitive inhibitor of KGA or GAC that functions by promoting the formation of an inactive tetramer of glutaminase.[7] This conclusion was recently confirmed by determination of the X-ray crystallographic structure of the GAC/BPTES complex.[8]

The benzophenanthridinone derivative, 5-[3-bromo-4-(dimethylamino)phenyl]-2,3,5,6-tetrahydro-2,2-dimethylbenzo[a] phenanthridin-4(1H)-one, or "compound 968", was first reported as a GAC inhibitor in 2010.[9] Similar to BPTES, 968 blocked the growth of transformed cells and was initially described as an allosteric inhibitor of glutaminase. However, the kinetic mechanism by which 968 inhibits GAC activity was not characterized. Therefore, the present study was designed to characterize the kinetics of 968 inhibition of GAC, with hopes that the resulting information would facilitate efforts to synthesize more effective analogues of 968.

Experimental Procedures

$hGAC_{\Delta 1}$ Expression

E. coli containing the $hGAC_{\Delta 1}$ plasmid, which encodes a truncated form of human GAC that lacks the N-terminal mitochondrial targeting signal, were grown in LB media.[10] The plasmid was isolated using a Qiagen Plasmid Maxi kit and it exhibited a 260/280-absorbance ratio of 1.6. BamH1 and Nde1 restriction digests were analyzed to confirm the plasmid. BL-21(DE3) competent E. coli cells were transformed with the purified $hGAC_{\Delta 1}$ plasmid and plated on agar/LB medium containing 0.25% ampicillin and 0.125% chloramphenicol to select for transformed cells. Single colonies from the plate were cultured for seventeen hours at 37oC in three separate tubes of 2xYT medium containing 0.01% ampicillin and 0.0025% chloramphenicol. Aliquots (500 μL) of each culture were transferred to three 1-L Erlenmeyer flasks containing 500 mL of 2xYT containing 0.01% ampicillin. Cell growth was promoted by shaking at 37oC and monitored by recording the absorbance

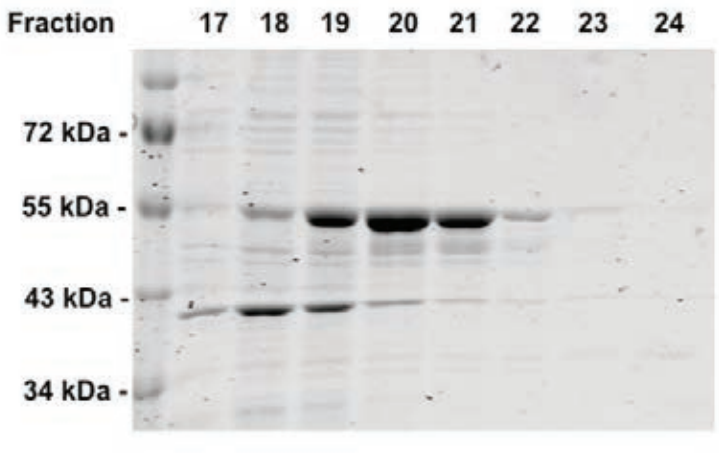

Figure 1: FLPC elution of hGACΔ1 from the Nickel-affinity column. Fractions 17-24 were separated on an SDS-10% polyacrylamide gel and stained with Coomassie-Blue. Fractions 20 and 21 were combined and used for the kinetic assays.

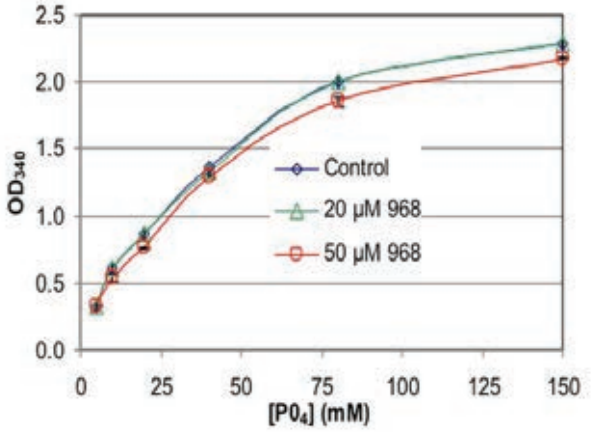

Figure: 2: Phosphate activation profiles of hGACΔ1. The hGACΔ1 activity was measured in the presence of increasing phosphate concentrations (2.5 to 150 mM) and in the absence and presence of 968. The data are the mean of three measurements for the control and 20 μM 968 and of two measurements for 50 μM 968.

at 600 nm. The cell cultures reached an OD_{600} value of 1.3 after five hours. At this point, the cells were cooled to 18oC and induced by the addition of 250 μL of 100 mM isopropyl β-D-1-thiogalactopyranoside (IPTG). After 18 hours of shaking at 18oC, the cells were pelleted by centrifugation at 2000 x g for fifteen minutes at 4oC and then stored at -80oC.

$hGAC_{\Delta 1}$ Purification

Cells containing the expressed $hGAC_{\Delta 1}$ were thawed and re-suspended in 20 mL of a lysis buffer consisting of 300 mM potassium chloride, 10 mM Tris chloride (pH 8), 10 mM potassium phosphate (pH 8), 10 mM imidazole, 10% v/v glycerol, and 1 tablet of complete Protease Inhibitor Cocktail (Roche Diagnostics) per 50 mL of buffer. The combined cell suspensions were subjected to four 30-second disruption cycles using an ultrasonic probe set to a 70% duty cycle and an output setting of 7. The cell suspensions were cooled in ice water during the sonication, with 1-minute pauses between cycles. Cell debris was pelleted by ultracentrifugation at 128,000 x g for thirty minutes at 4oC, and the supernatant was filtered through 0.22 μm syringe filters. Fractionation of His_6-tagged $hGAC_{\Delta 1}$ was performed by FPLC on a 5-mL HiTrap Chelating HP nickel-resin column using gradient elution with "Buffer A" (300 mM potassium chloride, 10 mM trishydroxyethylammonium chloride (pH 8), 10 mM potassium phosphate (pH 8), and 10% v/v glycerol), and "Buffer B" ("Buffer A" containing 1M imidazole). Tris-(2-carboxyethyl)phosphine hydrochloride (TCEP) reducing agent was added at a 50 μM level to both chilled buffers prior to fractionation, which was carried out in a cold room. Fractionation was achieved at a 3 mL/min flow rate while collecting 3 mL fractions. The first 8 fractions were eluted with 1% "Buffer B" (10 mM imidazole) and then stepped up to 5% and 10% "Buffer B" at fractions 9 and 11, respectively. Gradient elution was initiated at fraction 15 at a rate that would have reached 100% "Buffer B" composition over a period of 25 minutes, but was switched back to isocratic elution with 65% "Buffer B" at fraction 30. SDS-10%-PAGE followed by Coomassie Blue staining was used to determine which FPLC fractions contained the highest percentage of $hGAC_{\Delta 1}$.

Glutaminase Assay

$hGAC_{\Delta 1}$ activity was quantified using a modified version of the assay published by Curthoys and Weiss.[11] The standard glutaminase assay mixture contained 20 mM glutamine, 150 mM potassium phosphate, 50 mM Tris acetate (pH 8.6), and 0.2 mM EDTA. The standard glutamate assay mixture contained 80 mM Tris acetate (pH 9.4), 200 mM hydrazine, 0.25 mM ADP, 2 mM NAD+, and 0.2 mg/mL glutamic dehydrogenase. The assay was initiated by adding 2-5 μL of enzyme to 100 μL of glutaminase assay mixture in a test tube at 37oC. The reaction was stopped after ten minutes by the addition of 10 μL of 3 N HCl. Blanks were run for each experiment by adding the HCl to the glutaminase assay mixture before addition of enzyme. The amount of glutamate formed in the initial incubation was determined by adding 1 mL of glutamate assay mixture. After thirty-five minutes at room temperature, the absorbance at 340 nm was measured. Units of activity were then calculated using an NADH extinction coefficient of 6.27 mL μmol-1. Protein was determined using the Bradford dye-binding assay.[12]

Enzyme kinetics experiments were conducted in 96-well clear bottom plates using a Biotek Synergy 4 plate reader to measure path lengths and optical densities. Inhibition studies were carried out with 968 dissolved in dimethylsulfoxide (DMSO). The final DMSO concentrations in the assays ranged between 5 - 10%. Microplate assays were generally initiated by the addition of enzyme, but some assays were initiated by adding glutamine after addition of $hGAC_{\Delta 1}$. The kinetics of 968 inhibition in the presence of 150 mM or 10 mM phosphate was performed by adding 20 μL of either the standard glutaminase assay mixture or an assay mixture containing 10 mM phosphate to the 96-well plate. This was followed by adding 2.5 μL of 968 solutions in DMSO and either 1 μL of a 20-fold dilution of $hGAC_{\Delta 1}$ for wells containing 150 mM phosphate or 2 μL for the wells containing 10 mM phosphate. The reactions were incubated at 37oC for ten minutes and then quenched by the addition of 3 μL of 2 N HCl. The assay was completed by adding 200 μL of the glutamate assay mixture, incubating for thirty-five minutes at room temperature, and recording the absorbance of each well at 340 nm. A similar experiment was performed in the absence of added phosphate by charging a 96-well plate with 35 μL of a glutaminase assay mixture lacking both

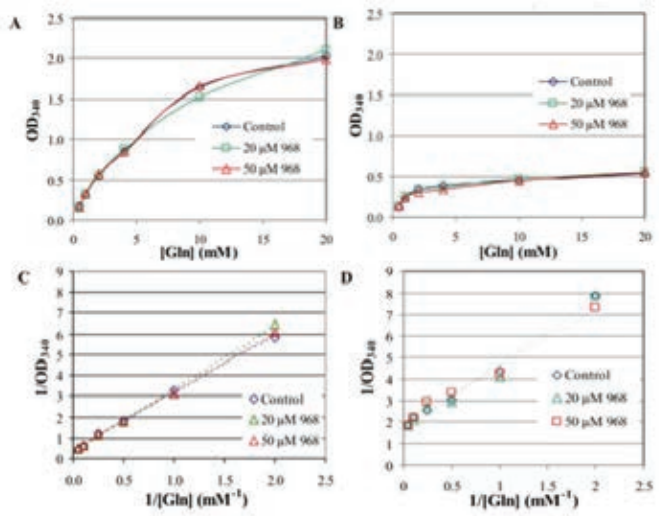

Figure 3: Glutamine saturation profiles for hGACΔ1 determined in the absence and presence of either 20 μM or 50 μM 968. The data are the mean of three measurements for the control and for 20 μM 968 and of two measurements for 50 μM 968. A) Assay at 100 mM phosphate; B) Assay at 10 mM phosphate; C) Lineweaver-Burk plot of 100 mM phosphate data; D) Lineweaver-Burk plot of 10 mM phosphate data.

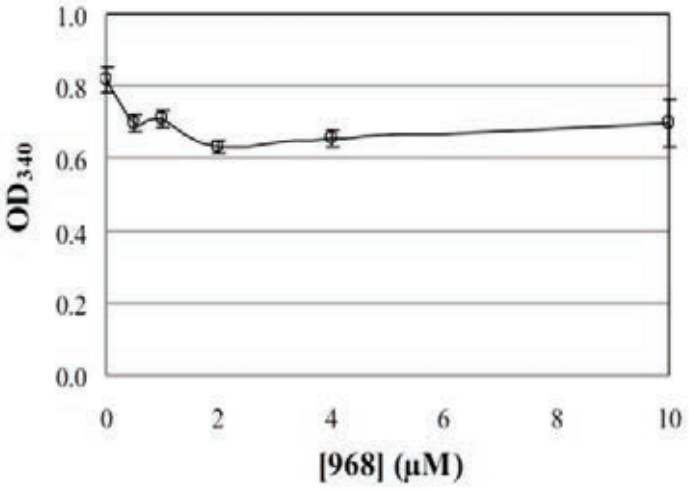

Figure 4: Effect of 0.5-10 μM 968 on hGACΔ1 activity in the absence of added phosphate. The data represent the mean of four measurements ± the standard deviation.

phosphate and glutamine. This was followed by the sequential addition of 5 μL of a 2-fold dilution of $hGAC_{\Delta 1}$, 4 μL of 968 solutions in DMSO, and 16 μL of 25 mM glutamine.

To replicate the previous experimental protocol, 75 μL of a solution containing 64 mM Tris acetate, 0.27 mM EDTA, and 4.4 μg of $hGAC_{\Delta 1}$ were added to each well of a 96-well plate.[9] Subsequently, 5 μL of various dilutions of 968 in DMSO were added and the samples were incubated at 35oC for 30 minutes. Then, 35 μL of 60 mM glutamine were added and the wells were incubated for another hour at 35oC. Glutamate formation was determined by transferring 10 μL of each sample to 200 μL of standard glutamate assay mixture and incubating for forty-five minutes at room temperature.

Glutamine saturation profiles were obtained by adding 10 μL of glutaminase assay mixture containing either 250 mM or 25 mM phosphate to the wells of a 96-well plate, followed by adding 10 μL of glutamine solutions ranging from 1.25 to 50 mM. To each well, 2.5 μL of either DMSO or 0.2 mM or 0.5 mM 968 solutions in DMSO were added. The reactions were initiated by the addition of 2.5 μL of a 5-fold dilution of $hGAC_{\Delta 1}$ and incubated for ten minutes at 37oC. The assays were completed by adding 200 μL of standard glutamate assay mixture, incubating for thirty-five minutes at room temperature, and then reading the absorbance at 340 nm.

The phosphate activation profiles were conducted by charging a 96-well plate with 20 μL of glutaminase assay mixtures in which the phosphate concentration ranged from 6.25 mM to 187.5 mM, followed by 2.5 μL of DMSO or either 0.2 mM or 0.5 mM 968 in DMSO. The reactions were initiated with 2.5 μL of a 5-fold dilution of $hGAC_{\Delta 1}$ and incubated for ten minutes at 37oC. The assays were completed as for the glutamine saturation profiles.

Mitochondria were isolated by differential centrifugation of rat brain that was homogenized in isotonic sucrose.[13] The purified mitochondria were diluted to a protein concentration of either 5.2 or 2.4 mg/mL. Samples containing 100 μL of the mitochondrial preparations were incubated for thirty minutes at room temperature with 2.5 μL of either DMSO or 968 solutions ranging in concentration from 0.22 to 10.9 mM. The wells of a 96-well plate were charged with 20 μL of glutaminase assay mixtures containing 150 mM, 10 mM, or 0 mM potassium phosphate followed by 5 μL of the 968/mitochondria mixtures (2.4 mg/mL levels for the wells containing phosphate and 5.2 mg/mL mitochondrial solution for the wells lacking phosphate). The plate was incubated at 40oC for ten minutes and the assay completed using 200 μL of glutamate assay mixture for each well as for the glutamine saturation profiles experiments.

Results

Human GAC (hGAC) is encoded by exons 1-15 of the *GLS1* gene that is located on chromosome 2.10 The enzyme used in this study, $hGAC_{\Delta 1}$, is a truncated version of hGAC that lacks the sequence encoded by the first exon, which contains the mitochondrial targeting signal. This modification prevents aggregation without affecting the glutaminase activity.[10] The recombinant $hGAC_{\Delta 1}$ enzyme includes an N-terminal His_6-sequence to facilitate its purification by nickel-affinity chromatography. Attempts to purify the $hGAC_{\Delta 1}$ by gravity elution with buffers containing imidazole proved to be surprisingly non-reproducible. However, gradient elution using an FPLC proved more effective. The enzyme required approximately 200 mM imidazole to elute from the nickel-affinity column. Analysis of the eluted fractions by SDS-PAGE demonstrated that considerable purification of $hGAC_{\Delta 1}$ was accomplished (Fig. 1). The bands corresponding to $hGAC_{\Delta 1}$ migrated slightly below the 55-kDa protein standard. The identification of this band as $hGAC_{\Delta 1}$ was confirmed by a Western blot using a rabbit polyclonal antibody to glutaminase (Aviva Systems Biology) that recognizes both the KGA and GAC isoforms. Protein concentrations for fractions 20 and 21 were determined to be 0.81 mg/mL and 0.69 mg/mL, respectively. The calculated specific activities for the two fractions were 102 units/mg and 95 units/mg, respectively. Based upon this analysis, fractions 20 and 21 were combined and used for the kinetic

analyses.

In the absence of phosphate, the purified glutaminase exists as a dimer that has little activity.[14] The addition of phosphate promotes the formation of a tetramer that is highly active. The phosphate activation profile of $hGAC_{\Delta 1}$ was determined in the presence of 20 and 50 µM 968 and compared to a control containing the same level of DMSO (Fig. 2). The control assay exhibited an activation profile that is characteristic of the enzyme purified from kidney mitochondria when assayed in the absence of DMSO.[14] The activation profiles for the control and the 20 µM 968 samples were identical. However, the profile obtained with 50 µM 968 exhibited a very slight inhibition at all concentrations of phosphate. Thus, 968 may be a weak inhibitor of the $hGAC_{\Delta 1}$ enzyme.

The potential inhibitory effect of 968 on $hGAC_{\Delta 1}$ activity was also characterized by analysis of its effect on activity with increasing concentrations of glutamine. The glutamine saturation profiles were determined with 10 mM and 100 mM phosphate and a five-fold dilution of the purified enzyme (Fig. 3A and B). The addition of either 20 µM or 50 µM 968 produced no inhibition at either concentration of phosphate. Michaelis constants for glutamine activation under high and low phosphate conditions were determined using Lineweaver-Burk double reciprocal plots (Fig. 3C and D) and Hanes-Woolf plots of glutamine concentration divided by activity vs. glutamine concentration (data not shown). This analysis indicated that the K_m for glutamine was decreased when the enzyme was assayed with the lower concentration of phosphate (Table I). However, the addition of either concentration of 968 had no effect on the apparent binding of glutamine to $hGAC_{\Delta 1}$.

As a follow up to the apparent lack of inhibition by 968, an assay was run in the absence of added phosphate (Fig. 4). In this experiment the $hGAC_{\Delta 1}$ was pre-diluted only by a factor of two, the final DMSO concentration was 7%, and the assay was initiated by the addition of glutamine. A modest inhibition by 968 was detectable without added phosphate. This analysis is not consistent with the previous report that 10 µM 968 was sufficient to produce a potent inhibition of GAC.[9] Therefore, the glutaminase assay was repeated exactly as described in the previous publication. In this assay, undiluted $hGAC_{\Delta 1}$ was pre-incubated for thirty minutes at 35oC with various concentrations of 968 in a phosphate-free buffer before initiating the assay through the addition of glutamine. This experiment produced greater variability, but showed no clear evidence of inhibition of $hGAC_{\Delta 1}$ by 968 (Fig 5).

The possibility that 968 inhibits glutamine transport into the mitochondria rather than glutaminase itself was examined by conducting an assay using whole mitochondria isolated from rat brain in place of the $hGAC_{\Delta 1}$ construct. In this analysis, the mitochondria were incubated with various concentrations of 968 for thirty minutes at room temperature before initiating the assay. A 30 % inhibition of the mitochondrial glutaminase activity was observed when assayed with 150 mM phosphate, but no inhibition was apparent when the assays were performed with 10 mM phosphate or with no added phosphate (Fig. 6).

Discussion

The principle goal of this study was to characterize the kinetics of 968 inhibition of a human GAC construct with the hope that this data might be used to direct future modifications to the chemical structure of 968. Although assays conducted in the absence of phosphate suggested that 968 produced a slight inhibition of $hGAC_{\Delta 1}$ (Fig. 4), the glutamine saturation profiles showed that 968 in fact had almost no effect on the activity of this glutaminase construct (Fig. 3). Furthermore, phosphate activation profiles gave no indication that 968 had any greater effect on $hGAC_{\Delta 1}$ at low phosphate

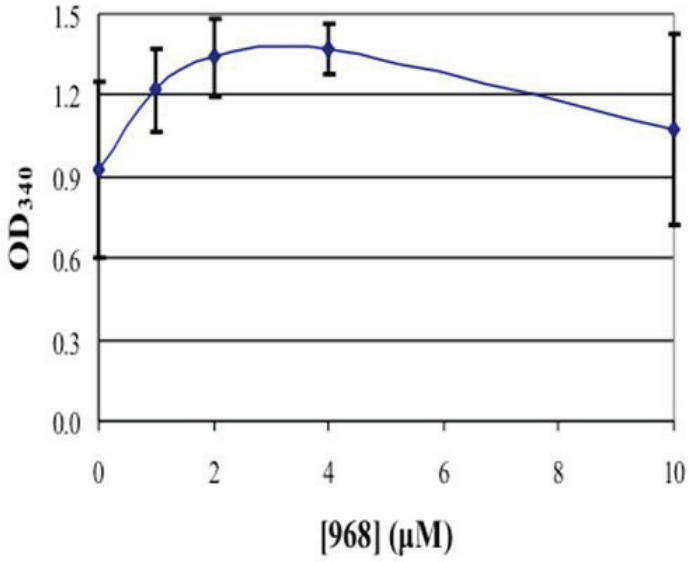

Figure 5: Effect of pre-incubating $hGAC_{\Delta 1}$ and the inhibitor 968 in the absence of added phosphate. The data are the mean of three measurements ± the standard deviation.

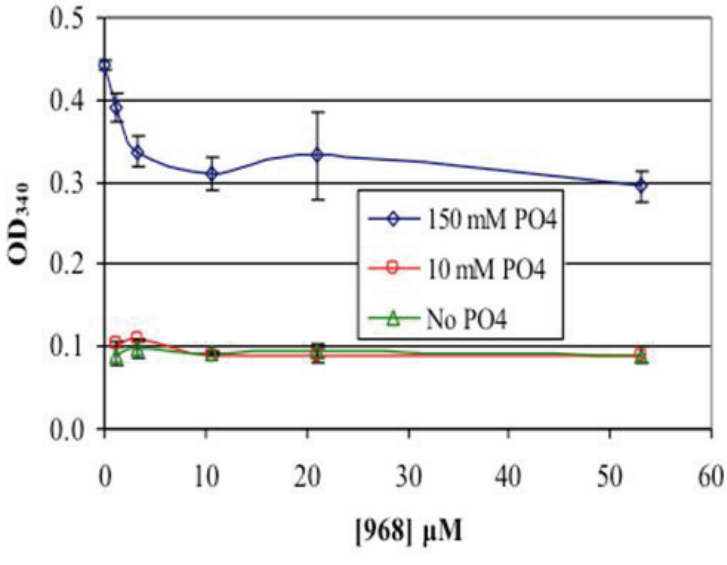

Figure 6: 968 inhibition of glutaminase activity in whole rat brain mitochondria at various phosphate levels. Freshly isolated rat brain mitochondria were pre-incubated with increasing concentrations of 968 and with three different concentrations of phosphate. The 150 mM phosphate data represent the mean of 6 measurements obtained from two experiments, while the 10 mM phosphate and no phosphate data are the mean of two measurements and three measurements, respectively, from a single experiment.

Table 1: Effect of 968 on the Michaelis Constants for Glutamine

	[PO4-3]	[968]	Slope	Intercept	R^2	K_m
Lineweaver-Burk:	100 mM	0 μM	2.74	0.419	0.9986	6.6 mM
		20 μM	3.01	0.313	0.9973	9.6 mM
		50 μM	2.84	0.357	0.9988	8.0 mM
Hanes-Woolf		0 μM	0.344	2.94	0.9914	8.6 mM
		20 μM	0.329	3.03	0.9943	9.2 mM
		50 μM	0.354	2.86	0.9889	8.1 mM
	[PO4-3]	**[968]**	**Slope**	**Intercept**	**R^2**	**K_m**
Lineweaver-Burk	10 mM	0 μM	3.01	1.71	0.9908	1.8 mM
		20 μM	2.97	1.66	0.9782	1.8 mM
		50 μM	2.64	1.93	0.9830	1.4 mM
Hanes-Woolf		0 μM	1.76	3.09	0.9954	1.8 mM
		20 μM	1.70	3.09	0.9967	1.8 mM
		50 μM	1.68	3.60	0.9909	2.1 mM

Linear transformations of the hyperbolic data from Fig. 3 were used to determine the Km values from the glutamine saturation profiles conducted at either 100 mM or at 10 mM phosphate in the absence or presence of either 20 μM or 50 μM 968.

concentrations than at the standard phosphate level used in glutaminase assays (Fig. 2).

The first disclosure of compound 968 was by Iconix Pharmaceuticals in a patent application filed in 2001 claiming its use as a modulator of Rho C activity.[15] The association of 968 with glutaminase inhibition first appeared in a publication focusing primarily on 968's ability to block transformation of cells by the oncogenic guanine nucleotide exchange factor Dbl.[9] Glutaminase was identified as the target of 968 by affinity purification experiments using 968 conjugated to biotin. Pre-incubation of GAC with 10 μM 968 produced greater than 80% inhibition of the glutaminase activity. Glutamine saturation profiles, presented as supplementary material, supported the authors' contention that 968 is not a competitive inhibitor with respect to either glutamine or phosphate ion, but rather functions as an allosteric inhibitor.

It is difficult to reconcile the discrepancies between the results of the current study and those from the original 968 disclosure. Even attempts to replicate the published protocol afforded no indication of $hGAC_{\Delta 1}$ inhibition by 968 (Fig. 5). One identifiable difference between the studies is the fact that the original report used the mouse ortholog of human GAC in assaying glutaminase inhibition. However, the inhibition of oncogenic activity in the earlier study was demonstrated using human cells. It should also be noted that the His_6-tag used to isolate cloned mouse GAC in the original study was cleaved prior to use in kinetic experiments, while the tag was left in place in the $hGAC_{\Delta 1}$ used in the present study.

Some understanding regarding the lack of agreement between the original 968 publication and the current study may be gleaned from the revelation that 968 is apparently ineffective at inhibiting glutaminase that has been activated by phosphate.[16] Although efforts were made in the present study to assess inhibition by 968 in the absence of added phosphate, the $hGAC_{\Delta 1}$ used in all experiments had been isolated using a buffer containing 10 mM phosphate. In this connection, it is interesting to consider the hypothesis that accumulation of phosphate in mitochondria is what triggers increased GAC-based glutaminase activity in cancerous cells.[17] Should this in fact be the case, the use of 968 in treating cancers characterized by high GAC activity might be futile.

A somewhat different account of the role of 968 in glutaminase inhibition can be found in a recent patent application filed by Cornell University.[18] It is stated in this application that 968 does not directly inhibit glutaminase catalytic activity. The patent presents data indicating that GAC isolated from cancer cells is more active in the absence of phosphate than GAC isolated from non-transformed cells. The patent also suggests that only in cancer cells is mouse GAC phosphorylated at Serine 103 (corresponding to Serine 95 in human GAC). The authors suggest that 968 may block this phosphorylation and thus prevent the phosphate-independent activation of GAC that is unique to cancer cells. The glutaminase construct used in this study, $hGAC_{\Delta 1}$, lacks the first 123 amino acids from the N-terminus. Thus, it lacks the predicted site of phosphorylation and its activation is highly dependent upon phosphate. However, this serine residue is conserved in the rat brain glutaminase. Thus, it is tempting to ascribe the limited inhibition of glutaminase activity from the rat brain assay of the current study to a portion of the glutaminase that is not phosphorylated (Fig. 6), although the question would remain as to why inhibition was only evident under high phosphate concentrations.

Another consideration with respect to the failure of this study to show GAC inhibition by 968 is that the inhibitory properties of 968 have been observed to diminish with time.[16] This is a surprising observation, since 968 might be expected to be quite thermally stable and show little propensity to react with water

or with oxygen. Precautions were taken to confirm the identity of the 968 sample used in the present study through electrospray time-of-flight mass spectroscopy. However, we could not reproduce the inhibition of GAC using the exact conditions described previously and could only show partial inhibition of the crude rat brain glutaminase. Thus, we cannot exclude the possibility that our sample of 968had undergone some non-detected change that resulted in loss of activity. In conclusion, 968 may yet prove to be a promising material from which therapeutic cancer treatments may be developed. However, it appears that studies directed towards improvements of the efficacy of 968 will not be able to rely on assays predicated on the direct inhibition of glutaminase activity.

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References

[1]Nelson, D.L. and Cox, M.M. (2008) *Lehninger Principles of Biochemistry,* 5th Ed., W. H. Freeman and Company, New York, NY.

[2]Erickson, J.W. and Cerione, R.A. (2010) "Glutaminase: A hot spot for regulation of cancer cell metabolism?" *Oncotarget* 1, Pg 734-740.

[3]Elgadi, K.M., Meguid, R.A., Qian, M., Souba, W.W. and Abcouwer, S.F. (1999) "Cloning and analysis of unique human glutaminase isoforms generated by tissue-specific alternative splicing". *Physiological Genomics* 1, Pg 51-62.

[4]Gao, P., Tchernyshyov, I., Chang, T., Lee, Y., Kita, K., Ochi, T., Zeller, K.I., De Marzo A.M., Van Eyk, J.E., Mendell, J.T. and Dang, C.V. (2009) "c-Myc suppression of miR-23a/b enhances mitochondrial glutaminase expression and glutamine metabolism". *Nature* 458, Pg 762-766.

[5]Shapiro, R.A., Clark, V.M. and Curthoys, N.P. (1978) Covalent interaction of L-2-amino-4-oxo-5-chloropentanoic acid with rat renol phosphate-dependent glutaminase. *Journal of Biological Chemistry* 253, Pg 7086-7090.

[6]Shapiro, R.A., Clark, V.M. and Curthoys, N.P. (1979) "Inactivation of rat renol phosphate-dependent glutaminase with 6-diazo-5-oxo-L-norleucine". *Journal of Biological Chemistry* 254, Pg 2835-2838.

[7]Robinson, M.M., McBryant, S.J., Tsukamoto, T., Rojas, C., Ferraris, D.V., Hamilton, S.K., Hansen, J.C., and Curthoys, N.P. (2007) "Novel mechanism of inhibition of rat kidney-type glutaminase by bis-2-(5-phenyladcetamido-1,2,4-thiadiazol-2-yl)ethyl sulfide (BPTES)". *Biochemical Journal* 406, Pg 407-414.

[8]DeLaBarre, B., Gross, S., Cheng, F., Gao, Y., Jiang, F., Jha, A.K., Song, J., Wei, W and Hurov, J. (2011) "Full-length human glutaminase in complex with an allosteric inhibitor". *Biochemistry* 50, Pg 10764-10770.

[9]Wang, J.B., Erickson, J.W., Fuji, R., Ramachandran, S., Gao, P., Dinavahi, R., Wilson, K.F., Ambrosio, A.L.B., Dias, S.M.G., Dang, C.V. and Cerione, R.A. (2010) "Targeting mitochondiral glutaminase activity inhibits oncogenic transformation". *Cancer Cell* 18, Pg 207-219.

[10]Hartwick, E.W., and Curthoys, N.P. (2011) "BPTES inhibition of hGA(124-551), a truncated form of human kidney-type glutaminase". *Journal of Enzyme Inhibition and Medicinal Chemistry.*

[11]Curthoys, N.P. and Weiss, R.F. (1974) "Regulation of renal ammoniagenesis: subcellular localization of rat kidney glutaminase isoenzymes. *Journal of Biological Chemistry* 249, Pg 3261-3266.

[12]Bradford, M.M. (1976) "A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding". *Analytical Biochemistry* 72, Pg 248-254.

[13]Haser, W.G., Shapiro, R.A., and Curthoys, N.P. (1985) "Comparison of the phosphate-dependent glutaminase obtained from rat brain and kidney". *Biochemistry Journal* 229, Pg 399-408.

[14]Godfrey, S., Kuhlenschmidt, T., and Curthoys. N.P. (1977) "Correlation between activation and dimer formation of rat renal phosphate-dependent glutaminase". *Journal of Biological Chemistry* 252, Pg 1927-1931.

[15]Sun, D., Perkins, E.L. and Tugendreich, S. *US Patent 6800634 (WO 2003/043582).*

[16]Katt, W.R., Ramachandran, S., Erickson, J.W., Cerione, R.A. (2012) "Dibenzophenanthridines as inhibitors of phosphate-activated glutaminase C and cancer proliferation". *Molecular Cancer Therapy* 11, Pg 1269-1278.

[17]Cassago, A., Ferreira, A.P.S, Ferreria, I.M, Fornezari, C., Gomes, E.R.M., Greene, K.S., Pereira, H.M, Garratt, R.C., Dias, S.M.G. and Ambrosio, A.L.B. (2012) "Mitochondrial localization and structure-based phosphate activation mechanism of Glutaminase C with implications for cancer metabolism". *Proceedings of the National Academy of Sciences, USA* 109, Pg 1092-1097.

[18]Cerione, K.W. and Cerione, R.A. *International Publication Number WO 2012/034123.*

From the Pupil's Perspective

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Abstract:

We suggest university anatomy outreach programs promote the personal and professional development of student presenters, better preparing them for healthcare professions. Through presentations to high school students, university student-led anatomy outreach programs provide opportunities to develop unique skills that may better prepare the university students to be successful future health care professionals. Some of these skills include time management, professional communication, scheduling, and working with emotional barriers. Importantly, delivering these presentations requires students to acknowledge and address their own weaknesses. Interacting with a variety of audiences, the university student presenters also learn how to communicate effectively by having to accommodate and adapt to varying knowledge levels. Ultimately, this better prepares them to convey difficult scientific information to a wide variety of individuals. These outreach programs benefit both presenters and audiences, allowing students to apply their knowledge, share their enthusiasm, as well as encourage an interest in science.

Introduction

The anatomy program at Colorado State University (CSU) is structured around a dissection course consisting of approximately fifty students and twelve cadavers. This course takes a regional approach to dissection and runs over the course of a 16-week semester every fall. The undergraduate human anatomy prosection course parallels the dissection course as well as provides access to ten additional cadavers dissected the previous fall. In this prosection course, there are five-hundred junior and senior level undergraduate students distributed among five laboratory sections allowing for a ratio of four to five students per cadaver. The prosection course is both laboratory and lecture based. The lecture portion is grounded in clinical applications strengthened by the intensive laboratory portion. To deepen this anatomical understanding, concepts from embryology, histology, anthropology, and physiology are also incorporated. In addition, digital supplementation with The Visual Human Dissector (VHD) and The Virtual Edge Anatomy software is used in the lab so students can compare live and virtual cadavers for an enhanced learning experience. This software also exposes students to cross-sectional images, vital for future healthcare professionals, and it serves as a valuable at-home study tool.

Accompanying the human gross anatomy prosection course is an outreach program that provides students in Colorado State University's honors program with unique opportunities. Through this program, students from the prosection course develop and deliver anatomy-based presentations to interested Colorado K-12 classes. The goal of our anatomy outreach program is to expose the K-12 classes to the field of science through interactive, hands-on anatomy presentations, and increase awareness of current health issues. Visual aids, which include healthy and diseased organs, are critical during these presentations to emphasize healthy lifestyles. They allow students to touch and manipulate preserved human organs. This haptic experience provides a greater impact as compared to purely oral presentations. These presentations also provide a venue to promote some of the unique opportunities at CSU; for example, CSU holds unprecedented access to twenty-two human cadavers.

The anatomy outreach program facilitates the student presenters' personal and academic growth. Student presenters have the opportunity to improve a variety of skills including: time management, scheduling multiple agendas, overcoming emotional barriers, and professional communication. These skills correlate to the general skills needed to succeed in science careers and in future clinical applications such as the patient-physician relationship. Current research has shown a linear relationship between involvement in educational outreach programs and successful clinical communication.[1] In this paper we examine the implications and impact of our anatomy outreach program. We have found that the presentations increase student presenters' understanding of the material and facilitate the development of a variety of unexpected skills which will be acknowledged and discussed in this paper.

Description

The core background knowledge enabling our students to be successful in delivering the anatomy outreach presentations is obtained largely from the Human Anatomy Prosection course. In addition, the student presenters involved in this outreach program are enrolled in a breakout honors section in which material from lecture is supplemented through the use of didactic case studies and the outreach presentations. While the case studies require students to do their own investigation and subsequently apply material that they have learned in lecture to diagnose hypothetical patients, the creative outreach presentations allow the anatomy students to share their knowledge with students from around the state.

First, the student presenters work in pairs to develop informative and entertaining presentations to be given in front of the honors breakout class. Then, using feedback from classmates and instructors, the student presenters improve their presentations for delivery to K-12 audiences. The student presenters are required to coordinate multiple aspects of the visit including room and date scheduling, distributing teacher guidelines, and ensuring specimen availability. The final product involves a group of visitors attending an hour-long presentation. During the first 30-40 minutes of the presentation, the student presenters use a collection of organs to advocate living a healthy lifestyle, spark an interest in science, and illustrate

key anatomical concepts from a clinical perspective. In the remaining 20-30 minutes, the K-12 audiences are given the opportunity to explore a cadaver and challenge the student presenters with questions. In some instances, if a group wishing to receive a presentation is not able to travel to CSU, student presenters will travel to a school with the collection of organs and deliver a 45-minute interactive oral presentation. The outreach presentations are centered on encouraging healthy lifestyles through exposure to diseased organs.

The success of these presentations hinges on the student presenters' mastery of a variety of skills such as thorough anatomical understanding, professional communication and scheduling, adept laboratory preparation, familiarity with the specimens, presentation skills, and dynamic teamwork. In addition, the student presenters are faced with the task of developing a presentation that is both engaging and informative to audiences of different ages with a variety of educational backgrounds. Student presenters are given significant freedom with their presentation content, leading to a multitude of creative outlets such as audience participation, metaphorical language, and the incorporation of personal anecdotes. One of the few requirements of the presentation is the discussion of body donation. This ensures all visitors that the cadavers and specimens were voluntarily donated and wished to contribute their body to learning. One major challenge to the student presenters is to leave the K-12 audiences with a greater understanding and/or interest in science, specifically involving the human body and how to properly care for it.

The anatomy outreach program requires a significant amount of preparation from student presenters and staff. A list of schools that have previously participated in the outreach program is given to the student presenters who are required to personally contact the teachers. If a teacher expresses interest in receiving a presentation, then the student presenters begin coordinating with the teacher. This entails sending teacher guidelines that encompass parking and building location, size limitations, fees involved, and the scope of the presentations. Typically, this communication continues over the period of a month. As the dates are being finalized, the student presenters compile a collection of diseased and healthy human organs obtained from prior dissections. Some of the interesting specimens include a brain, a melanoma liver, a cirrhotic liver, an emphysemic lung, and a heart with a pacemaker. A component of the student presenters' responsibility is to maintain the integrity of these specimens.

Several measures are taken to clean and prepare the laboratory to safely accommodate around 40 K-12 students plus two cadavers on the day of the presentation. Student presenters are responsible for ensuring adequate seating for all visitors and may encourage the visitors to sit down if they are feeling uncomfortable. After prepping the room, one presenter is responsible for greeting and directing the visitors to the laboratory, and the presentation begins.

Discussion

For over thirty-five years the Association of American Medical Colleges has recognized the importance of scientific outreach programs that serve to educate and recruit future students into the field of science.[2] The described presentations of the anatomy program acknowledge this importance of outreach and service learning. With initial hopes of educating K-12 schools in Colorado through awareness of science and the human body, the program today not only educates the K-12 audiences but it also allows the presenters to develop personal and professional skills. The program includes 22-28 student presenters per year who reach approximately 3,000 K-12 students from Colorado each year through their presentations. Student presenters' participation and the demand for presentations have increased since the program was first implemented in 2008. In this type of outreach, the student presenters engage in activities that meet community needs of coordinating with formal education to support an academic curriculum.[3]

Although the goals of the program are formally outlined (see addendum), from student presenters' perspectives, the development and delivery of the presentations is a dynamic process. Of the many challenges of this process, scheduling is at the forefront. Ideally, the visiting groups request a date and time that works with both the presenters' schedules and laboratory availability. However, this is rarely the case; instead, the scheduling tends to be a lengthy process requiring multiple emails until a final date and time is confirmed. This acquisition of time management skills and effective communication through a real-world application is essential to undergraduate students.[4]

The student presenters must anticipate the challenges that they will encounter on the day of the presentation. With cadavers on display, student presenters must be aware of the K-12 audiences' reactions to cadavers.[5] Encouraging students to sit down if they feel nauseated or to leave the room for water are essential elements of these presentations as they address the safety concerns of the K-12 audiences. The emotional responses range from sadness to fear, thus, student presenters learn to adapt and understand the individuals they are working with. This creates a sense of trust and establishes the presenters' roles as educators. Effective communication is a critical skill honed from this outreach, and it plays an important role in enhancing the student presenters' competitive skills in their chosen fields.[4]

Student presenters present to audiences with varying educational levels. Because of this, student presenters must adapt their presentation to each audience. For example, student presenters have the flexibility to emphasize healthy diet and exercise for middle school students, whereas older students' presentations place emphasis on physiological and anatomical effects of drugs, alcohol, and smoking. Furthermore, the student presenters are expected to utilize the age-appropriate tone and pitch for each K-12 audience. The ability to communicate scientific knowledge to others is a skill that student presenters can apply in future careers in which they must assess the needs of an individual and facilitate learning in a manner that is comprehensible to that individual.[6]

A personally challenging aspect of delivering these presentations is learning to acknowledge and address one's weaknesses. When learning through teaching, the thinness of knowledge is exposed to each student presenter, their peers, and instructors.[7] The obstacle is not to be intimidated by this exposure, but rather to use this transparency as fuel to master the material. Many outreach programs show that student presenters recognize that teaching material requires a deeper level of understanding in contrast to the fundamental college coursework.[7] This outreach program is no exception to that realization as student presenter pairs continuously practice and research the material on which they are presenting. Throughout the semester, there is a shift from apprehension to enthusiasm in the delivery of the presentations.

Feedback surveys were designed to assess the impact of the outreach presentations on both the visitors as well as the student presenters. The feedback survey of the student presenters includes questions regarding motivation, skills obtained, and attitudes towards the experience. Participation in these surveys is voluntary; therefore, the conclusions drawn do not

account for the total population involved in these presentations. According to the surveys, the outreach presentations have been well received by all of those involved. Many of the students participate in the optional survey and reflect upon their experience. A frequent theme cited by student presenters is enjoyment. This outreach program involves a role-reversal opportunity that gives the presenters the chance to share their passion and challenge others with science. Many students make a connection between the skills obtained and their future professional goals. A former student now at medical school states, "The program helped me to learn to better communicate with students effectively, and it also helped me to develop professionalism. It challenged me to really 'know' the material, because when you can teach it, then you really know it."

A separate survey was sent to the visiting K-12 teachers, which provided them the opportunity to critique the student presenters. The teachers unanimously indicated that student presenters were well prepared, knowledgeable, and able to appropriately communicate with their student audiences of varying educational levels. Teachers commonly note that in many cases the presentations exceeded their expectations. The overall attitude expressed by visiting teachers is reflected in this comment from an instructor who brought his class for a presentation, "The CSU cadaver lab is a terrific resource for instructors preparing students for health care careers. Aside from the information presented at the labs, encountering the cadavers is a way to prepare students for the rigors in the medical field on a psychological level." The psychological preparation noted is an item of importance for not only the K-12 audiences but also the presenters themselves.

Anatomy has historically served not only as a technical training tool but also as a means for training healthcare professionals to handle difficult emotions.[5] Undergraduate anatomy students in this program, many of which aspire to be physicians, are able to reap these benefits prior to matriculation in medical school. Students in the anatomy program become proficient anatomists, and additionally, they begin to understand their emotions in regard to death. It has been noted that one of the greatest challenges to first year medical students is dealing with the reality of facing death on a regular basis.[5] Practice in handling these complicated emotions allows students to develop a sense of professionalism that will impact their ability to confront dying patients in the future.[5] This outreach program takes this emotional component one step further by requiring student presenters to help their audiences cope with seeing and exploring a cadaver. This element corresponds to the responsibility that health-care professionals have in regard to communicating with the families of terminal patients. Ultimately, these presentations give student presenters a glimpse of their future professional role.[6]

Multiple roles have been assigned to physicians: expert, communicator, collaborator, manager, health advocate, scholar, and professional.[1] In order to address this array of responsibilities, many medical schools have implemented a curriculum involving the six core competencies adopted by the Accredation Council for Graduate Medical Education in 1999.[1] The competencies include patient care, medical knowledge, practice-based learning and improvement, interpersonal and communication skills, professionalism, and systems based practice.[1,8] This anatomy outreach program caters to each of these competencies. Student presenters use various specimens as well as cadavers to demonstrate certain pathologies, treatments, prevention, and to advocate for a variety of careers within the medical field. We believe this enables them to practice the competencies of patient care, medical knowledge, and systems-based practice respectively. Student presenters take part in self-directed learning in regard to the anatomy and pathology that they plan to emphasize in their presentation. Self-directed learning has shown to be a key component to professional development and the delivery of high-quality care.[1] The competencies of professionalism and adept communication are addressed through the student presenters' interactions with a variety of audiences, allowing the student presenters to master the material at hand via practiced-based learning. This type of teaching has also shown to be a valuable method for self-improvement.[1] The ability to acknowledge personal weaknesses and, furthermore, to address those weaknesses is perhaps one of the challenging parts of being a physician. Early exposure to this type of introspection is certainly advantageous to pre-medical undergraduates.[1]

There is no denying that teaching is a vital part of being a physician.[6] Through the delivery of anatomy outreach presentations, students are exposed to instances in which they must convey sensitive information. Teaching correlates to future patient education, specifically in learning how to explain things to someone who does not share the same educational background.[6] Additionally, evidence has shown a correlation between the successful communication of trained teachers versus those with no previous experience.[6] Perhaps by practicing the art of teaching via the anatomy outreach presentations, student presenters master a variety of skills better preparing themselves to ensure patient understanding and trust upon becoming a physician.

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References

[1]Gregory, J.K., et al. (2009) "Restructuring a basic science course for core competencies: An example from anatomy teaching." *Medical Teacher* 31.9. Pg 855-861.

[2]Houtz, L.E. and Quinn, T.H. (2007) "Build a Human Project: Improving Attitude and Increasing Anatomy Content Knowledge and Skills for Middle-Level Students." *Journal of Higher Education Outreach & Engagement* 11.2. Pg 21-32.

[3]Andrews, E., et al. (2005) "Scientists and public outreach: Participation, motivations, and impediments." *Journal of Geoscience Education* 53.3. Pg 281.

[4]Rao, S., Shamah, D. and Collay, R. (2007) "Meaningful Involvement of Science Undergraduates in K-12 Outreach." *Journal of College Science Teaching* 36.6. Pg 54-58.

[5]Arráez-Aybar, L.-A., Castaño-Collado, G. and Casado-Morales, M.I. (2008) "Dissection as a modulator of emotional attitudes and reactions of future health professionals." *Medical Education* 42.6. Pg 563-571.

[6]Amorosa, J.M.H., Mellman, L.A. and Graham, M.J. (2011) "Medical students as teachers: How preclinical teaching opportunities can create an early awareness of the role of physician as teacher." *Medical Teacher* 33.2. Pg 137-144.

[7]Elmendorf, H.G. (2006) "Learning Through Teaching: A New Perspective on Entering a Discipline." *Change* 38.6. Pg 36-41.

[8]ACGME. (2010) "Common Program Requirements". Accreditation Council for Graduate Medical Education <http://www.acgme.org/acgmeweb/>(Accessed 08/04/2012)

Evaluating Medical School Selection Criteria: Are we choosing the best candidates?

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What makes a good physician? What are some of the character traits or personalities that you look for in someone who is charged with examining, discussing, and deciding on some of the most personal and important aspects of your life? What characteristics make you trust someone to be scalpel-deep in the intimate details of the people you love? Do you consider yourself a valuable member of your health care team? How many times have you walked into your physician's office or a hospital and asked the receptionist where each of your potential physicians went to medical school? Or did their residency? Does the answer to the last two questions matter? If so, why? It's simple: you want to be served by the best physician available in a particular specialty. You want your spouse and your children and your parents to be treated by the most knowledgeable, the most skilled, and the highest regarded physician. That elucidates one last question: does our perception of "best" actually match those last three superlatives?

Best can be an arbitrary description. As a well-educated society, we tend to assign the distinction of "best" to the top tier in a statistical or performance category. The best 100m sprinter is Usain Bolt.[1] The best country in terms of math and science education is Singapore.[2,3] Inherently, we associate "best" physicians with top-ranked schools, top performance on board exams, and top salaries. But is that really the most appropriate application of the adjective "best" for a physician? Are we relegated to these few, tangible criteria to proclaim someone in charge of our health and often times our *life*, the best? Where is the set of rankings that rates physicians on their ability to explain complex medical conditions in a vernacular that the common man can understand? Where is the poll that groups clinicians on their bedside manner, on their ability to offer comfort and tacit reassurance in even the bleakest of times? Where is the list of physicians who are not ashamed or embarrassed to be faced with a complex condition, an anomaly in terms of pathology, look directly into your face, and say "I don't know, but I am going to do *everything* in my power to find the answer"?

When we take the time to think about it, the distinction of "best" does not seem as obvious as we would have thought. Unfortunately, these misconceptions of "best" permeate through the entire system of education; from the upper echelons of professional schools to acceptance into undergraduate programs out of high school. The focus of this article will revolve around the medical school admissions process.

It is pertinent to first start off with an overview of the medical school application process. There are two major types of medical schools in the country: allopathic and osteopathic. Students who attend allopathic programs will graduate with "M.D." (medical doctor) after their names and are what many people associate with the term "physician". Students who graduate from osteopathic programs carry the letters "D.O." (doctor of osteopathy) after their name. Both M.D.'s and D.O.'s are bestowed with the same credentials and have almost identical training. In most respects they are the same, and many patients wouldn't be able to tell which one is treating them.

The application process begins with a general, primary application that is sent to all of the schools a student chooses to apply to. This application contains demographic and transcript information, work experience, leadership roles, and other extracurricular activities. It contains the infamous personal statement which asks students to answer in 5300 characters, "Why do you want to be a physician?" It also contains information about your standardized test scores: the medical college admissions test (MCAT). The MCAT can be thought of as "the great equalizer", the one criterion that puts all candidates on a level playing field to gauge the competence of a candidate in relation to his or her peers. A 4-5 hour online examination, the MCAT serves to test students on the information they should have learned during their undergraduate studies necessary for beginning medical school. In short, it tests students on the carry-forward prerequisite courses for medical school curricula.

This initial screening is used to confirm minimum requirements and can determine whether or not individual schools will send the secondary or supplementary application. This application is school-specific and thus varies from school to school. The items that are rather ubiquitous on these applications, however, are essay questions designed to glean a better idea of a particular candidate. These questions range from "What unique characteristics would you bring to X school?" and "Describe a time in your life in which you faced adversity and discuss how you overcame that adversity". The secondary application also contains the letters of recommendation from faculty members that speak about the abilities of the candidate. After the secondary application is returned, the waiting period for the interview commences. Schools can choose to deny candidates right off the bat or can choose to call candidates for a face-to-face interview and offer them acceptance, waitlist them, or deny them.

An appropriate question at this point might be, "so what's wrong with the process?" It sure seems as if adequate measures are in place to identify strong candidates and rank them based on all of the information and essay responses provided. Students are given ample opportunity to provide detailed information about volunteer and clinical experiences, leadership roles, undergraduate research and publications, and a myriad of other résumé building blocks. They are afforded the opportunity to use their mastery of the English language to succinctly, yet effectively, answer why they are pursuing a career in medicine. They are even given an opportunity to appear in front of an admissions committee and, in a poised and professional manner, answer questions about ethics and motivations while coming off as amicable and competent. Isn't this sufficient?

I would argue that it is not. As a disclaimer, medical schools currently utilize the tools at their disposal and do their best to objectively judge candidates and select the best. The goal of these admissions committees is to find the candidates that will be successful, not to beleaguer applicants out of spite or malice. That being said, the system has some flaws, with the most tragic result being that superb and qualified candidates can fall through the cracks because of rather minor shortcomings in this process. Perhaps the greatest flaw in this process is

the transformation of a student candidate into an aggregate of numbers: GPA, MCAT score, number of credits taken, and number of volunteer hours collected. Unfortunately, when 40,000 applicants apply to an average of 15 schools each, admissions committees are forced to look at an aggregate of numbers.[4] The rationale behind candidate frustration with this aspect of the process is simple: a patient does not go see an aggregate of numbers; a patient goes to see a physician. Physicians are people who can feel emotion and empathize or can be cold and standoffish. Physicians are individuals who can converse with people from all different backgrounds and relate to each of their situations; or, they can be recalcitrant and far from personable. Clinicians are persons who discuss lab results, test values, and post-operative procedures and formulate a plan on how to proceed such that the patient's best interest is always in mind; they can also be persons that see little value in bedside manner and reassuring patients. My point is, how accurately can an aggregate of numbers gauge someone's personality, problem-solving skills, willingness to learn, and ability to work with and read people?

Sure, many steps in the application process attempt to illuminate these characteristics in a candidate. The essays allow for expression of ideals and morals that are not clarified elsewhere, the GPA gives some indication about the ability to handle copious amounts of scientific data, and the MCAT is an attempt to test aptitudes in basic science concepts. But in this time of grade inflation at all colleges and universities, does GPA paint an accurate picture? The MCAT tests retention from required courses, but does it accurately predict a student's ability to think critically? I would argue that critical thinking and application are the most important attributes of a candidate, as they estimate the ability to solve novel problems.

This invites a new question: have we as undergraduates been taught clinical application? One method of successfully accomplishing this is through clinical case studies. These involve applying basic science knowledge to solve unique and complex problems. Along with this application, students develop the ability to defend and explain their decisions. When proctored correctly, medical case studies turn into a mock differential diagnosis with a team of "specialists" discussing all possible causes for a particular condition. After generating a list of possibilities, the group or individual can systematically move down the list, describing tests or assays that should be performed to confirm or deny each condition. This type of approach involves some of the most evaluative and analytical types of critical thinking and requires the synthesis of concepts from the whole of the pre-med curriculum.

Students who possess the aforementioned skills may not be able to convey these attributes on the primary application. Someone who doesn't fall into the optimal range for GPA, the MCAT score, or a combination of both will not be offered an interview and possibly not even the secondary application. Interviews are only offered for the best applicants. Ah, and here we get back to the distinction of "best". Medical schools can only use the tools they are given to judge a particular applicant; that point is not being contested. GPA's can be a fairly accurate predictor of scholastic aptitude and it would be foolish not to factor in one's GPA in consideration for entrance into medical school. The MCAT, again, is the "great equalizer", the *single* part of all 40,000 candidates' applications that can be measured equally by Lady Justice and her scales. The effort made here by AAMC and AACOM, the two governing bodies of allopathic and osteopathic programs, respectively, is a good one, but it is not sufficient. They are limited, of course, due to the number of applicants.

The goal of the current admissions process is not strictly seeking students who will be good medical school students, they are looking for people who will be good physicians. I argue that this should involve searching for students with aptitudes that the MCAT cannot test: the ability to break complex medical jargon down into language that patients can understand and feel comfortable about, a bedside manner that is warm and reassuring, and critical thinking skills that lead to the best possible care. Students who score below or even in the lower tiers of a school's MCAT range may possess these aptitudes while other students who scored in the top percentiles may be severely lacking. A high score on the MCAT by no means guarantees a person who displays any of the aforementioned characteristics that we expect in physicians who care for ourselves and for our loved ones. The MCAT successfully measures some ability to perform well under stress, rote memorization of basic concepts in physics, biology, and chemistry, and some degree of critical thinking in the application of that knowledge to difficult questions asked on the exam.

One shortfall of the "great equalizer" is that it cannot test the skills that distinguish medical school graduates from great physicians. There are plenty of people who are intelligent enough to attend and do well in medical school. There are far fewer people that have the ability to face a patient and communicate on a level in which the patient understands exactly what is happening to them. The MCAT, although a great equalizer among candidates, cannot account for the social competencies and communication skills that compose the large gap between smart kids who can dictate the laws governing quantum physics and those students who can relate to the person who is the patient. The cases that physicians see each day are people; they are certainly more than an aggregate of signs and symptoms waiting to be treated. The essence of interaction, empathy, and the recognition of human dignity cannot be found in a scaled score. As such, the disqualification of a student based on his or her MCAT score would be a travesty.

So how do we solve the problem of admitting students who lack these skills yet have numbers on their application that suggest they might be the "best" candidates, while denying students who possess attributes we want in our physicians because they didn't score among the top tier candidates? This is not an easy question to answer. Interviewing all candidates would give committees a much clearer picture of candidates but is vastly implausible with the number of applicants for each school. Letters of recommendation should delineate these skills for a candidate, but these letters don't appear to carry the weight with a committee that they should. I suggest this: medical schools ultimately need an insider, someone who has observed and interacted with candidates in more than an academic capacity. They need someone who not only sees a student digest new information, but also identifies students with an aptitude for application. This is manifested best when this insider can observe the level of understanding a student has when that student teaches it to someone else. Seeing a student in a TA or GTA role or in a tutoring capacity can provide the insider with all the information they need about responsibility, owning their work, teamwork, being a leader, interacting with people, simplifying complex issues, breaking down concepts, and, perhaps most important, truly caring about others.

Unfortunately, systems like this are difficult to implement. Recently, however, a collaboration between Colorado State University (CSU) and Rocky Vista University College of Medicine (RVU) has begun which may serve as a model for future changes in this process. As part of this collaboration, a select few students are recommended for acceptance into the medical school after completion of a master's program at CSU.

This agreement provides the ability to assess a number of things: the degree to which students can accept responsibility, whether students are mature enough to say "I don't know" instead of guessing, the ability to work in a team setting with long hours and strict deadlines, and the ability to lead that team and bring members to a higher level of achievement. This collaboration allows CSU to recommend candidates they *know* are quality applicants because they have seen these applicants in numerous roles that distinguish them from their peers. Consequently, it ensures RVU that they are receiving the best possible, all-around candidates instead of potentially accepting students who look great on paper but lack crucial intangible skills that should typify a physician.

In short, collaborations like the CSU/RVU example allow medical schools to have an insider, someone who can pick out the students that would normally fall through the cracks yet would contribute an exceptional amount to that medical school, and someone who can rule out the candidates that would succeed in medical school but would flounder as physicians. Although it is not a system that can be changed overnight or even in a decade, any divergence from the path that we have been on for years could make a significant difference in the lives of candidates who would truly make the "best" physicians.

References

[1]Shelton, Gary. (2013) "Usain Bolt leaves no doubt he is the fastest man ever." *Tampa Bay Times*. <http://www.tampabay.com/sports/olympics> (02/25/2013)

[2](2011) "Average mathematics scores by 4th grade students, by education system 2011." Trends i n International Mathematics and Science Study. <http://nces.ed.gov> (02/22/2013)

[3](2011) "Average science scores by 8th grade students, by education system 2011." Trends in International Mathematics and Science Study. 2011. <http://nces.ed.gov> (02/22/2013)

[4](2012) " U.S. Medical School Applications and Matriculants by School, State of Legal Residence, and Sex, 2012." Association of American Medical Colleges. <https://www.aamc.org> (02/25/2013)

The Differentiation of Radial Glial Cell Line C6 in vitro

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Abstract:

Radial glia are cells found in the ventricular zone of the brain in the embryonic and neonatal neuroepithelia. These cells then differentiate into astrocytes, ependymal cells, and B-cells (sub-ventricular zone astrocytes) in the adult brain. Radial glia are hypothesized to have the capability to differentiate into oIPCs (intermediate progenitor cells that generate oligodendrocytes) and nIPCs (intermediate progenitor cells that generate neurons).[4] This research was an independent, experimental project to test these hypotheses. Using the radial glial cell line C6, cultures were grown to differentiate into astrocytes (as a control for cell potency) and oIPCs and nIPCs. This study showed that the C6 cell line does indeed have the potential for differentiation into oligodendrocytes and neurons in vitro, therefore lending evidence to their hypothetical role in the development of the central nervous system in vivo.

Introduction

Neuronal and glial cells were long thought to differentiate from different stem cell lines. Recent research, however, shows that these two cell types are much more closely related than previously thought.[4] Some glial cells, such as the sub-ventricular zone (SVZ) astrocytes, have shown the capacity to differentiate into neurons in the adult mammalian brain.[1] Radial glial cells (RG cells) give rise to these SVZ astrocytes, and it is hypothesized that RG cells are in fact pluripotent primary progenitor cells, meaning that they are capable of giving rise to a variety of neuronal and glial cell types. RG cells are derived from the neuroepithelium during development and are therefore found throughout the central nervous system (CNS) until birth. However, RG are thought to persist in the CNS through adulthood as well.[4] This work shows that radial glial cells have the capability to differentiate into astrocytes, oligodendrocytes, and neurons, and are therefore pluripotent cells. As these cells may be found throughout the central nervous system, exploiting their pluripotency could have powerful implications for brain and nerve repair. The capability to differentiate into almost any type of CNS cell could make repair to almost any CNS tissue possible.

Methods

Cell Culture

Cells were cultured in American Type Culture Collection (ATCC) complete growth medium, Dulbecco's Modified Eagle Medium (DMEM)/F12 (1:1) (ATTC), Fetal Bovine Serum (FBS) to a final concentration of 2.5%, horse serum to a final concentration of 15%, and Penicillin-Streptomycin to a final concentration of 1%. The cells were then incubated at 37.0°C in a 5% carbon dioxide (CO_2) incubator. Next, the C6 cells (ATTC CCL-107, radial glial cells isolated from the brain of a rat glial tumor induced by N-nitrosomethylurea) were passaged every 3-4 days at a 1:2 or 1:3 ratio. This was accomplished by removing and discarding the culture medium, adding 2.0 to 3.0 ml of Trypsin-EDTA solution to the flask, and incubating at 37.0°C for 5 -15 minutes. Following this treatment, 6.0 to 8.0 ml of complete growth medium was added and the cell suspension was aspirated by gently pipetting. Then, appropriate aliquots of the cell suspension were added to new culture vessels and incubated at 37° C. Stock cultures of the C6 cells were frozen in culture medium (95%) and Dimethyl Sulfoxide (DMSO) (5%) and stored at -150°C. All cells were taken through 13 passages before the first 24-well plate treatment. When cell cultures were split, the flask with higher viability was chosen for continued passaging. Viability was determined by a Nexcelcom

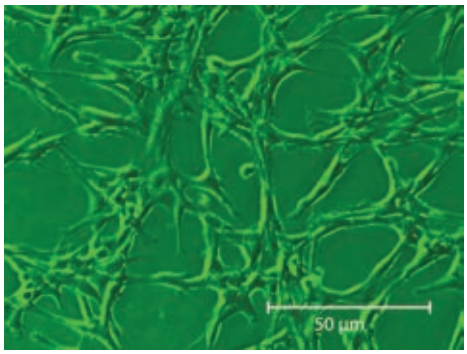

Figure 1: C6 cells cultured in a T-25 flask, observed on an inverted microscope and photographed. The elongated shape is typical of a fibroblast and the thin processes being characteristic of RG cells.

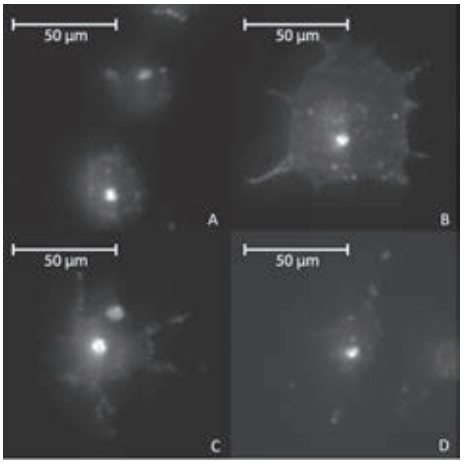

Figure 2: Calcein AM stain after 4 days of treatment. A) Cells cultured in DMEM/F12 (1:1) with 20ng/mL FGF-2 and 1% N2 for 2 days, then for an additional 2 days after withdrawal of FGF-2 for Oligodendrocyte differentiation. B) Cells cultured in DMEM:F12 (1:1) with 20ng/mL FGF-2, 50 ng/mL BMP-2, 50 ng/mL LIF, and 1%FCS for 4 days for Astrocyte differentiation. C) Cells cultured in DMEM/F12 (1:1) with 20ng/mL FGF-2, 1% N2 supplement, 1uM RA (300.4 ng/mL) and 5uM forskolin for Neuronal differentiation. D) Control cells cultured in normal culture media. Note the distinct morphological differences of A, B, and C when compared to D, the control. Each image is to scale respectively.

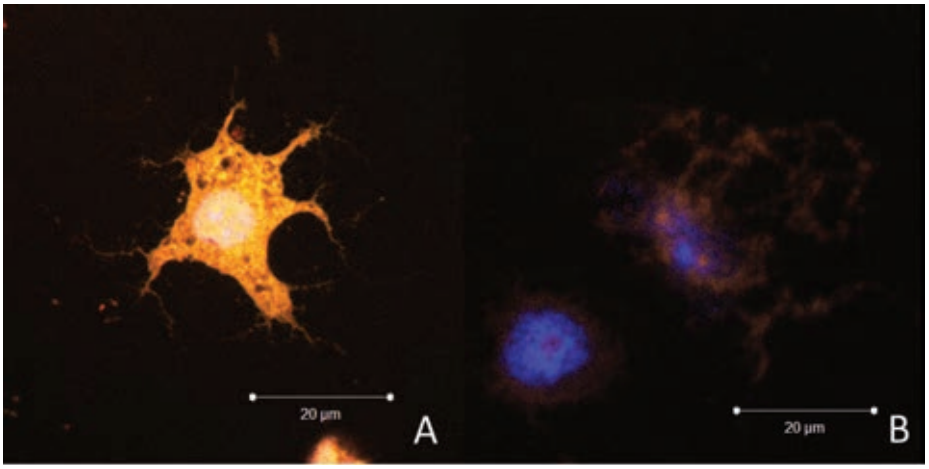

Figure 3: Immunofluorescent stain using phalloidin (red), anti-MAP2 (yellow), and DAPI (blue) visualized under a fluorescent confocal microscope. Cells cultured in DMEM/F12 (1:1) with 20ng/mL FGF-2 and 1% N2 for 2 days, then for an additional 2 days after withdrawal of FGF-2 for Oligodendrocyte differentiation. A) Characteristic morphology of an immature Oligodendrocyte. The cell appears yellow-orange due to high levels of MAP2. B) Characteristic morphology of a mature Oligodendrocyte. The cell body is reduced and is surrounded by many fibrous dendrites. The cell appears more reddish as MAP2 is no longer highly expressed after formation of the dendrites.

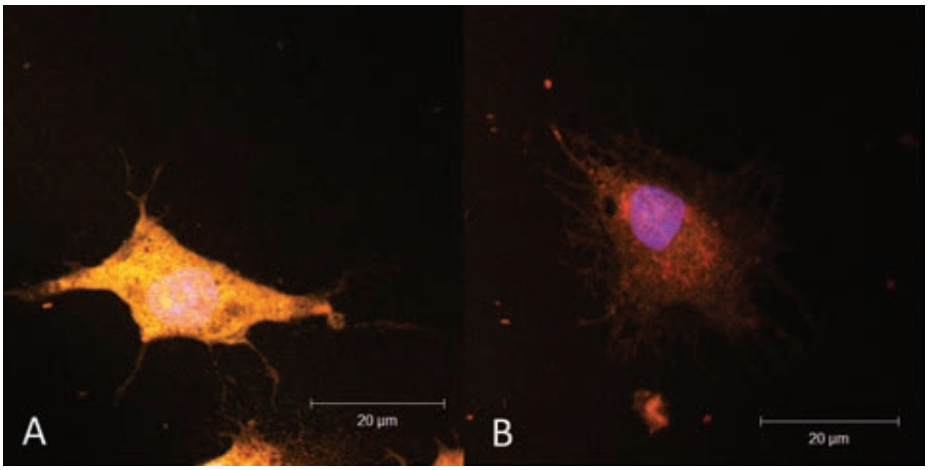

Figure 4: Immunofluorescent stain using phalloidin (red), anti-MAP2 (yellow), and DAPI (blue) visualized under a fluorescent confocal microscope. Cells cultured in DMEM:F12 (1:1) with 20ng/mL FGF-2, 50 ng/mL BMP-2, 50 ng/mL LIF, and 1%FCS for 4 days. A) Immature astrocytes expressing high levels of MAP2. B) Mature astrocytes with the characteristic bushy appearance.

Auto T4 cell counter. Once treatment began, the cells were observed each day using a stereomicroscope. Cells thawed from the tenth passage were used for the second 24-well plate treatments. These cells were treated in two 24-well plates. For astrocyte differentiation, cells were cultured in DMEM:F12 (1:1) with 20ng/mL Fibroblast Growth Factor 2 (FGF-2) (Sigma), 50 ng/mL Bone Morphogenic Protein 2 (BMP-2) (Sigma), 50 ng/mL Leukemia Inhibitory Factor (LIF) (Sigma), and 1% Fetal Calf Serum (FCS) for 4 days. For neuronal, differentiation cells were cultured in DMEM/F12 (1:1) with 20ng/mL FGF-2, 1% N2 supplement, 1µM Retinoic Acid (RA) (300.4 ng/mL) (Sigma), and 5µM forskolin (Sigma). For oligodendrocyte differentiation, cells were cultured in DMEM/F12 (1:1) with 20ng/mL FGF-2 and 1% N2 (R+D Systems) for 2 days, then for an additional 2 days after withdrawal of FGF-2.[5] The media was changed at the second day for all treatments.

Calcien AM staining

The media was removed with a micropipette and then the cells were washed with 150µL of Phosphate Buffered saline (PBS). Next, 200µL of 2µM Calcein AM solution was added to the cells, followed by incubation for 30 minutes at 37°C and 5% CO_2. The cells were rinsed three times with PBS, and then observed with a fluorescent microscope.

Immunofluorescence

Cells were cultured on poly-L-lysine coated coverslips in a 24-well plate, at approximately $1.74x10^4$ cells per well. After 4 days of treatment (described above), the cells were fixed with 4% paraformaldehyde. The cells were then permeabilized with 0.5% Triton X-100 in PBS for exactly 2 minutes. The primary antibody was rabbit α-MAP2 (1:500) (Sigma) in 1x block solution (20% goat serum, 2% BSA in PBS). The secondary antibody was Alexa Fluor 647 (Far Red) goat α-rabbit (1:1000) (Sigma) in 0.5x block solution. Phalloidin (1:1000) (Sigma) in 0.5x block solution was used to stain the actin cytoskeleton. Coverslips were mounted onto slides with Prolong Gold with DAPI. The cells were then observed with a Zeiss LSM 510 laser scanning confocal a microscope at 630x magnification. The slides were stored at 4°C.

Results

While in culture in the T-25 flasks, the cells remained highly confluent with typical fibroblast morphology (Fig. 1). Viability of the cells remained high throughout passaging, often well above 90% viability. When observed, the treated cells appeared to have lower confluency and viability than when cultured in the normal culture media. On the fourth day of treatment, the cells were stained with Calcein AM and observed. The living cells absorbed the Calcein AM stain and the morphology of the cells was easily observed (Fig 2). The cells treated to differentiate into oligodendrocytes displayed an oligodendrocyte like morphology from the original fibroblast. The oligodendrocyte like cells were rounded and considerably smaller, with many small processes along the periphery of the cell. The astrocytes displayed an enlarged cell body with several processes along the periphery of the cell. The neurons did not show a distinct change in the size of the cell body, but did however, possess morphologically distinct processes. The control cells retained the fibroblast morphology suggesting no differentiation took place. The Calcein stain confirmed the earlier observations of apparently lower viability, as many of the cells did not absorb the dye. DAPI was used to ensure that the Calcein was not auto fluorescing. The images in Figure 2 are not representative images and do not encompass all of the morphologies observed. In order to better observe the differences in morphology a Zeiss LSM 510

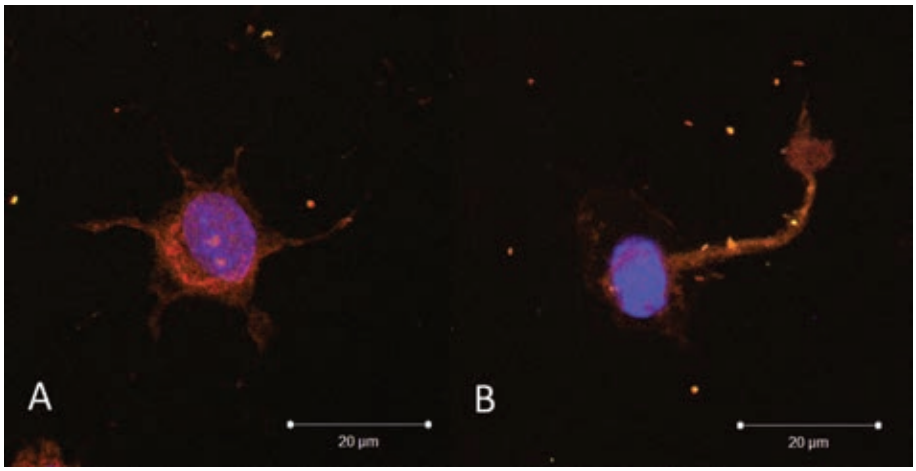

Figure 5: Immunofluorescent stain using phalloidin (red), anti-MAP2 (yellow), and DAPI (blue) visualized under a fluorescent confocal microscope. Cells cultured in DMEM/F12 (1:1) with 20ng/mL FGF-2, 1% N2 supplement, 1uM RA (300.4 ng/mL) and 5uM forskolin. A) Typical multipolar neuron morphology with dendrites extending radially from the cell. B) Bipolar neuron morphology; dendrites extending from the cell body near the nucleus and a long axon at one end.

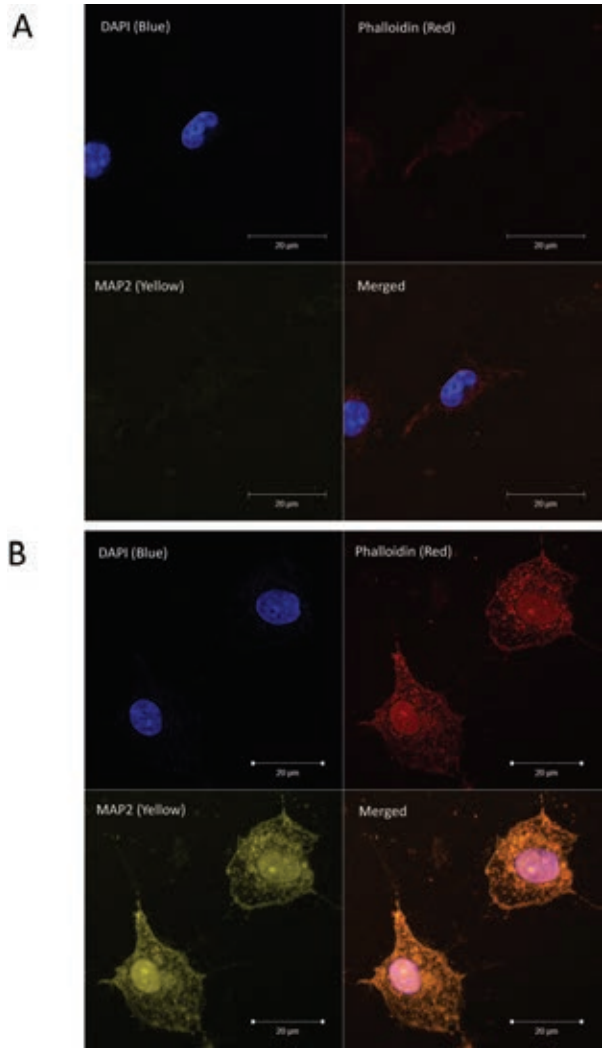

Figure 6: Immunofluorescent stain using phalloidin (red), anti-MAP2 (yellow), and DAPI (blue) visualized under a fluorescent confocal microscope.

A) Control cells cultured in DMEM/F12 (1:1) high glucose medium containing 2.5mM L-glutamine, 10% FBS, and 20ng/mL of FGF-2. Note the low levels of MAP2 (yellow) expressed and the retention of the fibroblast morphology.

B) Cells cultured in DMEM:F12 (1:1) with 20ng/mL FGF-2, 50 ng/mL BMP-2, 50 ng/mL LIF, and 1%FCS for 4 days. These cells are beginning to differentiate into astrocytes. Note the high levels of MAP2 (yellow) expressed and the distinct changes in morphology.

laser scanning confocal microscope at 630x magnification was used.

Under the confocal microscope, it was observed that the cells were in various stages of differentiation. Those in the midst of differentiation expressed high levels of Microtubule Associated Protein 2 (MAP2), as it is necessary in the building of microtubules, which are in turn necessary for the building of new dendrites. These cells appeared yellow-orange in color due to the superposition of the anti-MAP2 antibody (yellow) and the phalloidin (red) antibody (Fig 3 A). Those cells that displayed a mature morphology express lower levels of MAP2, thus appear redder in color (Fig 3 B). Like the oligodendrocytes in Figure 3, the astrocytes were observed in different phases of differentiation (Fig 4). The immature astrocytes also appeared yellow-orange in color due to high levels of MAP2 expression, with decreased expression in the mature cells.[7]

Unlike the oligodendrocytes and astrocytes, those cells treated for differentiation into neurons did not appear to be in different stages of differentiation. The neuronal cells were observed to have differing morphologies. Some neuron like cells displayed the morphology of a multipolar neuron, which is characterized by the axon and dendrites extending radially from the cell body (Fig 5A), whereas others displayed a more bipolar nerve morphology, characterized by the dendrites extending from the cell body near the nucleus with a long axon at the opposite end of the cell (Fig 5B).

While those cells that were treated for differentiation displayed distinctly different morphologies than the original fibroblast, and expressed high levels of MAP2, the control cells did not. Those cells that were grown on the poly-L-lysine coated coverslip in the normal culture media retained the fibroblast morphology. In addition, the levels of MAP2 were extremely low, especially when compared to an actively differentiating cell (Fig 6).

Discussion

The use of anti-MAP2 to detect the levels of MAP2 within the cells proved to be an effective way of testing for differentiation, and was actually more sensitive than expected. Those cells in the midst of differentiation expressed the highest levels of MAP2, as this is indicative of a cell actively building new dendrites.[8] Following differentiation the levels of MAP2 decreased, as no new dendrites were being made. This provided a method to estimate how mature

the cell type was based on morphology and MAP2 levels. The presence of immature oligodendrocytes and astrocytes suggests that longer treatments would be needed for complete maturation. The neurons however, appear to mature more rapidly as many of the cells had already matured by the end of treatment.

The lower viability observed during the Calcein staining would seem to suggest that the treatments may have been too harsh. However, the decreased viability was seen in all of the treated wells, including the control. Therefore, the decreased viability may have been due to another factor, perhaps leaving the cells exposed too long while changing media.

Despite a decrease in viability and many cells not achieving a mature morphology after treatment, this experiment suggests that radial glial cells are pluripotent cells capable of differentiating into not only astrocytes, but oligodendrocytes and neurons as well. Further experimentation will be needed in order to determine the appropriate lengths for treatments to ensure that the cells reach maturity. In addition to this, further studies with radial glial cells *in situ* and *in vivo* will be needed to determine the possibility, practicality, and applicability of the pluripotent nature of the radial glial cell in brain repair.

References

[1]Doetsch, Fionna, Isabelle Caille, Daniel A. Lim, Jose M. Garcia-Verdugo, and Aturo Alvarez-Buylla. "Subventricular Zone Astrocytes Are Neural Stem Cells in the Adult Mammalian Brain." *Cell* 97 (1999): 703-16.

[2]Gage, Fred H., Penelope W. Coates, Theo D. Palmer, H G. Kuhn, and Lisa J. Fisher. "Survival and differentiation of adult neuronal progenitor cells transplanted to the adult brain." *Neurobiology* 92 (1995): 11879-83.

[3]Hormigo, Adilia, Maria McCarthy, Jean M. Nothias, Koichi Hasegawa, and Wenchang Huang. "Radial Glial Cell Line C6-R Integrates Preferentially in Adult White Matter and Facilitates Migration of Coimplanted Neurons in Vivo." *Experimental Neurology* 168 (2001): 310-22.

[4]Kriegstein, Arnold, and Arturo Alvarez-Buylla. "The Glial Nature of Embryonic and Adult Stem Cells." *Annual Review of Neuroscience* 32 (2009): 149-84.

[5]Kuwabara, Tomoko, Jenny Hsieh, Kinichi Nakashima, Kazunari Taira, and Fred H. Gage. "A Small Modularity dsRNA Specifies the Fate of Adult Neural Stem Cells." *Cell* 116 (2004): 779-93.

[6]Kuwabara, Tomoko, Jenny Hsieh, Kinichi Nakashima, Masaki Warashina, and Kazunari Taira. "The NRSE smRNA specifies the fate of adult hippocampal neural stem cells." *Nucleic Acid Symposium Series* 49 (2005): 87-88.

[7]Wharton, S B., K K. Chan, and I R. Whittle. "Microtubule-associated protein 2 (MAP-2) is expressed in low and high grade diffuse astrocytomas." *Journal of Clinical Neuroscience* 9, no. 2 (2002): 165-169.

[8]Al-Bassam, Jawdat, Rachel S. Ozer, Daniel Safer, Shelley Halpain, and Ronald A. Milligan. "MAP2 and tau bind longitudinally along the outer ridges of microtubule protofilaments." *Journal of Cell Biology* 157, no. 7 (2002): 1187-1196.

The Glorification of Gluten-Free

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The last few years has seen an increase in dietary options available to people who have made the lifestyle decision to be gluten-free. This rise is due in part to the mainstream popularization of diets like *The Paleo Diet*, which advocate that many health problems are due to the modern human diet deviating from what humans have evolved to consume. According to this and other evolution-based diets, humans have not evolved to consume dairy, legumes, and especially grain products. Gluten is found in the majority of grains that are consumed in the standard Western diet. Furthermore, the sudden glorification of gluten-free diets can also be attributed to a growing body of scientific literature which has linked gluten consumption to more than a handful of common and debilitating autoimmune disorders.[1] In the scientific community, the precise mechanistic link between gluten consumption and autoimmune disorders is still being heavily investigated. At best, it can be said that there is an observable relationship between halting the consumption of grains and quantifiable changes in progression of certain autoimmune disorders. Meanwhile, in the mainstream media, gluten has been extensively vilified, and thus, people who do not have clinically diagnosed gluten sensitivity have made the decision to be gluten-free. In theory, having more people adopt the gluten-free lifestyle should be beneficial to everyone: those with clinically diagnosed gluten intolerances will have more gluten-free options, food manufacturers will be able to charge more for producing products verified to be free of gluten, and individuals without diagnosed gluten sensitivity can make decisions regarding their own health.

When it comes down to it, gluten is not a known toxin or a known poison to the human body. In fact, gluten is simply a storage protein found in wheat and many other commonly consumed cereal grains.[2] What makes gluten such a unique protein is that, in some individuals, consumption can trigger a sensitivity or intolerance. For the majority of people who consume a typical Western diet, this sensitivity is negligible. The often undiagnosed gluten sensitivity will present itself with mild feeling of being bloated, uncomfortable gas, or even a slight uneasiness in the stomach. For a select group of people, however, gluten sensitivity is a much more severe problem. This is true in the population of people who have been diagnosed with Crohn's or Celiac disease. These two clinical conditions are often incorrectly used interchangeably in the media, but they are two unique pathological conditions. In Celiac disease, consumption of gluten triggers an autoimmune reaction in the stomach. The individual's immune system begins attacking the villi. The villi are essentially the functional lining of the stomach and are absolutely imperative in the gastrointestinal chain for preliminary nutrient absorption. Over time, repeated damage to the villi impairs the ability to properly process essential nutrients. Thus, an individual with uncontrolled Celiac disease can show signs of malnourishment despite having a balanced diet.[3] In Crohn's disease it is unknown what exactly triggers the autoimmune reaction. Some research suggests that gluten is the cause of this immune system overreaction.[4] Additionally, in Crohn's the immune system attacks the intestines and not the stomach.[5] However, since both conditions are autoimmune disorders of the gastrointestinal system some experts recommend that both types of patients manage symptoms by cutting gluten from their diet.

Many researchers are starting to associate gluten sensitivity as a possible precursor to the chronic inflammation that is observed in the progression of many autoimmune disorders.[1] As this information makes its way into mainstream media, many people are making the leap in logic and are choosing to go gluten-free without any medical diagnosis. Similarly, individuals who subscribe to lifestyles such as *The Paleo Diet* or the *Specific Carbohydrate Diet* also go gluten-free without a medical diagnosis. The result of this is that more and more food manufacturers and restaurants are starting to label foods as gluten-free. While this would seemingly benefit those that made the transition to a gluten-free diet for personal reasons as well as those that have become gluten-free due to medical necessity, in reality this creates a problem for those individuals who do have an actual sensitivity. When restaurants cater to a large number of people who do not consume gluten for non-medical reasons, the employees may become relaxed about cross-contamination. Those individuals without true gluten sensitivity do not care if their gluten-free bread is cut on a surface that also touches bread-containing gluten. Similarly, I have witnessed employees at a major coffee chain spreading cream cheese from a communal container on both gluten-containing and gluten-free food. Breadcrumbs that end up on a knife can contain trace amounts of gluten, enough to trigger sensitivity in some patients. The popularization of gluten-free diets detracts from the necessity to prevent cross-contamination, as some employees see it as extra work without justification or reward. This puts those suffering from a medical condition at increased risk for gluten related outbreaks. Currently, the Food and Drug Administration (FDA) does not regulate what makes food gluten-free. Common sense would dictate that a food be considered gluten-free only if it is free from any minute traces of gluten. However, since it is not regulated, food manufacturers can label anything as gluten-free. It is really dependent on the integrity of a company to test their products to be free of gluten prior to advertising it as such. This creates a worry for those who are gluten-free for medical reasons since there is the fear that companies label their foods as gluten-free simply to appeal to those on gluten-free diets without insuring that there is no cross-contamination. Alarmingly, some companies have chosen to increase sales of products by explicitly marking them as gluten-free even though the product itself never contained gluten. For example, this can be seen in the supermarket when certain foods such as corn tortilla chips have the gluten-free label. While it is true that the chips are gluten-free, it is dishonest because it makes consumers seeking out gluten-free products pick that preferentially over comparable corn tortilla

chips that are not labeled as gluten-free but are inherently gluten-free. This can lead consumers to choose the product labeled as gluten-free to be safe. Products with the label are often more expensive. The popularization of gluten-free diets and the gluten-free lifestyle can be beneficial for everyone, but only if people understand the importance of keeping gluten-free products truly gluten-free.

Gluten-free products are appearing on store shelves, and it seems as if the gluten-free lifestyle is here to stay. The best way to address the negative consequences of gluten-free being mainstream is to regulate what can be labeled gluten-free. Currently, the FDA is reaching the end of a multiple year process to define what standard food must meet be to be labeled gluten-free. While that is an important step in the right direction, the solution is multifaceted. Individuals who choose to be gluten-free for reasons other than medical implications must begin to recognize the problems caused by cross-contamination. The preparation of gluten-free and conventional gluten products on the same surface must no longer be tolerated by customers, staff, and managers of restaurants. This is crucial in ensuring that gluten-free products in the service industry stay gluten-free until they reach the consumer. Anyone who favors gluten-free products over conventional food items has a responsibility to ensure that their food is indeed being handled properly, in order to prevent cross-contamination. Furthermore, the upcoming and long awaited regulation of the label gluten-free by the FDA will help ensure that those with true gluten sensitivity will reap only the positive benefits of the glorification of gluten-free.

References

[1]Petersen, V. (2012) "Is Gluten Intolerance the Cause of Autoimmune Disorders?" Health Now Medical Center <http://www.healthnowmedical.com> (03/06/2013)
[2]Freuman,T. (2012) "What is Gluten, Anyway?" US News Health. <http://www.health.usnews.com> (03/06/2013)
[3](2010) "Celiac Disease." PubMed Health. <http://www.ncbi.nlm.nih.gov> (03/06/2013)
[4](2013) "Crohn's Disease" PubMed Health. http://www.ncbi.nlm.nih.gov/pubmed/23477870 (03/06/2013)
[5](2012) "Crohn's Disease." PubMed Health. <http://www.ncbi.nlm.nih.gov> (03/06/2013)

Effect of capping ligand on serum protein adsorption and cell uptake of gold nanoparticles

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Abstract:

Gold (Au) nanoparticles (NP) exhibit plasmonic properties, which make them ideal for biosensing, imaging, and drug delivery. Due to the range of applications for Au NPs, it is necessary to evaluate their interactions in biologically relevant media. The objective of this study is to investigate protein adsorption and uptake trends for 60 nm Au NPs capped with citrate (CA) versus tannic acid (TA). To approach this objective, Au NPs capped in CA versus TA were dispersed in water or media supplemented with increasing amounts of serum protein (0-10%). The resulting dispersions were characterized using ultraviolet-visible spectroscopy, hyperspectral imaging, dynamic light scattering and laser Doppler electrophoresis. Significant agglomeration in media without serum and complete re-stabilization at 2% serum in media was observed for both CA or TA capped Au NPs, with no statistically significant change for increasing concentrations of serum (0-10%). NP characterization techniques indicated a statistically significant difference in size increase of about 21.4±1.8 nm and 27.2±1.8 nm for CA and TA capped NPs, respectively when dispersed in 10% serum. Cellular uptake into a lung epithelial cell-line (A549) was investigated qualitatively with darkfield microscopy and quantitatively via inductively coupled plasma-mass spectrometry. Uptake studies did not reveal a difference between the Au NPs capped with different ligands dispersed in 10% serum.

Introduction

Gold (Au) nanoparticles (NPs) exhibit plasmonic properties, which make them ideal for biosensing, imaging, and drug delivery.[1,2] Due to the range of applications for Au NPs, it is necessary to evaluate their interactions in biologically relevant media and with cells. Au NPs agglomerate in the presence of the salts found in cell culture media and are readily stabilized by the addition of serum proteins.[3] The agglomeration state for Au NPs has been shown to affect cell uptake, where the relative uptake of either single or agglomerated particles was unique for different cell types.[4]

It was previously shown that the capping agent can also play a role in cell uptake of 10 nm Au NPs.[3] Au NPs are often stabilized using electrostatically bound capping ligands, such as citric acid (CA) and tannic acid (TA). Additionally, many studies have demonstrated that regardless of size and capping agent, Au NPs adsorb serum proteins, which stabilize them in high conductivity media (i.e. cell culture media).[3,5] The adsorbed proteins serve as the primary interface for interaction with cells.[6,7,8,9]

In this study, the role of 2 different capping agents on protein adsorption for 60 nm Au NPs was systematically investigated for serum concentrations ranging from 0-10% in non-conductive (water) and conductive media (RPMI media). Cell uptake was then quantified for CA-Au NP and TA-Au NP dispersed in RPMI media containing 10 % serum.

Approach

Au NPs (60 nm) capped in CA or TA were used in this study. CA capped Au NPs (60 nm) were purchased from the National Institute of Standards and Technology (NIST) and TA capped Au NPs (60 nm) were purchased from nanoComposix. NPs were characterized using transmission electron microscopy (TEM) to determine size and morphology.

Au NPs were diluted to 10 µg/mL in either water or RPMI-1640 media (ATCC) supplemented with 1% penicillin/streptomycin and increasing concentrations of fetal bovine serum (0- 10%). Solutions were made by adding in order, serum, NPs, and water or media to prevent the aggregation of Au NPs in media without serum proteins. The resulting dispersions were characterized using ultraviolet-visible spectroscopy (UV-vis; Varian Cary 5000 UV-VIS-NIR Spectrophotometer), hyperspectral imaging (HSI; CytoViva, Inc.), dynamic light scattering (DLS) and laser Doppler

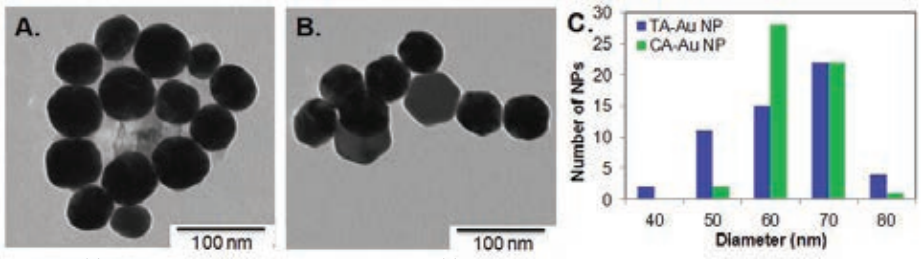

Figure 1: TEM data for 60 nm Au NPs. A. Image of TA capped Au NPs; B. Image of CA capped Au NPs; C. Histogram showing the size distribution for each NP.

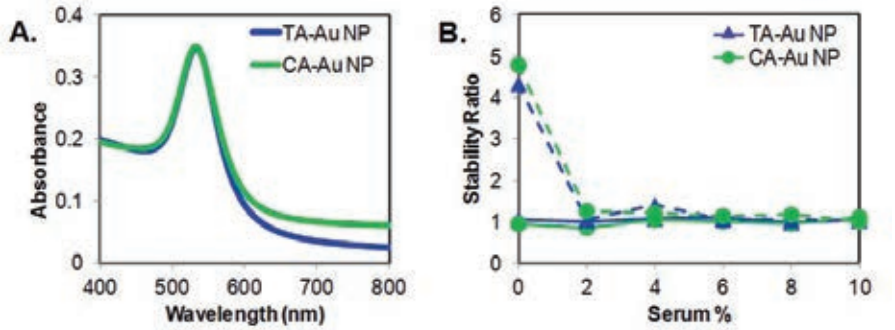

Figure 2: UV-vis data for 60 nm Au NPs. A. TA and CA capped Au NPs in water; B. Stability ratio for TA and CA capped Au NPs in water (solid lines) or media (dashed lines) with varying concentration of serum (0-10%).

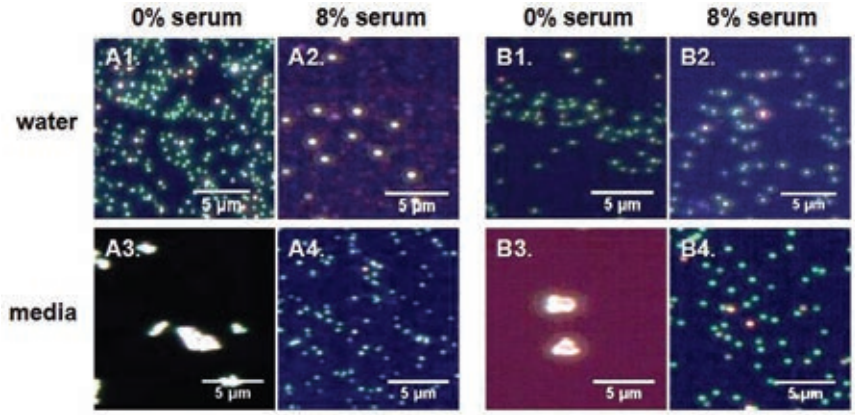

Figure 3: Darkfield images of Au NPs functionalized with tannic acid (A1-4) or citrate (B1-4) after dispersion in (1) water, (2) water + 8% serum, (3) media, or (4) media plus 8% serum.

electrophoresis (Malvern Zetasizer nano ZS). Due to the plasmonic properties of Au NPs, many characterization techniques can be used to investigate their interaction with serum proteins. Using more than one characterization technique is useful to validate and clarify results.

In order to compare the curves acquired by UV-vis, a stability ratio was calculated by dividing the area under the curve by the magnitude of the peak wavelength. The stability ratio was normalized to 1.0 based on the average stability ratio value for each sample. A drawback to the UV-vis technique is that the final curve is an average of the entire solution. This can be complimented by analyzing individual particles and clusters using HSI, which can measure the scattering spectrum from individual pixels in an image of NPs. Theoretical data for surface plasmon resonance was calculated based on an in-house program written in Matlab based on Mie theory.[10]

Optical microscopy is a useful tool in characterizing cell morphology and NP interactions with cells *in vitro*. Ultra resolution darkfield microscopy can be used to illuminate dense structures, such as cell membranes, organelles, and NPs without fluorescent tagging, but the identity of the dense structure cannot be confirmed using this technique alone.[11] Hyperspectral Imaging (HSI) is a spectroscopy technique that can be employed with both fluorescence and darkfield microscopy to confirm the 2-dimensional location of inorganic optically active NPs based on their spectral signatures. When HSI is employed in conjunction with ultra resolution darkfield microscopy, an image resolution of less than 150 nm and detection of less than 30 nm can be achieved.[11,12,13]

Hydrodynamic diameter and zeta potential data are useful for assessing changes in properties of NPs in different media. However, dynamic light scattering is limited to monodisperse spherical particles. Also, this technique is optimal within a specific concentration range and is not applicable for sub-10 nm size particles.

A human lung epithelial cell-line (A549) was used for uptake studies. Cells were cultured in RPMI-1640 media (ATCC) supplemented with 1% penicillin/streptomycin and 10% heat inactivated fetal bovine serum. Cellular interaction was studied qualitative using hyperspectral imaging, while quantitative Au NP uptake was analyzed using total metal analysis by inductively coupled Plasmon mass spectrometry (ICP-MS).

Results and Discussion

Au NPs were characterized using TEM (Figure 1). The Au NPs exhibited a mostly spherical morphology, with an average diameter of 58.1 ± 9.5 nm and 59.2 ± 6.5 nm for TA and CA, respectively, corroborating the values provided by the manufacturers. The average diameter was measured as described in materials and methods and a histogram for the size distribution is shown in figure 1C.

Au NP dispersions were characterized spectrally using UV-vis (Figure 2). The spectra shows slight variation in intensity for samples diluted to equal concentration (Figure 2A), but this difference is not expected to affect the results for stability ratios or dynamic light scattering. The peak absorption wavelength was 534 and 533 nm for TA- and CA-Au NPs, respectively, which correlates with the theoretical value for uncoated 60 nm Au NPs in water (536 nm).[14] The reason for the slight discrepancy is unclear, but may be related to the local refractive index variation caused by the presence of citric acid and tannic acid in solution or the slight size deviations observed by TEM.

The stability ratio is an analysis of the peak intensity and curve shape from the UV-vis data for TA- and CA- Au NPs in water or media with increasing concentration of serum protein. The results show that the samples exhibit reduced stability in exposure media without serum and that the samples are stabilized after the addition of 2% serum, remaining stable for increasing concentrations of serum in media and water (Figure 2B). There is no statistically significant difference for the stability ratio between samples from 2-10% FBS in either particle type.

Darkfield images are displayed in Figure 3. TA-Au NPs can be seen in Figure 3A1-A4, while CA-Au NPs are shown in Figure 3B1-B4. For each capping ligand, the trends remain consistent with results observed via UV-vis. When dispersed in water without serum or media supplemented with 8% serum, Au NPs appear to be green and are well-dispersed on the slide (Figure 3A1, 3A4, 3B1, 3B4). For Au NPs in water supplemented with 8% serum, TA-Au NPs appear yellow, and CA-Au NPs appear green (Figure 3A2

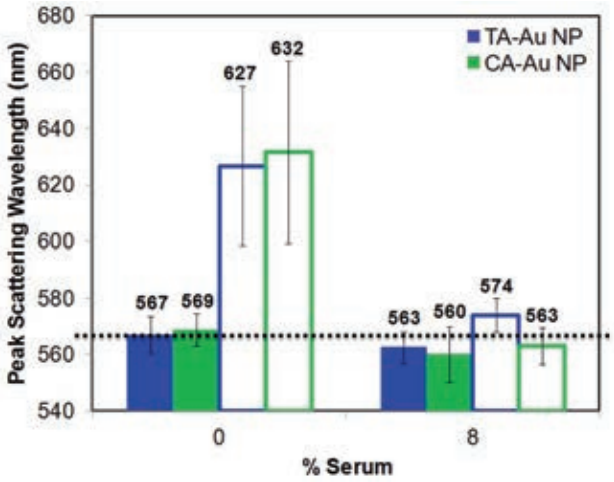

Figure 4: Peak scattering wavelength for TA- and CA- NPs in water (solid fill) and media (white fill) for 0% and 8% serum. Dotted line indicates theoretical plasmon resonance peak for 60 nm Au NPs with a local refractive index of glass or immersion oil (1.515).

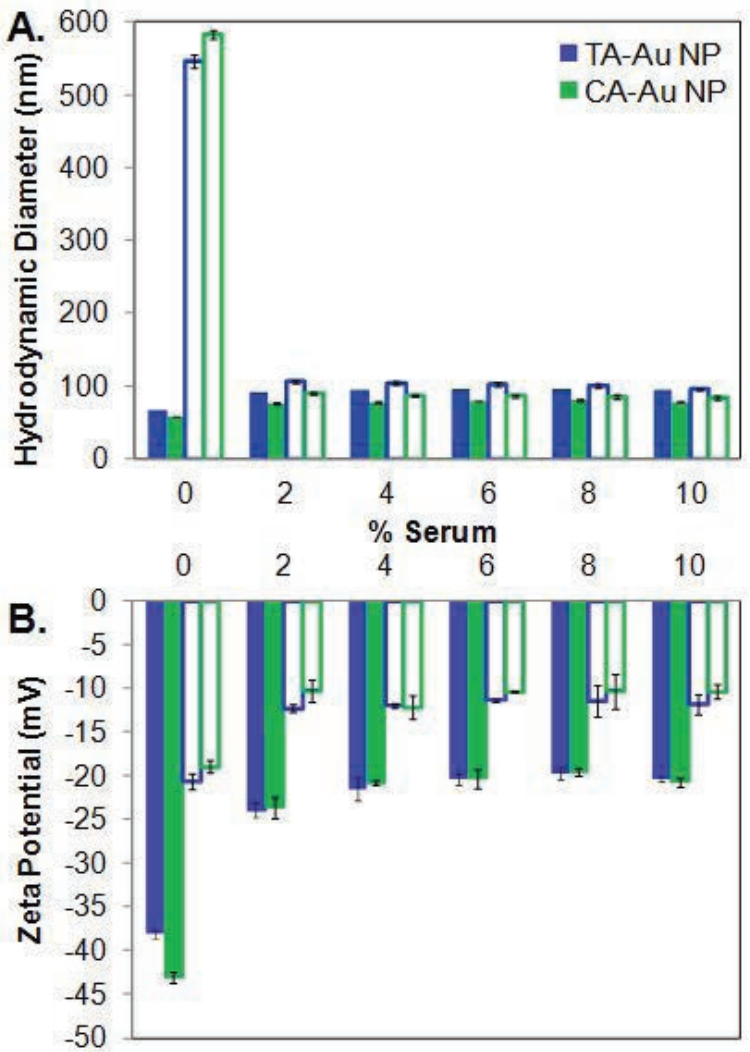

Figure 5: Hydrodynamic diameter (A) and zeta potential data (B) for increasing serum concentration in water (solid fill) and media (white fill).

and 3B2). The yellow appearance of the TA-NPs in water supplemented with serum may be caused by experimental error, as this indicates the formation of agglomerates, which is not supported by the UV-vis or HSI data. The significant decrease in stability observed via UV-vis is supported by visual observation using darkfield microscopy for both TA- and CA-Au NPs (Figure 3A3 and 3B3).

The scattering wavelengths collected from the darkfield images shown in Figure 3 are expressed in the bar graph in Figure 4. When dispersed in water without serum, the NPs scatter light with a peak wavelength of 567 and 569 nm for TA- and CA-Au NPs, respectively. This correlates well with theoretical data for the plasmon resonance peak of 60 nm Au NPs with a local refractive index of 1.515 (silica glass slide; immersion oil), which is 567 nm. The peak wavelengths are 563 and 574 for TA-Au NPs, and 560 and 563 for CA-Au NPs dispersed in serum supplemented water and media, respectively. These values are also close to the theoretical estimations. Wavelength values for the NPs dispersed in media correlate with the agglomeration observed in darkfield images. The peak wavelength for Au matches closely to the theoretical value, which is 545 nm in water.[10] The shift of about 10 nm is due the higher refractive index of glass and microscope oil, which must be accounted for when using HSI.[15,16]

Hydrodynamic diameter (A) and zeta potential data (B) for increasing serum concentration in water (solid fill) and media (white fill) are shown in Figure 5. DLS characterization data indicate a statistically significant difference in size increase of about 27.2±1.4 nm and 21.4±1.8 for TA- and CA- Au NPs, respectively when dispersed in serum. When dispersed in water, the difference in size increase is not statistically significant for any serum concentration (2-10%). Significant agglomeration in media without serum and complete re-stabilizatxion at 2% serum in media was observed for both NPs.

The zeta potential for TA-Au NP is about -38 mV versus -43.1 mV for CA-Au NP (Figure 5B). This is likely due to innate differences in the capping ligand chemistry. Once 2% serum is added, the value for zeta potential increases by 14.1 mV to -24.0 mV for TA-Au NPs. The value for CA-Au NPs increases by 19.4 mV for a zeta potential of -23.7 mV. The change in zeta potential with the addition of serum indicates protein adsorption; the similarity between the final values indicates the outer layer of proteins adsorbed to the Au NPs is similar regardless

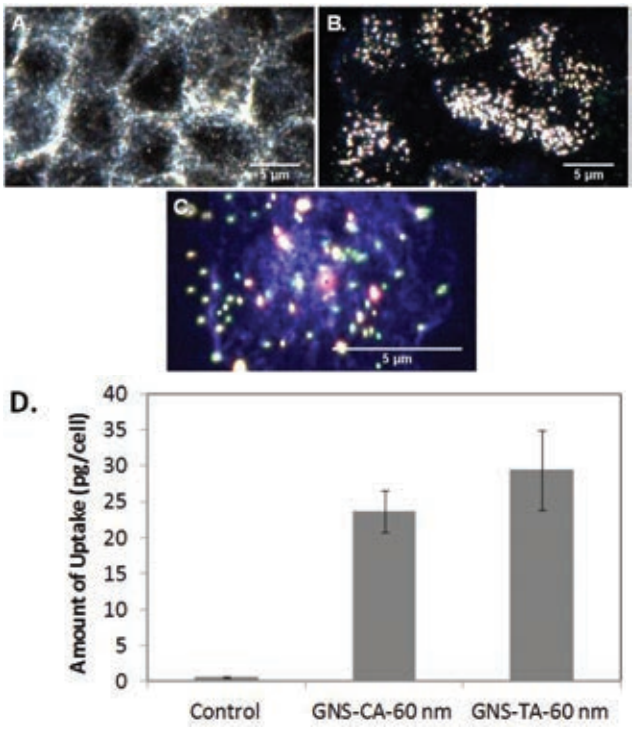

Figure 6: Interaction of Au NPs with A549 cells. A-C. Darkfield images; A. Control; B. Cells exposed to TA-Au NPs; C. Close-up image of cell exposed to CA-Au NPs; D. ICP-MS results.

of capping ligand. For Au NPs with both capping ligands, the change from 2% serum is statistically significant from 6, 8, and 10% serum conditions. The final value for these conditions is -20.2 mV for both TA- and CA-Au NPs, further indicating the similarity in protein adsorption trends for the different capping ligands.

The zeta potential data for conditions in media indicate a decrease in value for all samples compared to the 0% serum in water condition. The value for zeta potential in media with 0% serum is -20.7 and 19.0 mV for TA- and CA- Au NPs, respectively. This increase is likely related to the adsorption of Na+ to carboxylic acid groups on citric acid and tannic acid.4 After addition of serum, the value further increases to -11.8 mV and -10.7 mV, respectively, with no significant difference for the 2-10% serum concentration in either sample. This indicates that proteins may adsorb onto the NPs in a unique manner in media versus water.

NP uptake was investigated in cells using darkfield microscopy (Figure 6). The images show strong association of NPs will cells, and when compared to darkfield images of NPs in Figure 3, demonstrates the presence of primary particles and agglomerates. ICP-MS was used to quantify the particle uptake in the A549 cell-line. Results show that there is not statistically significant difference in cell uptake for CA- or TA-Au NPs (Figure 6D). This result is interesting because it has been previously shown that the capping agent can affect cell uptake for 10 nm Au NPs.[3]

Conclusion

The results of this work indicate that ≤ 2% serum is needed for stabilizing Au NPs in cell culture media. The adsorbed protein layer does not appear to change as the concentration of serum is increased; however, the composition of the adsorbed proteins is unknown. Based on preliminary research, questions arise, including: what concentration of serum will not stabilize the particles and does penicillin/streptomycin affect the stability. In conclusion, the capping ligand does not seem to strongly influence the adsorption of proteins or cell uptake of Au NPs.

Acknowledgements

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References

[1]Hao, R.Z., Song, H.B., Zuo, G.M., Yang, R.F., Wei, H.P, Wang, D.B., Cui, Z.Q., Zhang, Z., Cheng, Z.X., and Zhang, X.E. (2011) "DNA probe functionalized QCM biosensor based on gold nanoparticle amplification for bacillus anthracis detection." *Biosensors and Bioelectronics.* 26. Pg 3398-3404.

[2]Brown, S.D., Nativo, P., Smith, J.A., Stirling, D., Edwards, P.R., Venugopal, B., Flint, D.J., Plumb, J.A., Graham, D., and Wheate, N.J. (2010) "Gold nanoparticles for the improved anticancer drug delivery of the active component of oxaliplatin." *Journal of the American Chemical Society.* 132. Pg 4678-4684.

[3]Mukhopadhyay, A., Grabinski, C., Afrooz, A.N., Saleh, N.B., and Hussain, S. (2012) "Effect of gold nanosphere surface chemistry on protein adsorption and cell uptake *in vitro*." *Applied Biochemistry and Biotechnology,* accepted 29 March 2012.

[4]Albanese, A. and Chan, W.C.W. (2011) "Effect of gold nanoparticle agglomeration on cell uptake and toxicity." *ACS Nano.* 5. Pg 5478-5489.

[5]Murdock, R.C., Braydich-Stolle, L., Schrand, A.M., Schlager, J.J., and Hussain, S.M. (2008) Characterization of nanomaterial dispersion in solution prior to *in vitro* exposure using dynamic light scattering technique. *Toxicological Sciences.* 101. Pg 239-253.

[6]Lynch I., Cedervall T., Lundqvist M., Cabaleiro-Lago C., Linse S., Dawson K.A. The nanoparticle-protein complex as a biological entity; a complex fluids and surface science challenge for the 21st century. Advances in Colloid and Interface Science 2007, 134-135, Pg 167-174.

[7]Nel A.E., Madler L., Velegol D., Xia T., Hoek E.M.V., Somasundaran P., Klaessig F., Castranova V., Thompson M. Understanding biophysicochemical interactions at the nano-bio interface. Nature Materials 2009, 8, Pg 543-557.

[8]Alkilany A.M. and Murphy C.J. J. Toxicity and cellular uptake of gold nanoparticles: What we have learned so far? Journal of Nanoparticle Research 2010, 12, Pg 2313-2333.

[9]Ehrenberg M.S., Friedman A.E., Finkelstein J.N., Oberdörster G., McGrath J.L. The influence of protein adsorption on nanoparticle association with cultured endothelial cells. Biomaterials, 2009, 30, Pg 603-610.

[10]Yguerabide, J..; Yguerabide, E.E. Light scattering submicroscopic particles as highly fluorescent analogs and their use as tracer labels in clinical and biological applications: I. Theory. Analytical Biochemistry 1998, 262, Pg 137–156.

[11]Skebo J. E., Grabinski C.M., Schrand A.M., Schlager J.J., Hussain S.M. Assessment of metal nanoparticle agglomeration, uptake, and interaction using high-illuminating system. International Journal of Toxicology 2007, 26, Pg 135-141.

[12]Vodyanoy, V. High resolution light microscopy of live cells. Microscopy Today 2005, 17, Pg 26-28.

[13]Foster, B. Focus on microscopy: A technique for imaging live cell interactions and mechanisms. American Laboratory 2004, 11, Pg 21–27.

[14]Haiss W., Thanh N.T.K., Aveyard J., Fernig D.G. Determination of size and concentration of gold nanoparticles from UV-Vis spectra. Analytical Chemistry 2007, 79, Pg 4215-4221.

[15]White B., Strawbridge A., Grabinski C.M., Hussain S.M. Hyperspectral imaging (HSI) to evaluate the interaction of optically active nanoparticles in biological media and cells. Bios, accepted 2012 March 15.

[16]Mock, J.J., Smith, D.R., Schultz, S. Local refractive index dependence of plasmon resonance spectra from individual nanoparticles. Nano Letters 2003, 3, Pg 485-491.

Emergence of structures and forms in complex adaptive systems in nature

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Abstract:

Structure is an emergent property. The law of divergence (Second Law of Thermodynamics) proposes the increase of disorder with every spontaneous natural process. However, systems existing in nature constantly tend toward a state of increasing order. The self-organizing property present within these systems makes them robust and reliable to sustain changes in their surroundings. These systems inherently possess two intrinsic mechanisms. The first is a control mechanism that drives these systems away from a state of physical equilibrium or dead state, and the second is a feedback mechanism that enables these systems to adapt to the ever-changing environment through self-organization. The system elements organize themselves through various cycles of evolution by applying work on the system constraints in order to minimize them in the least possible time. Through this paper we investigate the cause of existence of an exergy gradient between the systems found in nature and their surrounding media. We relate the exergy flow current and constraint minimization to understand the existence of diverse forms and emergent complexity in nature, and we argue the existence of microscopic heat engines, which, by operating between two potentials, minimize physical constraints and optimize global exergy flow current in open adaptive complex dynamical systems, thereby giving rise to beautiful structural forms and varied species.

Keywords: accelerate, action, asymmetry, complex adaptive systems, complexity, constraint, minimization, emergence, entropy, exergy flow, feedback loop, fractal, microscopic heat-engines, organization, Principle of Least Action, self-organization, symmetry in nature.

Introduction

Nature has inspired man time and time again to design structures, imagine processes, and propound theories. Natural systems are beautiful and complex in construction, exhibiting enormous varieties of shapes and structures. Geometric perfection is rarely observed in natural systems. It is almost impossible to find perfect symmetry in animate systems (systems which are continuously self-organizing and intrinsically adaptive with the externally surrounding environment) in nature. There is a gate in Japan, Neiko, which is sometimes called by the Japanese the most beautiful gate in all of Japan. The gate is very elaborate, with many gables, beautiful carvings, columns, and dragon headed princes carved into the pillars. But when one looks closely, they see that in the elaborate and complex design along one of the pillars one of the small design elements is carved upside down; otherwise, it is completely symmetrical. The error was purposely put so that the gods would not be jealous of man's perfection.[1]A question arises, why is nature nearly symmetrical? What eludes that perfect geometry with sharp curves and faultless rounded circles that we spend time learning at schools?

Natural structures have living and evolving geometries that continuously optimize their struggle for better performance through progressive development.[2] Near symmetry in nature is a topic of thorough discussion at various interdisciplinary levels. It would require physicists, chemists, mathematicians, biologists, complexity theorists, astrobiologists, and engineers, all focused to develop a common world view, to decipher nature's enduring mysteries. Architecture in nature has been extensively investigated by the Constructal Law, widely regarded as the Fourth Law of Thermodynamics.[3] Natural structures evolve at various levels of hierarchy and become increasingly complex with time. Investigating such systems with continually optimizing geometries (architectures) becomes significantly difficult with passage of time. Also, with growth of complexity, systems become greatly organized at various hierarchical levels, namely physical, chemical, biological, societal, and technological.[4, 5, 6, 7, 8, 9, 10] However, in accordance to the Second Law of Thermodynamics, systems should come into a state of equilibrium with the surrounding by continuously dispersing energy. We need to develop certain fundamental laws and governing principles to identify, measure, engineer, and re-engineer such systems for the development of society to ensure our sustainable existence.[10, 11]

Prior to proposing any law or theory to investigate nature or natural processes, we must look into the most fundamental principles on which lies the foundation of all physical laws (i.e. the Principle of Least Action). The Principle of Least Action is an inherent law of nature which states that every spontaneous process tends to follow the path which will take least time to complete. Through this paper, we intend to present an idea on how the elements constituting a complex system obey the Principle of Least Action and hence, minimize the constraints by performing work on them. This is done through grouping and adding to the global exergy current flowing through the system, giving rise to various architectural structures and forms in nature. Exergy of a system is defined as the maximum possible work that it can perform. Mathematically, exergy is equal to Carnot efficiency times the heat contained by a system. In this paper, we present some analogies between the classical formulations of the motion of system elements, incorporating variational principles and thermo-dynamical aspects

of work, exergy and entropy. We believe every scientific theory must be broad in scope, present a constructive common worldview, be able to address a wide range of phenomena, and be able to sustain the tests of time like the laws of thermodynamics and quantum mechanics that have successfully explained countless natural phenomena. The long-range implications of our ideas have been presented in the discussion section.[7, 11, 12, 13, 14, 15, 16]

Methodology

A complex system is a system composed of many interacting elements, often called agents, which display collective behaviour that does not follow the behaviours of the individual parts.[17] The collective behaviour of the constituting elements is an emergent property. The word 'complex' has Latin origin, complexus, meaning consisting of many different and connected parts or not easy to understand. Nature inevitably consists of complex systems that are continuously evolving and optimizing themselves through various cycles of evolution to attain greater degrees of order; they recursively optimize their performance and get organized over time. Emergence is hard to quantify because, in a multi-element open system, there are infinite parameters controlling any emergent property of that system. Any change may cause that property to either totally disappear or appear altogether in a new form. Through this paper, we intend to present a new form of the Principle of Least Action to develop the idea as to how complexity evolves in multi-element systems, move into the domain of Constructal Theory, and eventually relate the two to describe the coherent interdependency between them.

Principle of Least Action: a multi-agent approach

The Principle of Least Action has emerged as the foundation for almost all physical laws, particularly those describing natural processes. There is not a broader and more fundamental principle in science than this. The Principle of Least Action has been refined time and again to describe a wide range of natural phenomena, be it the Gauss Principle of Least Constraint, Hertz's principle of Least Curvature, or the path integral formalism for quantum mechanics by Feynman. The conventional Least Action Principle is highly deterministic in nature and takes into account the variation in trajectory of a single system element between two pre-determined fixed points or states in space.[18] The cause of variation in trajectory of an element due to its interaction with other elements within the system needs to be taken into consideration. The rational choices of the system elements or agents are to pursue the shortest possible path in order to organize in the least possible time span, which drives the system towards greater entropy generation and irreversibility.[13, 19] In a networked complex system, each element will compute all possible paths from one node to another and thus will render the system towards a state of uncertainty.

In a one-dimensional state space, the equation of trajectory of a system element can be written as[20]:

$$(x(t))_{actual} = (x(t))_{shortest} + \varepsilon(t) \quad (1)$$

The above equation signifies that the actual path of a system element is greater than the shortest path by an amount 'ε(t)' termed as the 'variation parameter'. When a system element is free from mutual interactions, fields, and forces then, its actual path will always coincide with its shortest path, rendering the variation parameter to zero. For a two-element system, the elements in a system are labelled by α and β. So, the new trajectories of the two system elements due to mutual interaction are expressed as, for system element α:

$$x_{\alpha\beta}(t) = x_{\alpha\alpha}(t) + \varepsilon_{\alpha\beta}(t) \quad (2)$$

In eqn. (2), the left side represents the actual trajectory of the system element α due to its interaction with β.The right-hand side of the equation consists of two parts, $x_{\alpha\alpha}(t)$, the trajectory of element α in absence of any other interacting element which must inherently be its shortest path and the variation parameter, $\varepsilon_{\alpha\beta}(t)$, due to mutual interaction between the elements. Similarly, the trajectory of element β can be expressed similarly as:

$$x_{\alpha\beta}(t) = x_{\beta\beta}(t) + \varepsilon_{\alpha\beta}(t) \quad (3)$$

The variation parameter employed here is different from the one generally used in the analysis of calculus of variation to evaluate the shortest path. In open systems there is an incessant in-flux and out-flux of mass, energy, and information so the final state of such a system is often indeterminate. The variation parameter employed in eqn. (1, 2 and 3) has to be, thus, weakly constrained. A natural question arises here as to how this parameter can establish the growth of complexity in a system with time.

Exponential growth of complexity: an empirical relationship

A system can also be defined as a connected network. Systems found in nature are structurally complex. Complexity increases not with the amount of connections between the nodes that are present in a system but due to the numerous combinations of possible connections. In a system open to surrounding environment, the state of least action behaves as an attractor.[6, 7, 8, 9] The system elements progressively optimize their trajectories to achieve the least action state but ultimately fail to achieve that stationary (least action) state. The pursuit of the system elements to reach the stationary state causes the action of the system as a whole to increase and gradually diverges the system away from equilibrium, but at the same time, mutual interactions between the system elements induce internal irreversibility within the system that make the process of self-organization irreversible, causing dissipation of free energy and information from the system and the entropy to rise.[9, 21] The interaction or variation parameter thus plays a crucial role in complexation of a system.

According to the Principle of Least Action, the variation of the path is zero for any natural process occurring between two points of time, t_1 and t_2. Nature acts in the simplest way, in the shortest possible time.

Thus, the action integral is given by:

$$I = \int_{t_1}^{t_2} L dt = \int_{t_1}^{t_2} (T - V) dt \quad (4)$$

Where *L* is the Lagrangian, *T* and *V* are the kinetic and the potential energies of the system (respectively) and *L=T-V*. For the motion of the system between time t_1 and t_2, the Lagrangian, *L*, has a stationary value for the correct path of motion. This can be summarized as the Hamilton's Principle.[18] Rewriting eqn. (4) in multi-agent notation:

$$I_{\alpha\alpha} = \int_{t_1}^{t_2} L_{\alpha\alpha} dt = \int_{t_1}^{t_2} (T_{\alpha\alpha} - V_{\alpha\alpha}) dt \quad (5)$$

$I_{\alpha\alpha}$ represents the action of element α in absence of any other element. Eqn. (5) on solving will give the shortest possible path between two points in state space at times t_1 and t_2. In presence of a second element, β eqn. (5) gets modified into:

$$I_{\alpha\beta} = \int_{t_1}^{t_2} L_{\alpha\beta} dt = \int_{t_1}^{t_2} (T_{\alpha\beta} - V_{\alpha\beta}) dt \quad (6)$$

$I_{\alpha\beta}$ is the action of element α in presence of other interacting system elements. From eqn. (2, 3) we can observe that the trajectory obtained by solving eqn. (6) is greater than that obtained by solving eqn. (5) by an amount $\varepsilon_{\alpha\beta}$. Hence, $I_{\alpha\beta}$ is greater than $I_{\alpha\alpha}$. Neglecting the existence of any field, the potential energy term vanishes. So, we are left with:

$$(I_{\alpha\beta} - I_{\alpha\alpha}) = \int_{t_1}^{t_2} \frac{m_\alpha}{2}\left((\dot{x}_{\alpha\beta}{}^2) - (\dot{x}_{\alpha\alpha}{}^2)\right) dt > 0$$

$$(I_{\alpha\beta} - I_{\alpha\alpha}) = \int_{t_1}^{t_2} \frac{m_\alpha}{2}\left((\dot{x}_{\alpha\beta}{}^2) - (\dot{x}_{\alpha\alpha}{}^2)\right) dt =$$

$$\int_{t_1}^{t_2} \frac{m_\alpha}{2}\left((\dot{x}_{\alpha\alpha}{}^2 + 2\dot{\epsilon}_{\alpha\beta}\dot{x}_{\alpha\alpha} + \dot{\epsilon}_{\alpha\beta}{}^2) - (\dot{x}_{\alpha\alpha}{}^2)\right) dt > 0 \quad (7)$$

$$\int_{t_1}^{t_2} \dot{\varepsilon}_{\alpha\beta} dt > 0 \quad (8)$$

Eqn. (8) states that the integration of the time derivative of the variation parameter between times t_1 and t_2 is always positive. As it was discussed earlier, the interaction parameter induces irreversibility in a process due to mutual interactions and hence, generates entropy. Thus, the left side of the eqn. (8) represents the mechanical analogy of the entropy principle. For a system undergoing a process 1 →2, with entropy transfer across the system boundary due to heat transaction, the entropy generation $\dot{S}_{gen}$ must be greater than zero.[22]

$$S_2 - S_1 - \int_1^2 \frac{\delta Q}{T} = \dot{S}_{gen}, \text{ and } \dot{S}_{gen} > 0 \quad (9)$$

From the above equation it can be seen that the rate of change in entropy is a monotonically increasing function. Further, if we compare eqn. (8) and (9) we can find an analogy between them. Both the rate of change of the variation parameter and entropy are related. With increase in interactions due to multiple elements in the system the loss in information about each element becomes increasingly significant and contributes to overall complexity growth. Thus, the growth of complexity with time is profound and has been found to follow an exponential distribution.[7, 23, 24] The variation parameter controls the rational choices of the system elements to pursue specific trajectories to optimize their action.[12] Optimizing this parameter through cycles of evolution is the process of self-organization and complexity growth. We therefore present an empirical (although incomplete) expression for this parameter here.

$$\varepsilon_{\alpha\beta}(t) = \{\phi(x_{\alpha\beta}, \dot{x}_{\alpha\beta})\} x_0 e^{\lambda_{x\alpha\beta} t} \quad (10)$$

Eqn. (10) gives an expression for the growth of complexity in a system with time. Here, $\phi(x_{\alpha\beta}, \dot{x}_{\alpha\beta})$ is a function depending upon velocity and displacement, x_0 is the initial variation, i.e., at time t_1 and $\lambda x_{\alpha\beta}$ is the Lyapunov's exponent for the two element pair, α and β. In the metric formulation of complex systems action possessed and degree of orderliness are inversely related.[7,8,9]

$$\alpha = \frac{nm\hbar}{\sum_{i=0}^{n}\sum_{j=0}^{m} I_{ij}} \quad (11)$$

In the above equation α is the measure of organization in a complex networked system.[7] From the above equation it can be seen that action and organization are related inversely. Thereby, reduction in action with time and achieving a least action or maximum organized state is the elucidating motive of the system which it fails to achieve. This is because a maximum organized state for a system is also a state of maximum action.[8] Such a state thus acts as an attractor.[7, 8, 10, 11, 12] So, for natural systems the Lyapunov's exponent in eqn. (10) becomes negative. In a two-dimensional state space, the second Lyapunov's exponent should be positive in order to satisfy the Liouville'stheorem.[18]For a two-dimensional state space:

$$\varepsilon^x_{\alpha\beta}(t) = \{\phi(x_{\alpha\beta}, \dot{x}_{\alpha\beta})\} x_0 e^{-\lambda_{x\alpha\beta} t},$$

$$\varepsilon^y_{\alpha\beta}(t) = \{\phi(y_{\alpha\beta}, \dot{y}_{\alpha\beta})\} y_0 e^{\lambda_{y\alpha\beta} t} \quad (12)$$

Constructal Theory: optimizing physical constraints in a system

The Constructal Law states that if a system has freedom to morph, it develops so that the flow architecture provides easier access to the currents that flow through it.[2, 25] The system's purpose is global existence. It is present along with fixed global constraints which may include the space allocated to the system, available material and components, allowable temperature, pressure or stress ranges, etc. The system designer brings together all components and optimizes the arrangement in order to reach maximum performance. In this way, he "constructs" the optimal flow architecture. Therefore the flow architecture shape and structure are *deduced*, not assumed in advance. A design engineer, thus, designs a system by optimizing the constraints and maximizing the performance. A flow system is also characterized by "performance" (function, objective) and "flow structure" (configuration, layout, geometry, architecture). Unlike the black box of classical thermodynamics, which represents a system at equilibrium, a flow system has performance and especially configuration. Each flow system has a *drawing*.[26] Natural structures are flow structures through which heat, work, energy flow inwards and outwards with time. How do natural structures sustain and enhance their performances with time? How does nature create its structures?

The global exergy currents flowing through natural animate systems are fuel, food, or perhaps information. In a complex networked system the global currents flowing in the network could be exergy and information. A very interesting property of complex adaptive systems is that they operate a feedback loop by which they interact with the surrounding and self-organize them with time (a continuous learning process). Also, at the same time, they possess an intrinsic control mechanism that diverges the system away from equilibrium.[7, 11] It is due to the irreversibility generated because of mutual interaction (eqn. (8, 10)). The self-organizing process operating through a feedback loop is analogous to parallel computation processes by which information gets stored into the physical memory of the system.[7, 27]

In the earlier sections we analysed the cause of internal irreversibility and related it to entropy generation (eqn. (8, 9 and 10)), but we did not discuss the existence of any physical constraints present within the system. The physical constraints can be the physical boundary of the system, distance between nodes in a networked complex system, physical hindrance to the motion of the system elements within, or can be any energy barrier. In addition to the generated irreversibility due to motion and mutual interaction, the physical constraints provide another challenge for natural systems to organize, to sustain, and also enhance their performances with time. So, what mechanism does a natural system employ to organize itself with time? How does the system morph and transit from one level of complexity to the other?

Emergence of geometry in nature and enhancing performance through constraint minimization and parallel computation

It was discussed in earlier sections that geometries in nature are alive and imperfect. The existence of physical imperfections and asymmetry are signs that, they are continuously evolving and are living.[2] Geometry and design are weakly connected in the sense that geometry is concerned with the shape and the form of a system, whereas design is concerned with its function and achievable performance. Thus, geometry represents the evolving system and design represents the sustaining system. In the empirical function to denote complexity we had multiplied a function $\phi(x_{\alpha\beta}, \dot{x}_{\alpha\beta})$ with the exponential term. The function depends upon the inter-nodal distance and instantaneous velocity of the elements crossing the nodes. Continuous self-organizing processes aim towards minimizing action and reducing the inter-nodal distance or displacement. With

self-organization the distances between the nodes shrink and the system's geometry varies continuously with time.[7] With shrinkage, the action of individual element is reduced and the elements evolve through time by approaching the shortest path. The continuous variation (geometrical) of a system or morphing would provide multiple pathways for the exergy currents to flow. Since a natural system is open, the search for shortest path for the exergy currents to flow through it will cause the system to continuously morph. On one hand, this would cause the system to change its shape by enlarging or shrinking its boundaries along its different axes of symmetry. On the other hand, the continuous search for shortest paths for currents to flow would cause internal differentiation of the paths at finer levels leading to alveoli structure of lungs, veins for blood circulation, a flowing river with numerous branches and tributaries, etc. Thus, the combined effect is seen in the form of asymmetrical structures showing self-similarity at finer levels. This "self-similarity" is signature in fractals.

Nature is full of fractals. Everything around us, animate or inanimate; for example, a leaf, a tree, the human body, a mountain range, snowflakes, etc. are all fractals. Fractals possess a great deal of self-similarity when examined successively at finer scales. The fractal dimension (d_F) of the least state attractor can be deduced from the Lyapunov's exponents; the negative exponent ($-\lambda x_{\alpha\beta}$) denoting shrinkage along the x-axis and the positive exponent $\lambda y_{\alpha\beta}$ denoting elongation along the y-axis.[18]

$$d_F = 1 + \frac{\lambda y_{\alpha\beta}}{\lambda x_{\alpha\beta}}, \qquad 1 < d_F < 2 \tag{13}$$

Thus, the growth of complexity in natural systems along with progressive development or self-organization generates fractals in nature, the reason of enormous varieties of beautiful structural forms, asymmetry, and geometric imperfections. Sustenance and never-ending struggle for achieving better performance takes place by two proposed ways:

1. Allow better access to the flow of exergy currents within the system through constraint minimization.
2. Through numerous simultaneous (parallel) self-organizing processes operating between nodes within the networked system (Accelerated Natural Computation).

Interestingly, both the processes are intrinsically linked. The system elements apply work on the constraints and minimize them to make way for efficient exergy flow within the networked system. The flow of exergy currents through the system establishes a gradient between the system and the surrounding. Exergy of the surrounding is null, i.e. the surrounding environment can be considered as a dead state compared to the system. The existence of this exergy gradient between the system and the surrounding causes the system elements to act as microscopic heat engines. The work output of these heat engines (system elements) is used up in minimizing constraints. The energy rejected while performing work, by the Second Law, is dissipated into the surrounding media. According to the exergy principle, exergy of a system can never increase. The rate of decrease in exergy of a system is equal to difference between the internal irreversibility and cumulative exergy of the system elements.

$$\frac{dA}{dt} = \Sigma_j \left(1 - \frac{T_0}{T_j}\right) Q_j - I < 0 \tag{14}$$

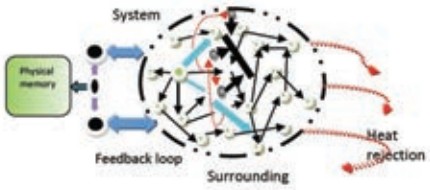
$$I > \Sigma_j \left(1 - \frac{T_0}{T_j}\right) Q_j \tag{15}$$

So, eqn. (15) can be rewritten as:

$$T_0 \dot{S}_{gen} > \Sigma_j \left(1 - \frac{T_0}{T_j}\right) Q_j,$$

$$\dot{S}_{gen} > \frac{\Sigma_j Q_j}{T_0} - \Sigma_j \frac{Q_j}{T_j} \tag{16}$$

From the above expression it can be deduced that internal irreversibility due to rise in complexity is greater than the difference between global entropy of the system and summation of local entropy for the all constituting system elements due to heat (energy, information) exchange with the surrounding media. Internal irreversibilities or inherent complexities restrain the system elements from performing maximum work to minimize constraint and limits the system from achieving least action state. The work lost due to irreversibility is the exergy destruction. Accelerating the self-organizing process causes the system elements to perform cumulative work on the constraints in a parallel arrangement. The parallel arrangement gives multiple simultaneous paths for exergy currents to flow by minimizing constraints along them. This also enhances the processing speed of the system by accelerating the feedback mechanism loop. This induces rapid decrease in action of the system as a whole and organizes the system at an accelerated rate. Thus, complexity present in the system generates irreversibility, preventing the system elements from minimizing the constraint to a possible minimum, thereby preventing the system from reaching the least action state. Grouping causes multiple processing within the system thus accelerating the rate of self-organization. These two processes are constantly in operation, preventing any complex-adaptive system reaching the dead state and also allowing the system to grow and develop in time and progressively enhance its performance. The figure below shows an evolving complex networked system with various nodes (concentric circles, green), inter-nodal trajectory of system elements (thin arrows, black), the exergy flow current (thick arrows, cyan), and system elements (solid spheres, black). The system elements work between the exergy gradients of system and surrounding (dotted red arrows) and perform work (thick black arrows) to minimize physical constraint (thick black line). In the figure below, three elements group, and together they apply cumulative efforts to modify the constraint. This causes simultaneous work and accelerates the feedback loop. The history (in the form irreversibilties in shape or structural from) gets stored in the physical memory of the system.



Discussion

The aim of this section is to discuss the long-range implications of the ideas presented in this paper and the future work to be done using them.

1. In the future. we can work on defining the function $\phi(x_{\alpha\beta}, \dot{x}_{\alpha\beta})$ and investigate complexity growth in greater detail. We can also work to formulate the multi-agent approach of the Principle of Least Action and quantify organization, parallel computation, and accelerated self-organization.[7, 13]
2. According to the Space Time Energy Matter (STEM) compression, systems increasingly get localised in space and increase their performance efficiency.[10] We have seen earlier how self-organization processes shrink inter-nodal distance in a system. Thus, STEM compression and approach to a least action state are analogous. Both appear to be unrealized attractors for the leading edge of complexity development (of emergent hierarchical intelligence) in the

universe.[10]

3. A debatable question has many a time erupted in our minds: are we alone in this universe? The Search for Extra Terrestrial Intelligence (SETI) project, Black hole intelligence, XRB's[11], the Cosmic Contact Censorship[14] can be explained by the idea of exergy flow and constraint minimization.

4. Using geometry in nature to design more efficient structures that allow for better exergy flow can optimize our resources and allow our sustainable existence.

5. Are Cosmological Natural Selection (CNS) and natural selection of rational choices of system elements in game-theoretic formulation of complex systems related?

Conclusion

Through this paper, the key features of complex systems, their continuously evolving geometries, the process of self-organization (by operating a feedback loop), the growth of complexity with time, and accelerated rate of organization by minimization of physical constraints through parallel processes have been addressed by making use of the Constructal Law and Principle of Least Action for multi-agent systems. We believe these ideas will open up new gateways to engineer and redesign our future structures and technologies. Finally, in the words of Feynman, *"God made the laws only nearly symmetrical so that we should not be jealous of His perfection!"*

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References

1. Feynman, R. P., Leighton, R. B. and Sands, M. (1963) *The Feynman Lectures on physics Vol. 1*. Addison – Wesley. Pg 664–665.
2. Reis, A. H. (2006) "Constructal Theory: From Engineering to Physics, and How Flow Systems Develop Shape and Structure." *Applied Mechanics Reviews* 59. Pg 269–280.
3. Bejan, A. (1997) *Advanced Engineering Thermodynamics*. John Wiley &Co.
4. Bar – Yam, Y. (1997) *Dynamics of Complex Systems*. Addison – Wesley.
5. Chaisson, E. J. (1998) *Order, Form and Complexity*. Harvard University Press.
6. Georgiev, G. and Georgiev, I. (2002) "The Least Action and the Metric of an Organized System." *Open Systems and Information Dynamics* 9.4. Pg 371-380.
7. Georgiev, G. (2012) "A quantitative measure, mechanism and attractor for self-organization in networked complex systems." *Sixth International Workshop on Self-Organizing Systems*, Delfts University, The Netherlands.
8. Chatterjee, A. B. (2012) "Action, an Extensive Property of Self-Organizing Systems." *Int. J. of Basic and App. Sciences* 1.4. Pg 584-593.
9. Chatterjee, A. B. (2012) "Intrinsic Limitations of the Human Mind." *Int. J. of Basic and App. Sciences* 1.4. Pg 578-583.
10. Smart, J. M. (2002) "Answering the Fermi Paradox." *J. of Evol.and Tech.*.
11. Vidal, C. (2010) "Black Holes: Attractors for Intelligence?" *Towards a scientific and societal agenda on extra-terrestrial life,* Kavli Royal Society International Centre.
12. Chatterjee, A. B. (2012) "Game – theoretic formulation of complex systems." *Proc. of the Sixth International Conference on Game Theory and Management*, St. Petersburg State University, Russia.
13. Chatterjee, A. B. (2012) "Principle of Least Action: a multi – agent approach." *Foundations of Physics, (under review)*.
14. Vaidya, P. G. (2007) "Cosmic Contact Censorship." *Arxiv Physics*. http://www.arxiv.org/. (Accessed 04/12/1990).
15. Vaidya, P. G. (2007) "Are We Alone in the Multiverse?" *Astrobiology Newsletter* 3. 4. Pg 4-6.
16. Vidal, C. (2010) "Computational and Biological Analogies for Understanding Fine-Tuned Parameters in Physics." *Foundations of Science* 15.4. Pg 375-393.
17. Heylighen, F. (1999)The Growth of Structural and Functional Complexity during Evolution, *The Evolution of Complexity*, eds. F. Heylighen, J. Bollen& A. Riegler, Kluwer Academic, Dordrecht, Pg 17 – 44.
18. Goldstein, H. (1980) *Classical Mechanics*. Addison – Wesley.
19. Wang, Q. A. (2004) "Maximum path information and the principle of least action for chaotic system." *Chaos, Solutions & Fractals* 23.1253.
20. Feynman, R. P., Leighton, R. B., Sands, M. (1963) *The Feynman Lectures on Physics Vol. 2*. Addison – Wesley.
21. Annila, A. et al (2009) "In the Light of Time." *Proc. R. Soc.* A465. Pg 1173.
22. Nag P. K. (2007) *Engineering Thermodynamics*. Tata McGraw Hill.
23. Chaisson, E. J. (1998) "The cosmic environment for the growth of complexity." *BioSystems* 46. Pg 13–19.
24. Chow *et al.* (2011) "Nonlinear pedagogy: Learning design for self-organizing neurobiological systems." *New Ideas in Psychology* 29. Pg 189–200.
25. Bejan, A. (2005) "The Constructal law of organization in nature: tree-shaped flows and body size." *The J. of Exp. Biology* 208. Pg 1677–1686.
26. Bejan, A. and Lorente, S. (2004) "The Constructal law and the thermodynamics of flow systems with configuration." *Int. J. of Heat and Mass Transfer* 47. Pg 3203–3214.
27. Devezas, T. C. (2005) "Evolutionary theory of technological change: State–of–the art and new approaches." *Tech. Forec. & Soc. Change* 72. Pg 1137-1152.

Detritus, Water Volume, and pH in Epiphytic Bromeliads' Central Tank

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Abstract:

Tropical plants are known for their highly efficient nutrient cycles; some of the best examples of this are epiphytic bromeliads. Epiphytic bromeliads rely solely on precipitation and leaf detritus for nutrients; such as nitrogen and phosphorous. However, little is known about the impact of detritus and water volume on the central tank chemistry in these bromeliads. Using pH as a bioindicator, this study examined this relation in epiphytic bromeliads along the Continental Divide in Monteverde, Puntarenas, Costa Rica. The collected data showed no statistical significance in pH when compared by four categories: (1)Detritus Dry Weight, (2)Total Water Volume, (3)Ratio of Dry to Wet Detritus Weight and (4)Ratio of Dry Detritus Weight to Total Water. Detritus was also ranked qualitatively on a scale from one to five based on decomposition level. The pH of these groups was compared by Kruskal-Wallis test and found not to be significant. Differences in pH imply either synergistic effect between two or more of these groups or influence from physiological characteristics of the bromeliad itself.

Key words: bromeliad, detritus, epiphyte, pH, water volume

Resumen

Los plantas tropicales son conocidas por sus eficientes ciclos de nutrientes; algunos de los mayores ejemplos son las bromelias epifítas. Las bromelias epifitas cuentan con la precipitación y los detritos solamente de hojas como nutrientes; tales como nitrógeno y fósforo. Sin embargo poco se conoce sobre el impacto de los detritros y volumen de agua en la química del tanque dentro de estas bromelias. Al usar pH como un indicador biológico, este estudio examinó la relación entre bromelias epifitas en la division continental en Monteverde, Puntarenas, Costa Rica. Los datos recogidos no mostraron una diferencia siginificativa en pH cuando se compararon cuatro categorías: (1)Peso Seco del Detrito, (2) Todo Volumen de Agua, (3) Relación de Detrito Seco a Mojado y (4) Relación del Peso Seco de Detrito con todo el Volumen de Agua. Los detritos fueron clasificados cualitativamente en una escala de uno a cinco basado también en el grado de descomposición. El pH de los grupos fue comparado con la prueba de Kruskal-Wallis y no se encontró ninguna diferencia significativa. Las diferencias en pH implican ya sea un efecto sinérgico entre dos o más de estas grupos o una influencia de características fisiológicas de la bromelia en sí misma.

Introduction

Epiphytic Bromeliads are often called "'aerial marshes"', but unlike terrestrial water bodies which get nutrients from silt and soil, these plants must rely solely on nutrients from detritus, specifically decaying leaf material, to survive.[1] Epiphytic bromeliads are a highly specialized group of vascular plants from the Bromeliaceae family. They are important in Neotropical forests in nitrogen and phosphorous cycles, as well as increasing biomass and providing unique habitats. By growing leaves in a tight, overlapping rosette pattern bromeliads form a central tank for nutrient and water collection. Their morphology also allows them to grow on trees and branches with no contact to the ground. Since epiphytic bromeliads do not absorb nutrients from their substrate, they are highly dependent on detritus and precipitation trapped in their central tank for sustenance.[2]

Detritus, as well as the bacteria, fungi, and protozoa that consume it, live in the bromeliad's central tank and make up the basic trophic level of bromeliad habitats.[4,5] These organisms perform anaerobic and aerobic respiration, and release CO_2 as a byproduct of detritus decomposition.[6] Dissolved carbon dioxide reacts with water to form dissociated carbonic acid (Fig. 1). This reaction can lower the pH of the water. The actions of the biota present in the central tank therefore affect the chemistry of the water present in the central tank.[7]

Research has been done on the microfuana of epiphytic bromeliads and on how detritus affects terrestrial bromeliads.[7,9,10,11,12] However, little is known about effects of detritus on the chemistry in the central tanks of epiphytic bromeliads. The focus of this experiment is to test whether detritus and water volume amounts influence epiphytic bromeliad tank environment. This study uses the pH of the water in the central tank as a bioindicator of respiration and decomposition. This research is important in identifying and exploring the nutrient cycling processes of the unique habitats formed by epiphytic bromeliads.

Figure 1: Example of epiphytic bromeliad.[3] Bromeliad depends solely on water and nutrients stored in its central tank for survival. Breakdown of detritus is the source of nutrients for central tank.

$$CO_2 + H_2O \leftrightarrow H^+ + HCO_2^-$$

Figure 2: Reaction of aqueous dissolved carbon dioxide to dissociated carbonic acid. pKa=6.35.[7]

Methods

Sampling Site

This study was conducted in the Monteverde Cloud Forest, in Puntarenas, Costa Rica along the Continental Divide near Cerro Amigos and the Monteverde Biological Station property (Fig. 2). All samples were taken on the Pacific slope (10° 19' N, 84° 47-8' W) during the wet season, (July 23rd-31st 2012) at an altitude of 1845 asl. The site is classified as lower montane wet forest by the Holdridge life zones; with an average yearly rainfall of 3.5 meters.

Bromeliad Selection

Individuals were sampled only along the Pacific slope of the continental divide. This insured similar exposure to conditions such as wind, precipitation, and sunlight. The Continental Divide was selected because strong winds keep trees shorter, making them easier to work with. Within the selected area, there was variability in the microhabitats where the epiphytes grew. For this experiment a strict set of criteria were imposed on possible samples. This was done to minimize any confounding variables that might impact the study. Only specimens found at eye level or below in areas of at least partial shade during the day were sampled. Any specimens found in light patches or on downed trees not overshadowed were avoided. There were no restrictions placed on species, genus, or morphospecies of bromeliads used. Only bromeliads with a tank diameter of 4 cm were sampled (Fig. 3). There was also no specification of the substrate on which the individual grew.

Sampling Procedure

Once a bromeliad had been selected, detritus was remove and bagged. Latex gloves were worn to protect the material removed. After any major debris was removed, a pipette was used to siphon off any water in the central tank and surrounding rosette. This liquid was then transferred to a water tight container. The pH of the solution was measured using a portable pH meter. The vial was then labeled with the sample number and pH reading. Any further detritus was collected from the specimen. The leaves' crevices were probed with a finger and any available material was removed. The sample was then examined for extent of detritus decay. A qualitative scale was used to rank the detritus from one to five, with a one corresponding to solid detritus, a five corresponding to complete decomposition, and a three being about fifty-fifty (Fig. 4). The amount of detritus was not considered, only the level of decomposition.

Detritus and Water Weighing

Detritus and water samples were kept refrigerated overnight in the lab. The samples were then removed from storage and their individual bags. The detritus was compacted by hand in the bag and any excess water was removed through applied pressure. The matter was removed by hand and placed in a paper towel of a standardized weight (1.45 g). The inside of the bag was then cleaned to remove any additional debris. The paper towel and detritus were weighed using a standard scale. The detritus was placed in a hot box at 66°C for twenty four hours. The detritus was removed and weighed again. This revealed the dry weight of the detritus and the weight of the water that had been sequestered in the decomposition process. Sequestered water was assumed to be the difference between wet weight minus dry weight. After this, the detritus was discarded.

Water samples were centrifuged to remove suspended debris. The free water was transferred to a container, weighed and discarded.

Results

Detritus and water volume amounts were found to have no significant impact on epiphytic bromeliad tank water. Five parameters were investigated in this study: (1) Dry Weight, (2) Total Water Volume, (3) Ratio of Dry to Wet Detritus Weight, (4) Ratio of Dry Weight to Total Water and (5) Detritus Decomposition Level. The first four sets were log transformed to create normal distributions. This allowed for regression comparisons with pH, which was found to be normally distributed already.

The regression between pH and log Dry Weight (ANOVA F=2.020, p>.05, DF=34) showed no statistical significance. Dry weight did have the greatest influence on pH (correlation coefficient=0.24) but was still not significantly different than zero. A coefficient between 0.1 and 0.3 is considered to be a small relationship between the variables.[13] The three parameters (Total Water Volume, Ratio of Dry to Wet Detritus Weight and Ratio of Dry Weight to Total Water) also fit this trend, but had lower correlation coefficients (0.13, 0.09, and 0.06). All four showed wide spreading (Fig. 6), and all four have low F statistics (Table 1).

The pH was investigated at different Detritus Decomposition Levels. The samples were grouped by decomposition level (1, 2, 3 and 4+) (Fig. 7). Categories 4 and 5 were

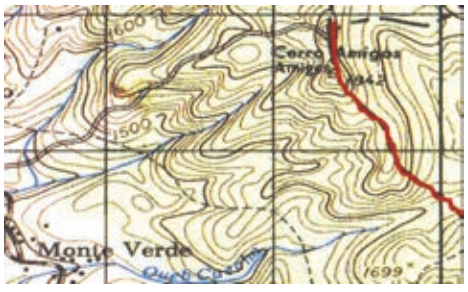

Figure 3: Topographic map of Monteverde, Puntarenas, Costa Rica. Red line indicates Pacific slope of Cerro Amigos.

Figure 4: Example of epiphytic bromeliad sampled. Specimen grew on main trunk of a tree along the Continental Divide in Monteverde, Costa Rica. Habitat conditions included strong winds, precipitation from clouds and being shaded for part of the day.

Figure 5: Detritus removed from a sample epiphytic bromeliad. A qualitative scale was used to classify each sample by decomposition level. From one to five the scale progressed from solid matter to complete decay. This sample was ranked a three out of five.

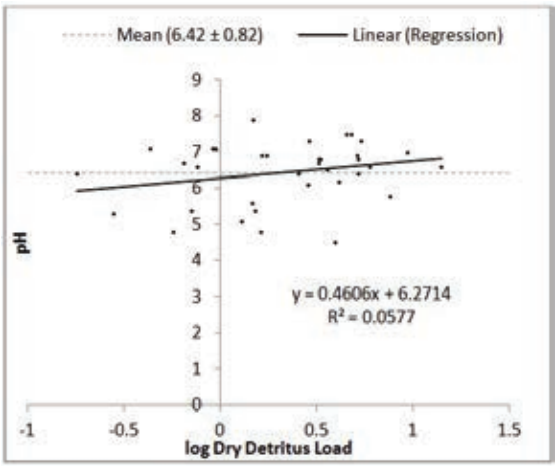

Figure 6: The effect of detritus load (mean weight 0.46 ± 0.32) on water pH in the central tank formed by the leaf rosette of epiphytic bromeliads. Samples were taken along the continental divide in Monteverde, Puntarenas, Costa Rica. pH ranged from 4.8-7.9 with a mean of 6.42 ± 0.82. A non-statistically significant positive correlation is present in graphs depicting pH as a function of the log transformed data of Total Water Volume, Ratio of Dry to Wet Detritus Weight and Ratio of Dry Weight to Total Water (0.12 ± 0.33, -0.59 ± 0.77 and 1.9 ± 2.6 respectively.

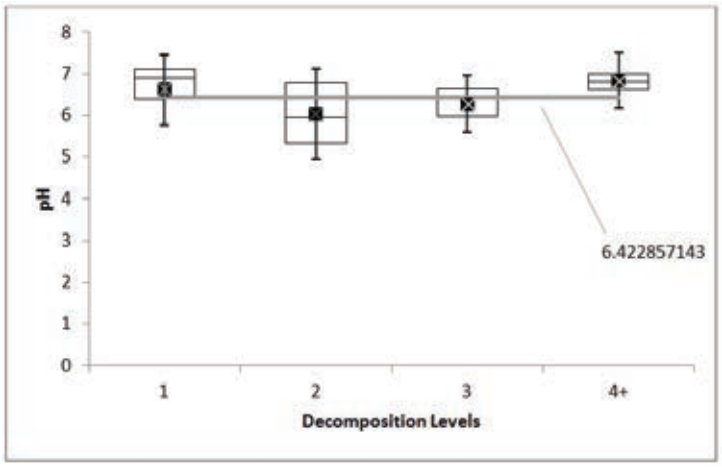

Figure 7: Box and Whisker plots of pH and detritus decomposition level. Level 4+ is a combination of samples with highest decomposition levels. Whiskers represent one standard deviation from the mean. Level 4+ had the most basic average pH of 6.83 ± 0.41. The lower levels (1, 2, 3) all have slightly more acidic pH averages (6.62 ± 0.84, 6.03 ± 1.09, 6.27 ± 0.67). The overall mean pH was 6.42. Samples were taken along the continental divide in Monteverde, Puntarenas, Costa Rica.

combined to 4+ to meet minimum sample size to run a non-parametric test. The Kruskal-Wallis test returned no statistical difference in mean pH between the groupings ($\chi 2=5.08$, $p>.05$, $DF=3$).

Discussion

These investigations showed no statistically significant relationship between detritus amount, decomposition level, and the central tank's pH for epiphytic bromeliads. This implies that the pH is not solely dependent on either the decomposition or on the amount of leaf material in the central tank. This finding is supported by research done by Benzing *et al.* in which terrestrial bromeliads were found to create stable microhabitats with little variation based on external factors.[7] It was also thought that because bromeliads are exposed to varying precipitation levels, the central tank pH would simply be a function of dilution. To test this, pH was compared to Total Water Volume and was found to have no statistical significance. This indicates that pH depends on more factors than precipitation input.

It was hypothesized that the decomposition level of matter in the bromeliad would influence the central tank's water pH. As heterotrophs consume the detritus for respiration, they release CO_2.[5] It was thought that this byproduct would lower pH as carbon dioxide reacts with water. Higher decayed matter was assumed to have been present in individuals longer and that more of its carbon would have been reduced and released as CO_2. This should result in a lower pH of the aquatic environment. Data from this showed that litter decomposition level had no statistical effect on pH.

Table 1: Test statistics from the four linear regression correlation tests. All four had low F statistics and high p values. Only dry detritus weight had a small correlation with pH value; all others had coefficients that signified no impact.

Regression of pH by	F statistic	p value	dF (Model,Error)	R^2 value	Correlation Coefficient
Dry Detritus Weight	2.020	>.05	1,33	0.057	0.24
Total Water Volume	.1315	>.05	1,33	0.004	0.06
Ratio of Dry to Wet Detritus Weight	.5384	>.05	1,33	0.019	0.13
Ratio of Dry Weight to Total Water	.5382	>.05	1,33	0.10	0.09

The more degraded leaf matter corresponded to more basic water (Fig. 7). Level 1 detritus, which corresponds to no decomposition in the matter, had an average pH close to that of measured rainwater pH (6.42) in Monteverde.[14] Then for Level 2, the water had the lowest pH. The water became more basic in Level 3 and Level 4+ had the most basic pH. This could be due to an initial surge of degradation thus releasing carbon dioxide from the material. In a study of terrestrial bromeliads, the microhabitat was characterized as dominated by respiration consumers.[11] This appears to hold true, at least at low decomposition levels, for the epiphytes in the family. After the initial break down, there seems to be a period of stabilization. This effect could come from the host bromeliad itself in an attempt to keep a favorable central tank environment.[11] This could signify that the bromeliad processes are most efficient at a specific pH, and that the plant has pathways to maintain this equilibrium.[15]

The investigation of the relationship between pH parameters of detritus and water volume revealed no significant results. Neither the detritus nor the water volume seemed to affect the habitat of the bromeliads. The amount of dry detritus had the biggest impact on pH. Biologically this implies that the amount of available material for degradation does play some role in the chemistry of the central tank water, even if it is a minor one. However, since there was some noticeable difference in pH for all four comparisons, maybe there is a synergistic

effect between two or more of these categories. This opens up the possibility of research that investigates the relationship between epiphytic bromeliads, habitat, and tank water.

Epiphytic Bromeliads offer a unique research opportunity. Their evolution has turned them into little microcosms of life. More research needs to be done to better fully understand how these plants are able to survive, and thrive, under conditions that seem inhospitable.

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References

[1]Richards, P.W. (1981) The tropical rainforest. Cambridge University Press, Ames, Iowa. Pg 84.

[2]Utley, J.F. and Burt-Utley, K. (1983) "Species account of bromeliads *In* Costa Rica natural history." Janzen, D.H., ed. The University of Chicago Press, Chicago. Pg 412-414.

[3]Tristram, Peter. (2013) "Vriesia." Photograph. *Coffs Harbour Bromeliads*. Forest Drive Nurse. <http://ornamentalplants.com.au> (02/24/2013)

[4]Juliano, S.A. AND YEE, D.A. (2006) "Consequences of detritus type in an aquatic microsystem: effects on water quality, micro-organisms and performance of the dominant consumer." *Freshwater Biology*. 51: 448–459.

[5]Cardelús, C.L. and Mack, M.C. (2010) "The nutrient status of epiphytes and their host trees along an elevational gradient in Costa Rica." *Plant Ecology*. 207: 25-37.

[6]Rich, P.H. and Wetzel, R.G. (1978) Detritus in the lake ecosystem. *The American Naturalist*. 112:57-71.

[7]Benzing, D.H., Derr, J.A., and Titus, J.E. (1972) "The water chemistry of microcosms associated with the bromeliad Aechmea bracteata." *American Midland Naturalist*. 87:60–70.

[8]Baird, N.C., Gillespie, R.J., Humphreyes, D.A., and Robinson, E.A. (1986) *Chemistry*. Allen and Bacon, Inc, Newton, Massachusetts.

[9]Goffredi, S.K., Kantor, A.H. and Woodside, W.T. (2011) "Aquatic microbial habitats within a neotropical rainforest: bromeliads and pH-associated trends in bacterial diversity and composition." *Microbiology Ecology*. 61:529–542.

[10]Bogusch, W., Hietz, P., Ketteler, N., and Zotz, G. (2010) "Growth of epiphytic bromeliads in a changing world: the effects of CO2, water and nutrient supply." *Acta Oecol*. 36:659-665.

[11]Bento, L., Enrich-Past, A., Estevese, F.A., Farjalla, V.F., Guimaraes-Souza, B.A., Marotta, H., Mendes, G.B., and Santoro, H. (2006) "Limnological parameters of the water collected in tropical bromeliads." *Acta Limnologica Brasiliensis*. 18:47-53.

[12]Benzing, D.H. (1998) "Vulnerabilities of tropical forests to climate change: the significance of resident epiphytes." *Climatic Change*. 39:519-540.

[13]Cochran, W.G. and Snedecor, G.W. (1982) *Statistical methods*. The Iowa State University Press, Ames, Iowa.

[14]Miller, Rebecca. (2010) "Epiphyte composition effects on through fall nutrients and pH." *CIEE Summer 2010 Research*. Pg 156-182.

[15]Laessle, A.M. (1961) "A micro-limnological study of Jamaican bromeliads". *Ecology*. 42:499-517.

Effects of caffeine on the growth and post-embryonic development in Manduca Sexta

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Abstract:

This study evaluated the impact of caffeine on the growth, metamorphosis, and adult development of Manduca sexta larvae. A range of dosages were administered in an artificial diet according to insect weight. Corresponding growth and progress through development stages of the animals yielded a negative correlation between caffeine ingestion and maturation. Data indicated that caffeine caused significantly decreased growth rates and hindered development in Manduca sexta. Ingestion of caffeine retarded growth rates and reliably culminated in defective development and/or death of the insect. These results support the potential use of caffeine as a means for control of insect development.

Introduction

Over the past half century, the xanthine alkaloid compound, caffeine (1,3,7, trimethylxanthine) has been heavily ingested by humans.[1] Concerns have arisen as a result of observed negative effects on development and possible toxicity of the compound.[2,3] This knowledge, and the potential development for use as a pesticide, prompted an evaluation of how the continuous ingestion of caffeine, in varying dosages, impacts the growth, development, and metamorphosis of the tobacco hornworm, *Manduca sexta*.[4]

Caffeine is known as a stimulant drug that inhibits the enzyme cyclic AMP phosphodiesterase (E.C. 3.1.4.53). Cyclic AMP is released in the human body when it is in an excited state. In a typical adrenaline response, cyclic AMP is broken down by phosphodiesterase and the body returns to a resting state. The presence of caffeine causes a prolonged alerted response because cyclic AMP is not broken down. In humans, caffeine has also been found to cause the antagonization of adenosine receptors, increased cytosolic calcium levels, blocked stimulation of glucose transport, and inhibited intrinsic protein kinase activity.[5] These symptoms can have many negative implications for the human body, especially under long term exposure to caffeine.

The fact that caffeine has several mechanisms of action can help to explain its pleotropic effects and physiological properties. Studies of the silk moth, *Bombyx mori*, have shown that the enzyme cyclic AMP phosphodiesterase is active throughout the growth and development of the moth and that it is inhibited in the presence of caffeine.[6] In hornets, *Vespa orientalis*, and bees, *Apis mellifera*, significant sensory and physiological changes were observed when the insects were exposed to caffeine. These effects included but were not limited to alteration in motor abilities, sensory responses to light and noise, increased irritability, and changes in appetite, copulation, hibernation, and longevity. The insects did not development any observable tolerance or addiction to caffeine.[7] These studies give reason to consider that caffeine does impact insect behavior, growth, and development.

Several invertebrate studies have shown that growth and metamorphosis of the organism display a negative correlation with respect to caffeine ingestion through dietary means. For example, caffeine is known to be a potent inhibitor of adult development in the moth *Hyalophora cecropia* by disrupting the synthesis or release of a brain hormone among impacting other crucial events.[8] Caffeine arrested growth and severely affected pupae formation in *Musca domestica* (common house fly) larvae.[9] In the larvae of the moth fly, *Telmatoscopus albipunctatus*, caffeine is known to cause retardation of growth and development and along with a high mortality rate.[10] Additionally, caffeine was shown to prevent the initiation of adult development in various silk moth pupae, *Samia cynthia, Callosamia promethea*, and *Bombyx mori*.[6,11]

Caffeine has been shown to reliably inhibit growth and metamorphosis in a number of insects. However, the mechanism by which this effect manifests itself is unknown and may vary between species. Studies have produced data that suggests some reasonable possibilities. One potential explanation, suggested by the results of a study with moth flies, proposed that caffeine may affect larval growth by retarding DNA synthesis.[10] Administration of caffeine to moth larvae, *Hyalophora cecropia*, indicated that the synthesis or release of a brain hormone was disrupted and that the xanthine compound likely acts at other sites within the sequence of events leading to adult development.[8] In *Callosamia promethea*, giant silk moths, it was shown that caffeine caused extensive degenerative damage in the brain and segmental ganglia, and resulted in arrested adult development through augmentation of the central nervous system.[11] These studies illustrate the multiple mechanisms and sites of action that the caffeine compound has on invertebrate systems.

The brain and associated neural hormones are crucial to normal insect development. For example, caffeine induces changes in gene expression in the honey bee, *Apis mellifera*, brain that exert both cognitive and developmental effects.[12] The *Hyalophora cecropia* silkmoth exhibits a change in the levels of cyclic AMP prior to the initiation of normal adult development, potentially providing a significant opportunity for caffeine to impact subsequent development.[13] Additionally, caffeine has been shown to specifically attack the central nervous system in the giant silk moth, *H. cecropia*, resulting in changes that successfully inhibit growth and metamorphosis.[11]

Manduca sexta (tobacco hornworm) serve as an important model for the study of the

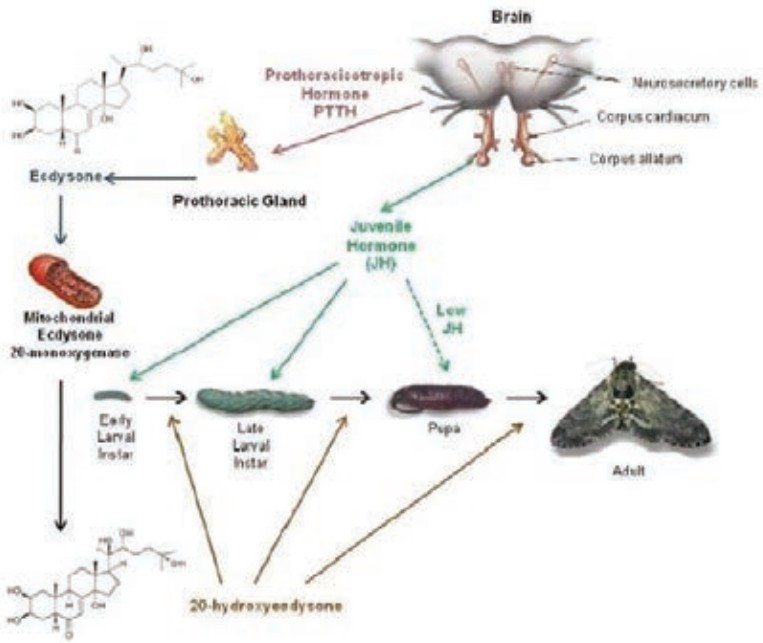

Figure 1: Regulation of metamorphosis in Manduca sexta by key developmental hormones.[24] The insect brain secretes PTTH (prothoracicotropic hormone) and JH (juvenile hormone). PTTH acts on the prothoracic gland to promote the synthesis of ecdysteroids such as ecdysone. Ecdysone is converted to the active form of the molting hormone by the mitochondrial ecdysone 20-monooxygenase. Together 20-HE (20-hydroxyecdysone) and JH work synergistically to regulate larval molts as the insect grows through each instar. Ultimately, the presence of ecdysone relative to the absence of JH results in the formation of a pupa and eventually the emergence of an adult.

growth, development, and metamorphosis of insects. They are an ideal candidate due to their pest reputation, rapid growth, large size, and ease of laboratory rearing.[14]*Manduca sexta* have a complete life cycle lasting 30-50 days with several larval stages.[14] When the larvae have reached the fifth instar, the process of metamorphosis begins. A variety of hormones are responsible for the steps involved in this process. An endocrine cascade begins once the larvae have reached a critical weight.[15] Once the process has been initiated metamorphosis is continually regulated by ecdysteroid hormones which ensure that each step occurs in a coordinated fashion.[16] The brain controls the secretion of the hormones controlling development based on the action of its tropic hormone (prothoracicotropic hormone) on the prothoracic glands.[17] The secretion of the brain peptide hormone prothoracicotropic hormone (PTTH) positively regulates the production of ecdysteroids by activation of ecdysone synthesis.[18,19] Subsequent conversion of the molting hormone ecdysone to the active form 20-hydroxyecdysone (20-HE) by the NADPH-utilizing ecdysone 20-monooxygenase (E20-M; E.C.1.14.99.22) is essential to the development from larvae to pupae.[20] Lower concentrations of JH result in an ecdysone-induced molt that produces a pupa.[21] Following the final larval-pupal molt, the larvae develop into opaque green pupae that gradually tan.[22] Juvenile hormone (JH) levels are responsible for the induction of larval molting and regulate the cuticular melanization of the new endocuticle formed when the pupa is generated.[23] Eventually there is no active JH present and the pupa gives rise to an adult.[24] The roles of these hormones in the process of insect development are displayed in Figure 1.

Considering the reviewed examples and the paucity of information linking caffeine to specific developmental pathways, the synthesis and activity of these brain-associated hormones could potentially be affected by caffeine ingestion. The results of this study seek to clarify and characterize the impact of caffeine on insect larval development.

Materials and Methods

Manduca sexta Rearing

Manduca sexta eggs and artificial diet were purchased from Carolina Biological (Burlington, NC). *Manduca sexta* were grown on an artificial diet in a Percival Intellus Environmental Controller (Perry, IN) at a constant temperature of 27°C at 63% humidity with a 12-hr light/dark cycle. Caffeine powder was purchased from Sigma-Aldrich (CAS# 58-08-2). Caffeine was carefully mixed with the artificial diet and administered through dietary ingestion. Each insect was raised in an individual specimen cup. Evaluation and maintenance of insects was performed on a daily basis as per Hatakoshi, Nakayama, and Riddiford.[22] All food prepared with caffeine was retained with that particular larva until completely consumed. Caffeine allotments were recalculated periodically as animals increased in size in order to maintain correct proportions to respective lethal dose values.

Experimental Design

Three experimental trials were conducted. The first trial was designed to establish the lethal dosage (LD) of caffeine in *Manduca sexta* and consisted of five experimental groups: control, LD_{25}, LD_{50}, LD_{75}, and LD_{100}. Insects used in this trial ranged from early third instar to mid-fourth instar. Dosages for each experimental group were developed with the goal of simulating mammalian toxicity equivalents. Calculations were performed in accordance to the lethal dosage recorded in humans where LD_{50} in humans is approximately 150-200 mg/kg.3 Caffeine doses were calculated for each experimental group based on an average of 175 mg/kg for LD_{50}. Values are listed in Table 1.

Dosages were calculated based on the initial weight of the animal. The amount of caffeine was increased by one order of magnitude in order to encourage more pronounced results as it became apparent in initial trials that *Manduca sexta* are not as sensitive to caffeine as humans. Insects naturally have higher levels of P450 enzymes that allow them to metabolize harmful compounds at a faster rate than humans.[25] As a result, increased LD values were established as a baseline for *in vivo* assessments. Doses were measured and incorporated into two grams of the artificial diet.

The second trial was designed based upon the results of the first trial with the goal of obtaining a higher degree of fatality. The dosage was increased by another order of magnitude in order to account for the high P450 metabolism of toxic compounds noted in insects and with hopes of obtaining consistent lethal results. Three experimental groups were established: control, LD_{50x100}, and $LD_{100x100}$. Insects used for this experiment were in late second to early third instar. In this age range insects were large enough to receive a measurable caffeine dosage, but also young enough to provide sufficient time to observe long-term

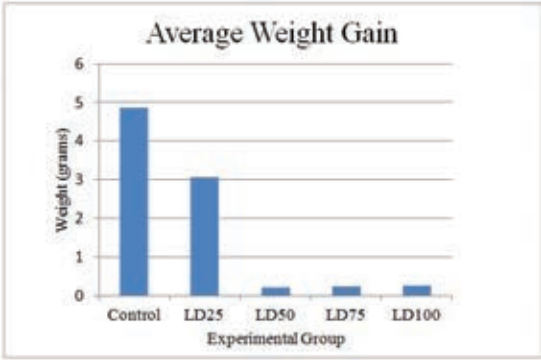

Figure 2: Average weight gain in Manduca sexta upon exposure to caffeine in trial 1. Average weight gain values were determined as per the protocol described in the Materials and Methods section under Evaluation and Analysis. The experimental groups LD_{50}, LD_{75}, and LD_{100} receiving higher dosages of caffeine experienced significantly lower levels of growth than the Control and LD_{25} groups.

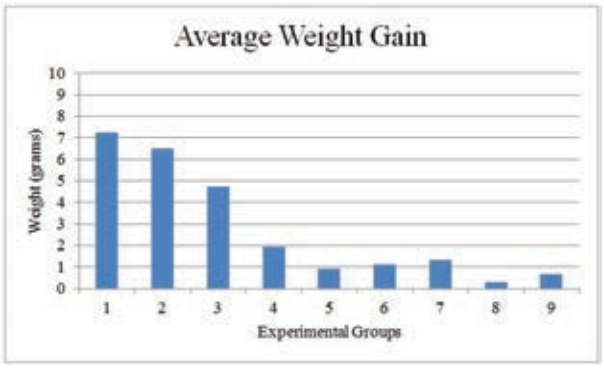

Figure 3: Average weight gain in Manduca sexta upon exposure to caffeine in trial 3. Average weight gain values were determined as per the protocol described in the Materials and Methods section under Evaluation and Analysis. Experimental groups are designated as control (1), LD_{25} (2), LD_{50} (3), LD_{75} (4), LD_{100} (5), LD_{25x100} (6), LD_{50x100} (7), LD_{75x100} (8), and $LD_{100x100}$ (9). The following experimental groups receiving higher dosages of caffeine, LD_{75}, LD_{100}, and LD_{25x100}, LD_{50x100}, LD_{75x100}, $LD_{100x100}$, experienced significantly lower levels of growth than the control, LD_{25} and LD_{50} groups.

effects.

The purpose of the third trial was to further test the effects of various caffeine dosages on *Manduca sexta* larvae in terms of lethal dosage and developmental impacts. Nine experimental groups were established in the third trial: control, LD_{25}, LD_{50}, LD_{75}, LD_{100}, LD_{25x100}, LD_{50x100}, LD_{75x100}, and $LD_{100x100}$. Insects chosen for this trial were also in the late second to early third instar. Dosages were calculated as previously described using increases of one and two orders of magnitude to account for increased P450 metabolism.

Evaluation and Analysis

Animals were maintained and evaluated through pupation. All resulting pupae were stored in the incubator following the experiment for comparison between experimental groups. Weight, behavioral observations, and physical observations were recorded daily. Insects were weighed on a Fisher Scientific scale (Model S-300D). Behavioral and physical observations consisted of an overall assessment established by determination of the level of relative activity (based on reaction to touch and amount of movement on the scale), the presence/quantity of feces, whether or not food was consumed, and the condition of the animal in terms of size and coloring as per Hatakoshi, Nakayama, and Riddiford.[22]

Table 1: Established caffeine dosage for each experimental group. This table shows the relative dosages used to calculate measured amounts of caffeine. Each amount was used to determine the amount to feed each individual and corresponds to a certain experimental group.

Experimental Group	Dosage (mg/kg)
Control	0.0
LD_{25}	87.5
LD_{50}	175.0
LD_{75}	262.5
LD_{100}	350.0

Results from each trial included a quantitative analysis of overall weight gain and growth rate. Experiments were replicated in order to obtain a compilation of accurate and consistent results. Behavioral and physical observations were also noted. For all trials the change in total weight for each insect was calculated and then averaged to determine an average change in total weight for a particular experimental group. This number is referred to as average weight gain. For an individual insect, the change in weight was calculated by subtracting the initial weight from the final weight at the end of the trial. Chi-squared analysis was performed for the first and the third trial on the average weight gain data to determine significance of the values.

For each experimental group in the third trial, the maximum change in growth was taken for each animal and averaged. The maximum change in growth for an individual was designated as the day in which the insect gained the most weight.

Results

The results of the first trial indicated that increased dosages of caffeine corresponded to decreased overall weight gain. This was especially notable in the LD_{50}, LD_{75}, and LD_{100} groups as shown in Figure 2. In terms of general post-embryonic development, *Manduca sexta* in the LD_{50}, LD_{75}, and LD_{100} groups exhibited difficulty accomplishing normal developmental molts between instars in the final molt prior to pupation. If the insects do not reach a critical weight, they cannot mature correctly.[21] A number of insects tanned without attempting the final larval-pupal molt or forming a pupa. The majority of the animals that were fed caffeine died before reaching maturity and did not proceed through development in a successful manner.

In the first trial, control animals developed normally and gained an average of 4.85 grams. LD_{25} animals exhibited an average weight gain of 3.05 grams. In the LD_{50} experimental group, the average growth was 0.22 grams. In the LD_{75} group, the average growth was 0.24 grams. In the LD_{100} group, average growth was 0.26 grams. Physical effects of caffeine ingestion manifested themselves primarily in the final stages of adult development and varied between individuals. Symptoms included failure to complete the final molt prior to pupation and premature tanning with no final larval-pupal molt and no pupa formation. In general, insects either produced malformed pupae or failed to complete the last larval-pupal molt prior to pupation hindering any further development. Some successful pupation was noted, but resulting pupae appeared squished or were malformed. *Manduca sexta* were observed repeatedly to have difficulty molting at each instar. This difficulty resulted in constrictions of molted skin that could have contributed to premature death. Several individuals in the LD_{75} and LD_{100} groups died with no significant growth and no sign of any progress toward adult development. Those insects in the higher dosage experimental groups that did accomplish some weight gain tanned with no final molt and did not

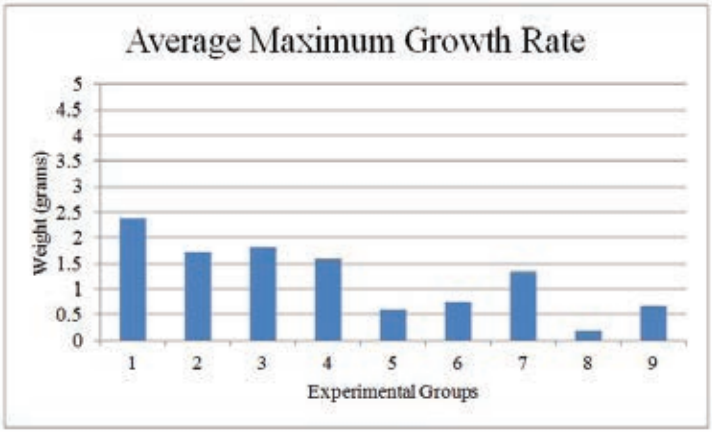

Figure 4. Average maximum growth rate of Manduca sexta upon exposure to caffeine in trial 3. Average maximum growth rate values were determined as per the protocol described in the Materials and Methods section under Evaluation and Analysis. Experimental groups are designated as control (1), LD_{25} (2), LD_{50} (3), LD_{75} (4), LD_{100} (5), LD_{25x100} (6), LD_{50x100} (7), LD_{75x100} (8), and $LD_{100x100}$ (9). Control and lower caffeine dosage groups had higher maximum growth rates overall.

survive. Chi-squared analysis showed a significant decrease in average weight gain for the LD_{50}, LD_{75} and LD_{100} groups.

Results of the second trial are replicated in the third trial and are therefore not shown. Results from the third trial were obtained in much the same way as the first trial. It is apparent from these data that the animals receiving a diet with caffeine did not achieve the same degree of weight gain as the control. Increasing caffeine dosage corresponded to decreasing overall weight gain. Excluding a few animals from LD_{25x100} and LD_{50x100} groups, respectively, all of the animals in the LD_{100}, LD_{25x100}, LD_{50x100}, LD_{75x100}, and $LD_{100x100}$ groups showed no significant growth and died prior to reaching the final stages of development before they could even have a chance to attempt pupation. Control, LD_{25}, and LD_{50} experimental groups experienced significant overall weight gain throughout development as seen in Figure 3.

General observations regarding how the final stages of development for the insects in the third trial progressed revealed a low level of successful pupation and a high level of mortality. Many of the animals that received caffeine did not achieve successful adult development and could not form a normal pupa. Results were similar to observations made in the first trial. Control insects developed normally. In the LD_{25} group, animals tanned without pupating and some failed to complete the final larval-pupal molt as indicated by white stripes on the side.[21] In the LD_{50} group, all the insects in the group formed an unhealthy pupa. Pupae typically had a clear, fragile membrane area on the underside or had misshapen hooks. In the LD_{75} group, all animals failed to complete the final larval-pupal molt and died. Insects in the groups above LD_{75} died prior to achieving any successful development. Any individuals that did manage to accomplish some weight gain reached the same fate as those in the LD_{75} group. Chi-squared analysis found the decrease in average weight gain values to be significant for LD_{75}, LD_{100}, $LD_{25 x 100}$, $LD_{50 x100}$, $LD_{75 x 100}$, and $LD_{100 x 100}$.

As expected, the control group had the largest maximum growth rate. These data did not show as much of a change in rate as might have been expected, but it was clear that insects fed caffeine grew significantly less each day and reached an overall smaller weight as shown in Figure 4. Average maximum growth rate for control animals was 2.38 grams. The average maximum growth rate for animals received caffeine ranged from 0.2 to 1.59 grams.

Throughout the experiment, it was observed that animals that received caffeine often had an aggressive or irritable response to touch. This was exhibited with flailing and biting motions. Some individuals showed a high level of activity during handling and when placed on the scale. A few insects sat on the scale with their heads up in the air swaying back and forth. In comparison, the animals in the control group were relatively docile. They did not significantly react to touch and were not overly active.

Discussion

As reported, caffeine retarded the growth of *Manduca sexta*. Overall, feeding caffeine via artificial diet to *Manduca sexta* appeared to lower the rate of growth, decrease the extent of weight gain, and hindered the ability to complete healthy development. Insects receiving caffeine administered through their diet did not match the growth rate or size of control insects. Indeed, total growth was significantly decreased in all experimental trials along in correlation with increased caffeine ingestion and exposure. As caffeine administration was increased, weight gain was substantially lessened and eventually caused mortality. In addition to decreased weight gain, it was also apparent that caffeine slowed down the growth rate of the larvae. Decreased observed weight gain and growth rate could be the result of diminished appetite in treated animals; the cause for this could be two-fold. The first possibility is the detection of caffeine, resulting in rejection of the artificial diet and depreciated nutrition. The second is that their ingestion of the caffeine brought about a decreased appetite response that also resulted in lowering overall food consumption. These reasons are reviewed by John Glendinning.[26]

Behavioral modifications were also noted for animals treated with caffeine. *Manduca sexta* that were fed and exposed to the intermediate amount of caffeine on a regular basis had more extreme reactions to stimulation via touch or noise and were more active overall during observation periods. Consequently, these results suggest that caffeine impacts the animals in a similar way to how it impacts humans as described by Isaac et al., and causes to some degree a heightened state of alert or hyperactivity.[27]

It was also observed that the animals receiving caffeine had difficulty completing the final stages of larval development. *Manduca sexta* from the highest dosage groups perished before reaching the fourth and fifth instars and therefore never pupated. However, animals from the lower dosage groups typically did survive until this stage and several different observations were made. Typically, one of two things happened: mortality was observed prior to the final larval-pupal molt or they exhibited premature tanning with no indication of complete pupation. Animals that showed such mortality developed pronounced lateral white striping. The animals that tanned prematurely during the fifth larval instar did so without pupating and did not display these white strips. These results suggest that the overall impact of caffeine administration lead to the inhibition of *Manduca sexta* growth and post-embryonic development. It is possible that caffeine contributes to the activity or inactivity of important developmental hormones, for instance

different ecdysteroids like ecdysone.[16] Another potential is that the inhibited growth of the organism overall negatively impacts the stages of its development. If the animal is moving through stages of development too quickly or too slowly, an early or delayed response by hormones, such as PTTH (prothoracicotropic hormone), ecdysone, and JH (juvenile hormone), could be causing these kinds of results. If the animal is not at a critical weight, then the release of the developmental hormones is delayed as described by Nijhout and Williams.[28]

It is known that various flavonoids, other plant allelochemicals, and compounds such as caffeine have an effect on insect growth, development, and reproduction. However, the mechanisms for these effects require more study. The inhibition of post-embryonic development in insects by allelochemicals and other compounds such as caffeine is considered. It is recognized that these compounds could potentially function as biopesticides that act to control insect development.[24] By preventing the insects from reaching sexual maturity, the pest can be effectively controlled. The mechanism of how ingested caffeine affects the insect, as well as how it impacts humans, mammals, and other insects, must first be understood in order to determine the impact of the compound on subsequent development. These evaluations would yield information that should be taken into consideration when considering caffeine and any other compound as a potential biopesticide. Further investigations are required to determine the feasibility of this application due to the high concentrations of caffeine required to achieve a negative effect.

The results of this *in vivo* study demonstrate that caffeine causes significantly decreased growth rates and hinders development in *Manduca sexta*. While the exact biochemical mechanisms of this inhibition have yet to be elucidated, a correlation between caffeine and invertebrate development is evident. Certainly further study of this model system may lead to a better understanding of the effects of caffeine on insect development.

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References

[1]Gilbert, R. (1984) "Caffeine consumption." *Progress in Clinical and Biological Research* 158. Pg 185-213.
[2]Bracken, M., Triche, E., Belanger, K., Hellenbrand, K. and Leaderer, B. (2003) "Association of Maternal Caffeine Consumption with Decrements in Fetal Growth." *American Journal of Epidemiology* 157.5. Pg 456-466.
[3]Peters, J. M. (1967) "Factors Affecting Caffeine Toxicity: A Review of the Literature." *Journal of Clinical Pharmacology* 7. Pg 131-141.
[4]Kim, Y., Uefuji, H., Ogita, S. and Sano, H. (2006) "Transgenic tobacco plants producing caffeine: a potential insect pest control." *Transgenic Research* 15.6. Pg 667-672.
[5]Foukas, L., Daniele, N., Ktori, C., Anderson, K., Jensen, J. and Sheperd, P. (2002) "Direct Effects of Caffeine and Theophylline on p110δ and other phosphoinositide 3-kinases. Differential effects on lipid kinase and protein kinase activities." *The Journal of Biological Chemistry* 277.40. Pg 37124-37130.
[6]Morishima, I. (1973) "Cyclic AMP phosphodiesterase activity during the development of the silkworm, *Bombyx mori*." *Journal of Insect Physiology* 19.11. Pg 2261-2265.
[7]Ishay, J. and Paniry, V. (1979) "Effects of caffeine and various xanthines on hornets and bees." *Psychopharmacology* 65.3. Pg 299-309.
[8]McDaniel, C. and Berry, S. (1974) "Effects of caffeine and aminophylline on adult development of the Cecropia silkmoth." *Journal of Insect Physiology* 20.2. Pg 245-252.
[9]Srinivasan, A. and Kesavan, P. (1977) "Toxic effects of caffeine on growth and metamorphosis of the larvae of *Musca domestica*." *Journal of Toxicology and Environmental Health* 2. Pg 569-576.
[10]Sehgal, S. Simoes, L.G. and Jurand, A. (1977) "Effects of caffeine on growth and metamorphosis of moth fly *Telmatoscopus albipunctatus* (Diptera, Psychodidae)." *Entomologia Experimentalis Et Applicata* 21.2. Pg 174-181.
[11]Blaustein, M. and Schneiderman, H. (1960) "A brief survey of potential antimetabolites and enzymes on the development of giant silkmoths." *Journal of Insect Physiology* 5.2. Pg 143-159.
[12]Kucharski, R. and Maleszka, R. (2005) "Microarray and real-time PCR analyses of gene expression in the honeybee brain following caffeine treatment." *Journal of Molecular Neuroscience* 27.3. Pg 269-276.
[13]Rasenick, M., Neuburg, M. and Berry, S. (1976) "Brain cyclic AMP levels and the initiation of adult development in the Cecropia silkmoth." *Journal of Insect Physiology* 22.11. Pg 1453-1456.
[14]Kingsolver, J. (2007) "Variation in growth and instar number in field and laboratory *Manduca sexta*." *Proceedings of The Royal Society in Biological Sciences* 274.1612. Pg 977-981.
[15]Callier, V. and Nijhout, H. (2011) "Control of body size by oxygen supply reveals size-dependent and size-independent mechanisms of molting and metamorphosis." *The National Academy of Sciences of the United States of America* 108.35. Pg 14664-14669.
[16]Schwartz, L. and Truman, J. (1983) "Hormonal control of rates of metamorphic development in the tobacco hornworm *Manduca sexta*."*Developmental Biology* 99.1. Pg 103-114.
[17]Marchal, E., Vandermissen, H., Badisco, L., Van de Velde, S., Verlinden, H., Iga, M., Van Wielendaele, P., Huybrechts, R., Simonet, G., Smagghe, G. and Vanden Broeck, J. (2009) "Control of ecdysteriodogenesis in prothoracic glands of insects: A review." *Peptides* 31.3. Pg 506-519.
[18]Rybczynski, R., Snyder, C. A., Hartmann, J., Sakurai, S. and Gilbert, L. (2009) "*Manduca sexta* prothoracicotropic hormone: evidence for a role beyond steroidogenesis." *Archives of Insect Biochemistry and Physiology* 70.4. Pg 217-229.
[19]Rountree, D. and Bollenbacher, W. (1986) "The release of the prothoracicotropic hormone in the tobacco hornworm, Manduca sexta, is controlled intrinsically by juvenile hormone." *Journal of Experimental Biology* 120. Pg 41-58.
[20]Smith, S. (1985) "Regulation of Ecdysteroid Titer: Synthesis." In: *Comprehensive Insect Physiology, Biochemistry, and Pharmocology*. 7. Kerkut, G.R. and Gilbert L.I., eds. Pergamon Press. Pg 295-341.
[21]Reynolds, S., Brown, A., Rakesh, S., Riddiford, L. and Hiruma, K. (2009) "Induction of Supernumerary larval molting in the tobacco hornworm *Manduca sexta*: Interaction of bisacylhydrazein ecdysteroid agonsits with endogenous juvenile hormone." *Physiological Entomology* 34.1. Pg 30-38.
[22]Hatakoshi, M., Nakayama, I. and Riddiford, L. (1988) "The induction of an imperfect supernumerary larval molt by juvenile hormone analogues in *Manduca sexta*." *Journal of Insect Physiology* 34.5. Pg 373-378.
[23]Curtis, A., Hori, M., Green, J., Wolfgang, W., Hiruma, K. and Riddiford, L. (1984) "Ecdysteroid regulation of the onset of cuticular melanization in allatectomized and Black mutant *Manduca sexta* larvae." *Journal of Insect Physiology* 30.8. Pg 597-599.
[24]Vandock, K. (2010) *Mitochondrial Transhydrogenations in Manduca sexta:* Relationship between Reversible NADPH□ NAD+ Transhydrogenase and Ecdysone 20-Monooxygenase in Fifth Instar Larvae. Ph.D. Dissertation, Department of Biological Sciences. Bowling Green State University.
[25]Scott, J. and Wen, Z. (2001) "Cytochromes P450 of insects: the tip of the iceberg." *Pest Management Science* 57.10. Pg 958-967.
[26]Glendinning J., Davis A. and Ramaswamy S. (2002) "Contribution of Different Taste Cells and Signalling Pathways to the Discrimination of "Bitter" Taste Stimuli by an Insect." *The Journal of Neuroscience* 22.16. Pg 7281-7287.
[27]Isaac, R., Chenxi, L., Leedale, A. and Shirras, A. (2010) "*Drosophila* male sex peptide inhibits siesta sleep and promotes locomotor activity in the post-mated female." *Proceedings of the Royal Society in Biological Sciences* 277.1678. Pg 65-70.
[28]Nijhout, F. and Williams, C. (1974) "Control of moulting and metamorphosis in the tobacco hornworm, Manduca sexta: growth of the last instar larva and the decision to pupate." *Journal of Experimental Biology* 61. Pg 481-491.

Paula Giovanini-Morris

Colorado State University

P. Giovanini-Morris, *Windows & Doors,* 2009
Hand embroidery with bead embellishments. DMC floss.
Beads; 10.5"x10.5"

Embroidery is the medium I choose to create narratives on the human condition and my journey through it. The vibrant colors of the floss and the textures I am able to create utilizing different thickness of floss and various stitches create a dialogue that invites the viewer to look deeper into the work to explore the basics of technique and the implied messages in the imagery. The stitching process is meditative, repetitive, tedious, and ultimately rejuvenating. By the time of completion, I have arrived at a satisfactory answer to the question I was contemplating.

Much of my work explores relationships and biological processes that occur in the body and brain. The human emotions of joy, hope, grief, loss, and psychiatric pathologies are issues I continue to encounter and contemplate.

My Mother, I Am concerns the relationship of mothers and daughters, specifically my relationship with my mother, a repeating theme in my work. Mothers can be extremely loving and supporting as well as critical, cruel, and competing.

Windows and Doors is another piece that was sparked by my elderly mother's decline into Alzheimer's dementia. This work explores the concept of memory. The biochemical explanation of the release of neurotransmitters at the neuron synapses in the brain allows for registration and encoding of the event, retainment, and ultimately the retrieval of the memory. Metaphorically, the "windows and doors" in this brain speak visually to the places I imagine my "snapshots," or memories, of personal life events are placed for safe keeping, allowing for later reflection and meaning in my life journey.

Going Under for the Third Time explores the concept of hope in the face of sadness and despair. The work speaks to the forces occurring on the earth—the pollution of the land, waters, and atmosphere, as well as personal hardship and loss. I believe in a higher power in the universe, a creator and destroyer, the human need to call on the divine in times when one is unable to control the forces around you, and on the hope that this intervening force will restore balance, harmony and peace.

Shattered is a piece about psychiatric illness and culminated from my experiences working in a psychiatric facility with adolescents. These individuals suffered from major depression, schizophrenia, and personality disorders. While working on this piece, I was exploring the various pathologies these children exhibited and their inability to control what lies inside them.

In closing, I invite the viewer to explore the colors, textures, and variety of stitches which create the imagery embedded in my embroidery. My greatest desire is for the viewer to be able to connect with my work.

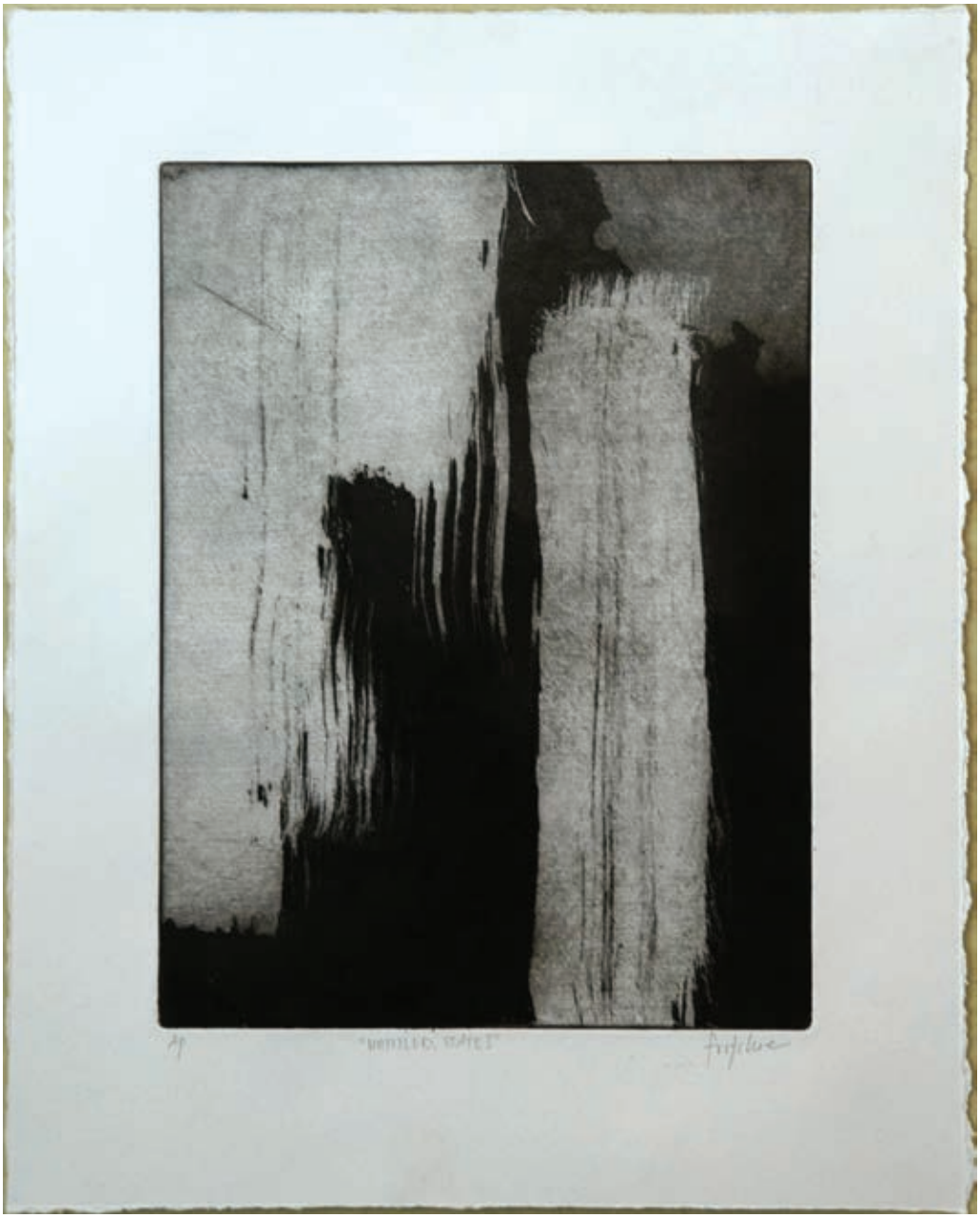
P. Giovanini-Morris, *Shattered,* 2005
Hand embroidery with bead/mirror/ acrylic paint embellishments. DMC embroidery floss; 6.5" X 6"

P. Giovanini-Morris,
Going Under for theThird Time, 2007
Hand embroidery with bead embellishments. DMC floss, beads. Gold purl; 6" X 7"

P. Giovanini-Morris,
My Mother, I Am. 2011
Hand embroidery.
Machine embroidery.
DMC floss, silk ribbon,
gold cord; 7" x 10"

Frances Ritchie

Colorado State University



F. Ritchie, *Untitled,* 2012
Aquatint on Paper;
12"x18"

For me, this work was an investigation into the freedom of experimentation. Many times artists get caught up in the specifics of what we are creating and why, but with this piece I was determined to let that go. As children, we explore art through the process of play. We scribble with a crayon, sketch with a marker, poke at clay, pull a brush down on a page, and there is a fulfilling joy in that process. Art emerges through experiment. Ultimately, through this piece, I reverted back to the childhood process of play, acknowledging the beautiful forms that transpired.

Zachary Heil

Colorado State University

Z. Heil, *Untitled,* 2009
Acrylic paint; 2'x3'.

This painting is based off of a trip I was fortunate enough to go on to the Oregon coast with a good friend and his family. At the time, I was inspired by how dynamic the landscape around the area was and decided to make a picture plain that contained unique scenes all of the same beach area. Outside of wanting to re-create the distinct atmospheres of each scene, the piece is about orchestrating colour in different ways, whether in a landscape or abstraction, and seeing how the pigments and styles affect each other.

Adrian R. P. Brown

Oregon College of Art & Craft

A. Brown, *June 22, 2009,* 2010
Sterling silver, nickel-plated copper and brass;
8”x2”x3”.

Adrian R. P. Brown is a metalsmith in Portland, Oregon. She holds a Bachelor of Environmental Design from Texas A&M University and a Bachelor of Fine Art in Craft from the Oregon College of Art & Craft (expected 2013) with an emphasis in metalsmithing. These two disparate courses of study inform her most recent body of work as she explores the relationship between place and identity. Her pieces frequently confront issues of utility, gender, and sexual identity while investigating scale, material, and craft in a digital age. She is the recipient of numerous scholastic awards, a two-time Niche Awards finalist, and the winner of the 2013 Niche Awards Student Fashion Accessory category.

June 22, 2009 depicts scattered motorcycle parts under a street sign at the intersection of NW Glisan Street and NW 14th Avenue in Portland, Oregon. This closely personal piece represents the largest obstacle Brown ever faced: a near-fatal motorcycle accident. In her lifelong recovery, art became the most effective tool for restoring her wellness, both in body and mind. Born and Raised is a more cheerful piece, celebrating her upbringing in Bossier City, Louisiana, and her fond memories of Mardi Gras celebrations. Secrets (Belt) embodies the ambiguity of gender that is often kept behind closed doors. The hinged members can be displayed one at a time or together, though any pieces folded inside are not hidden but magnified. The wearer of the belt is never identified as wholly male or female, always a combination of the two.

A. Brown, *Secrets (Belt)*, 2012 Sterling silver, nickel, copper, flocking, found object (lens); 3”x35” x1”

A. Brown, *Born and Raised*, 2011 Electroplated copper, feathers, enamel paint; 24”x15”x8”.

Books or Stories? The Changing Value of Social Education in Rural Morocco

By Elena C. Robertson
Occidental College

Formalized, secular education is generally regarded by the western world as the "cure-all" for the developing world's problems and under many circumstances this is true: religious fanaticism, child and mother mortality rates, violent crime, and general human rights abuses have been shown to decrease in educated communities. In recognition of the positive impacts education can have upon developing nations, the United Nations has included several provisions for universal education in the Millennium Development Goals, created in 1990 and meant to be achieved by 2015. Due in part to the United Nation's Millennium Development Goals (UNMDG), the global spotlight has begun to focus on increasing the universal education rate, particularly in the developing world.[1] Much of the focus has been on the African continent, especially Sub-Saharan Africa because the continent plagued by disease, high mortality rates, and some of the world's worst human rights abuses. By focusing on the regions of the world that are most desperate for development, the United Nations has been able to identify and begin to solve some of the root causes of much the world's problems.

While much of the emphasis of the UNMDGs has been on Sub-Saharan Africa, the North African region has made some notable strides in achieving the UNMDGs: Morocco is likely to achieve goals 3 and 4, may achieve 2 and 5 with some changes, data is unavailable for goals 1, 6, and 8, while they are unlikely to achieve goal 7; Algeria is on track for goals 2-7 and data is unavailable for goals 1 and 8; Libya is likely to achieve goal 4, may achieve goals 1, 2, 3, 5, and 7 with some changes, and data is unavailable for 6 and 8; Egypt is likely to achieve goals 1, 2, 4, 5, and 6, may achieve goal 3 with some changes, and data is unavailable for goals 7 and 8.

Out of this entire region, Morocco proves to be an especially interesting country to study in the context of the UNMDGs for several reasons: unlike it's North African neighbors, Morocco did not undergo a massive political revolution following the Arab Spring, thus making its position in the context of the UNMDGs easier to analyze since the government has not been overthrown. This observational study aims to do two things: first is to examine Feriyat, a village in rural Morocco in the context of the UNMDGs and how a village that exists with virtually no chance of achieving all of the MDGs functions; second is to show that while this village is relatively 'un-modern,' the women of Feriyat lead very beautiful, meaningful lives.

Examining Feriyat through the lens of the UNMDGs

Goal 1: End Extreme Poverty and Hunger: Halve the proportion of people whose income is less than $1 a day; Achieve full and productive employment and decent work for all, including women and young people; Halve the proportion of people who suffer from hunger.

The United Nations Human Development Index (HDI) ranks Morocco at 123 out of 177 countries with regards to poverty. The HDI is composed of many factors, all of which are used in tracking countries' progress towards achieving the UNMDGs. According to the United Nations Development Program's Multidimensional Poverty Index (MPI), Morocco has an index of 4.8%. The MPI is calculated by taking household size and examining those households having no household member who has completed five years of schooling and having at least one school-age child (up to grade 8) who is not attending school; having at least one household member who is malnourished and having had one or more children die; not having electricity, not having access to clean drinking water, not having access to adequate sanitation, using "dirty" cooking fuel (dung, wood or charcoal), having a home with a dirt floor, and owning no car, truck, or similar motorized vehicle while owning at most one of these assets: bicycle, motorcycle, radio, refrigerator, telephone, or television.[2] The maximum MPI score is 100, with the poverty threshold being 33.3—that is, any household with a MPI score greater than 33.3 is categorized as impoverished. A rating of .048 means that 4.8% of households in Morocco fall into the UNDP's "poor" category. 2.5% of Moroccans live below the international poverty line of $1.25 purchasing parity power/day, down from 8.4% in 1985 and 9.7% of the total population is unemployed while around 20% of Moroccans between 15-24 are unemployed.[3]

Feriyat, in the context of this goal, is very interesting. Throughout this project, observational research was conducted on the El Amm family. Within the El Amm family, the parents received no education while the younger generations of children and grand children have completed or are enrolled in primary schooling. There were no school-aged children who did not attend school. From my observations of families living in rural areas, meals consist mainly of bread and potatoes, and a truly extraordinary amount of sugar is consumed on a daily basis. Because of cultural norms, women receive very little meat, should any be available. Unlike other families in Feriyat, the El Amm family possessed only male/bull cows. Because a cow must produce a calf to produce milk, and male cows do not birth calves, bull cows do not give milk.[3] Milk is a major source of protein, vitamins, and vital nutrients, especially when meat is unavailable. The El Amm family does not have access to sanitized drinking water or "adequate" sanitation (both of which shall be discussed later). They do, however, have several televisions, several cellular phones, and a bicycle. The floors are cement and while propane is the main source, both coal and wood are also used for cooking.

Feriyat is primarily an agricultural village where men work with livestock or in the fields while the women cook, clean, and take care of all domestic tasks. The proportion

of unemployed people is astronomically high. Not only could most people be found sitting idly throughout the day, but it seems that the only realistic option for employment, at least in the eyes of male Feriyat citizens, is immigrating to Spain to do menial labor and sending money home. In the El Amm family, two of the four sons live and work in Spain and another is in the process of obtaining the necessary paperwork and visas to immigrate and join his brothers. As there is no solid definition of "meaningful employment," it cannot be determined with the available information whether or not domestic tasks are considered "meaningful." In examining Feriyat through the context of this goal, it becomes obvious that much work needs to be done in order to meet this particular UNMDG, particularly with regards to diets.

Goal 2 and 3: Universal Education: Ensure that children everywhere, boys and girls alike, will be able to complete a full course of primary schooling; eliminate gender disparity in in all levels of education

These goals are two of the most interesting and important UNMDGs, and are the main focus of this paper: not only do they state the importance of universal access to education, but emphasize the fact that mere physical access to a school is not enough. In developing nations around the world, there are sometimes enough schools but not enough teachers or supplies. In some cases, the mere physical presence of an education system is not enough. Children in developing nations, especially females, are usually burdened with a large load of domestic responsibilities from a young age. They are expected to assist in household chores and take care of younger siblings as soon as they are old enough to do so. These societal expectations prove to be a challenge to achieving universal education. Morocco has made great strides towards the goal of universal education. Between 1990 and 2011, Morocco's Education Development Index ranking for education rose from .254 to .447.[4] The education index measures a country's relative achievement in both adult literacy and combined primary, secondary, and tertiary gross enrollment. First, an index for adult literacy and one for combined gross enrollment are calculated. Then, these two indices are combined to create the education index, with two-thirds weight given to adult literacy and one-third weight to combined gross enrolment.[4] In fact, the entire Arab region has seen a drastic increase in HDI: in 1980, when the HDI was introduced as a more comprehensive means to analyze development than previously existing factors such as GDP/GNP, the overall HDI of the Arab world increased from .444 (1980) to .641(2011).[5] Morocco's HDI, according to the most recent data published by the UNDP, is .582, contributing to an overall HDI rank of 130 out of 187 countries and below the average HDI in comparison to the rest of the Arab world. One of the contributing factors to Morocco's fairly dismal HDI ranking is its Gender Inequality Index (GII) ranking: Morocco's GII ranking has gone from .706 (1995, first available ranking) to .510, reflecting an increase in female achievements in reproductive health, empowerment, and participation in the labor force. The Gender Inequality Index (GII) reflects gender-based disadvantage in three dimensions: reproductive health, empowerment, and the labor market. The index shows the loss in potential human development due to inequality between female and male achievements in these dimensions. It varies between 0, when women and men fare equally, and 1, where one gender fares as poorly as possible in all measured dimensions.[5] As this explanation shows, having a ranking closer to 0 is desirable. Over the last fifteen years, Morocco has made many changes to its society that have contributed to its overall improvements in the HDI. There are now more women in parliament than ever before and the recent Moudwanna reforms greatly increased women's rights regarding marriage and divorce and shared responsibility of the household.[6] There has been an influx of government support in sending rural girls to school, with the development of live-in dormitories/student houses at little or no cost (as subsidized by the government or various NGOs) to accommodate the need for housing nearer to secondary schools. UNICEF's data shows that girls' education in Morocco, in light of the UNMDGs, has been doing very well: (all data 2005-2009) primary school attendance ratio of females to males (gross) is 88; survival rate to last primary grade administration data is 76%; secondary school enrolment ratio of females to males (gross) is 51; secondary school attendance ratio among females to males is 36; and literacy rate for females 15-24 years of age is 68%.[7]

Feriyat is an excellent example of recent change in the global effort to reach universal education. Approximately ten years ago, according to Dr. Lahcen Haddad, a primary school was built to cater to the villages' young children. Both boys and girls attend the school, with the age range being larger than the typical school as some children began their schooling at a much older age than is standard. In Feriyat, there are undoubtedly more boys in each classroom than there are girls, whether this is due to more boys of primary school age living in the region or due to societal expectations regarding girls' education is unknown. However, it is of note that every teacher in Feriyat's lone school is male, which speaks to the history of inequality in education in rural Morocco. The mere creation of a primary school in Feriyat within the last decade shows initiative, and with the increase in financial support from both the government and various NGOs, of girls' secondary education, Morocco is dedicated to achieving universal education.

Goals 4 and 5: Child and Mother Mortality Rates: Reduce, by two-thirds, Infant Mortality Rate for children under 5; Reduce, by three-quarters, Mother Mortality Rate; Achieve Universal Access to Maternal Care

According to UNICEF, the infant mortality rate for children under 1 year in 1990 was 69/1,000 and, as of 2009, is 33/1,000 live births; and for children under 5 years was 89/1,000 (1990) and is now 38/1,000(2009). The decreases in infant mortality rate are on track with the goals of the UNMDGs: mortality for children less than 1 has been reduced by 47% and less than age 5 by 43%. Morocco is 74th in world for infant mortality, with each woman having a 1/360 chance of dying due to pregnancy/birthing related complications, and 66th for maternal mortality: one of the reasons for this relatively high ranking could be that there are only .62 physicians for every 1,000 persons (in comparison with, as a base, the U.S'. 2.672 physicians/1,000 persons and Egypt with 2.83 physicians/1,000 persons).[6] Because there has been a low physician to person ratio for a long period of time, Moroccans have become adept at home-birthing in areas where doctors are not readily available. That is reflected by the relatively low maternal mortality rate. This being said, it is important to note that many women prefer to give birth at home. Giving birth in the home is preferable to some because the home is seen as a warm atmosphere where a woman can be surrounded by her family members. "Giving birth is perceived as a natural event and not a sickness," so many women do not understand why they should go to a health center. Norms among rural Moroccan communities have shifted: no longer are women expected, because of cultural/religious reasons, to go through pregnancy unaided by medical professionals. However, after speaking with several pregnant women, it was ascertained

that while traveling to nearby Bejaad for regular check-ups is now the norm, it is still expected that a woman will give birth in her home. Despite this societal expectation, the increase in hospital visits during pregnancy has resulted in a decrease in both mother and infant mortality rates in rural Morocco.

Goal 6: Combat HIV/AIDS: achieve universal access to treatment for HIV/AIDS for all those who need it; have halved by 2015 and begun to reverse the incidence of malaria and other major diseases

Morocco does not have a serious problem with HIV/AIDS: according to UNICEF, only 19,000-34,000 people are living with HIV/AIDS as of 2009, and the nation-wide prevalence rate is .1%. Other major infectious diseases such as Typhoid, Malaria, and Encephalitis are similarly a non-issue in Morocco. The biggest challenge faced by HIV/AIDs prevention and education organizations is social stigma. In the conservative Muslim culture of Moroccan society, discussing sex is taboo, thus the discussion on how to prevent HIV and other STD transmission does not occur in a formal setting. As a result of this, only 12% of girls between 15-24 have a "comprehensive understanding of HIV".[7] High risk groups such as truck drivers, sex workers, and prisoners are typically more prone to contracting HIV and other STDs than other people. As a result of this, organizations such as Moroccan Association for the Fight Against Aids and Pan-African Association for the Fight Against Aids have begun programs that target at risk individuals. One of the main forums for teaching safe sex practices is through sessions that teach sex workers how to properly use condoms. According to a 2010 article written on AIDs awareness in Morocco, because Moroccans see themselves as living in a conservative culture, there is strong resistance against HIV/AIDs education because it necessitates discussing sex outside of marriage.[8] While no data was available on the attitudes of the Feriyat education system towards HIV education, it can only be assumed that in this extremely conservative community the issue is not discussed, partly because it may seem irrelevant or because it is taboo.

Goal 7: Environmental Sustainability: Integrate the principles of sustainable development into country policies and programs and reverse the loss of environmental resources; Reduce biodiversity loss, achieving, by 2010, a significant reduction in the rate of loss; Halve, by 2015, the proportion of the population without sustainable access to safe drinking water and basic sanitation; By 2020 to have achieved a significant improvement in the lives of at least 100 million slum dwellers

In light of the global population reaching 7 billion, the need for environmental sustainability will become even more important. In the context of Feriyat, however, the idea of environmental sustainability is virtually non-existent. Feriyat is a community that survives mostly off of agriculture and because of that there is rampant over-harvesting and illegal clearing of land in-order to make way for more useful or profitable crops. As of 2008, 81% of Moroccans have access to improved drinking water sources, with 98% of urbanites having access and 60% of rural persons having access. 69% of Moroccans have access to improved sanitation facilities, with 83% of urban persons and 52% of rural persons having access to improved sanitation facilities.[7] It should be noted that traditionally, Moroccans (along with many other countries around the world) use Turkish/squat toilets instead of western toilets. The possession of Turkish toilet that connects to a septic system is considered an "improved sanitation facility," thus the lack of a Turkish toilet or septic system would be considered unimproved. In Feriyat, toilets of any kind are far and few between and there is no septic system—the majority of families use nature as their bathroom. Plumbing is similarly unheard of or very few families have even squat toilets and those that do lack any form of plumbing. Thus, their waste goes directly into the ground. Most families in Feriyat are dependent upon the rains for their water, as they get water from illegal wells that collect rainwater. Running water is scarce: at the time of writing, there is only one known family in Feriyat with running water. Because of the vague nature of 'significant improvements in the lives of 100 million slum dwellers' it is unknown whether or not this applies to Morocco.

Goal 8: Global Partnership: Develop further an open, rule-based, predicable, non-discriminatory trading and financial system; Address the special needs of the least developed countries; Address the special needs of landlocked developing countries and small island states; Deal comprehensively with the debt problems of developing countries; In cooperation with pharmaceutical companies, provide access to affordable essential drugs in developing countries; In cooperation with the private sector, make available benefits of new technologies, especially information and communication

As Morocco is not a landlocked country, small-island state, or classified as a "least developed country," and has a strong import/export relationship with the Western world and is not a country with a high risk of major infectious diseases, many of aspects of this particular UNMDG are to some extent irrelevant. Furthermore, many of the goals of "Global Connectedness" are geared towards improvements that can be made by the government and large corporations, and as this paper focuses on the grass roots level, inappropriate to discuss here. There are, however, some aspects of this UNMDG that can begin at a grass roots level. The use of internet and social media websites, such as Facebook, played a major role in the recent Arab Spring. The worldwide popularity of such websites speaks to the interconnectedness of the entire world, as people of all ages living in any country are capable of connecting with one another over the Internet. Even people living in rural areas have "profiles" on Facebook, despite having extremely irregular access to the Internet. In Morocco, the prevalence of social media networks and cell phones is a nationwide phenomenon: 72/100 people have cellular phones while 33/100 have regular access to the Internet. It should be noted that the cellular phone statistic is skewed; many urban Moroccans have two or more personal cellular phones, thus increasing the density of cellular phones throughout the country. In Feriyat, all men were observed to have cellular phones while their female counterparts did not have personal phones. Phone service in Feriyat is extraordinarily limited: in order to make or receive calls, one must climb up a hill and continually adjust his or her position to receive a signal. Another aspect of global connectedness that is not addressed in the UNMDG but should be noted is the international quality of Feriyat. As has already been mentioned, many of the native inhabitants of this region immigrate to Spain in order to find lucrative work, and a few times each year to see their families. Another exceptional characteristic of Feriyat is that American students come for a weeklong "Village Stay" through the SIT study abroad program. Some of these students elect to return later in the semester and, in one case, in their post-collegiate lives. This introduces an interesting dynamic into the village, as the visiting American students are expectedly unaccustomed to village life, and force the citizens of Feriyat to examine how their lives differ from Americans' lives.

Conclusions on UNMDG Status

As can be seen from the brief examination above, Morocco as a whole is doing fairly well in the context of the UNMDGs, partially because some of the issues the UNMDGs aims to change have already been achieved in Morocco. However, the UNMDG that rural Morocco in particular is having the most difficulty with are the ones concerning universal education and gender equality. The remainder of this research examines the historical and contemporary challenges related to these issues, and will look at the viability of non-formal education as a means to create a meaningful society among those who are unable to receive the formal, secular education the UNMDG aim for.

Recent History of Education in Morocco

In order to understand the current situation in rural Morocco, it is important to have a background that provides context for the recent happenings and the societal structure. In 1912, Morocco became a French protectorate under General Louis H. Lyuatey as the resident general.[9] Lyuatey created an intricate and extremely hierarchical system of education. Five different types of schools were created for each social class and, as a student progressed from primary to secondary school, family lineage determined whether or not he could continue his education. In addition to his schools that separated children by their social class, Lyuatey created an entirely different school system for the Berbers as well; "the French regarded the Berbers as a group that needed taming". This belief ultimately led to the creation of a legal separation between the Berber and Moroccan Arab populations in a document called the Berber Dahir. While the dream of a "Franco-Berber" education never came to pass, this strict separation of nations within a state caused deep harm to Moroccan culture as a whole. In 1940, approximately 3% of the school-aged population was in school, a statistic that caused Lyuatey to drastically increase the number of schools throughout Morocco. However, "Lyuatey never envisioned educating the greater mass of Moroccans becausehe wanted only to create an educated Moroccan elite," a fact that is painfully obvious through his policies. Despite exacerbating the socio-economic tensions with his new system of schooling, Lyuatey did a great deal to advance women's education in his tenure as resident general. In 1933, the first all girls' school opened followed by another in 1939. These schools did not, however, instruct their female students in academic subjects, but only in the skills necessary to run a home (this type of education increased a girls' bride price). Prior to Lyuatey's "rule" of Morocco, young girls were generally unable to attend school as learning academic subjects decreased their bride price. While Lyuatey can be accredited with greatly advancing the status of women's education, it was only after Morocco's independence that girls were truly given the opportunity to learn. In 1956, Morocco gained tangible independence from its former colonizer, France. This does not, however, mean that Morocco gained ideological independence from France. At the time of independence, business, politics, and all aspects of life considered "upper class" were conducted in French, while Arabic/Darija were reserved for the "lower class" and more rural communities. Under French rule, education was non-compulsory; at the time of independence a mere 10% of children were enrolled in primary school, 15,000 boys and zero girls were enrolled in secondary school, and 350 students were enrolled at the university level.[10] Thus, despite Morocco's political independence from France, the vestiges of French supremacy and colonialism remained. Lyuatey's system of education was, again, geared only towards educating extremely wealthy Moroccan families and the European community of Morocco, as there were very few corresponding Arabic schools for the general Moroccan populace. Immediately following the formal decolonization of Morocco, the administration's effort to improve agricultural techniques and expand educational opportunities was an indication that it was more concerned with processes rather than simple forms. Numerous other programs have been evolved to bring greater benefits to Morocco's rural population, including the scores of work centers, community development pilot stations (in conjunction with an expert United Nations staff), and the national anti-illiteracy campaign for adults.[11] In 1963, education was made compulsory and mandatory for all children between ages 6 and 13. This mandate led to an immense overhaul of the education system. The Ministry of National Education (MOE) put in place two policies called Moroccanization and Arabization. Moroccanization refers to the replacing of expatriate teachers with Moroccan teachers, a feat that proved particularly difficult as there were very few trained Moroccan educators as a result of Lyuatey's former education system.[9] It was not until the mid-1970s that Moroccans filled the majority of teaching positions. Arabization was the process by which Arabic would replace the French language in all of Morocco's schools. Again, this proved extraordinarily difficult: Arabic, unlike French, is a highly impractical language for education "because of a lack of technical vocabulary in the language." Additionally, the Berber population had "no knowledge of French and very little to no knowledge of Arabic" as a result of its former segregation. These obstacles are what make Morocco such a unique case: in Northern Africa, there were no other states that faced these exact issues, as the native tongue was usually relevant to education.

Despite the many challenges facing Morocco with regards to educational reforms, Morocco has made great strides in improving overall literacy. In 2006, UNESCO awarded Morocco the Confucius Prize for Literacy as a result of the success of Morocco's Non-Formal Education Program. According to UNESCO's press release regarding this award, the Non-Formal Education program in an "innovative national literacy initiative designed specifically for marginalized adolescents in rural areas," coupled with training for recent dropouts who wish to return to school. The mere creation of this program speaks volumes about Morocco's desire to have universal and democratized education for all children. While Morocco's literacy rate is still unfortunately low (hovering around 54%), the initiative to improve education and eventually achieve universal literacy has been shown by the government.[6] In requiring all children regardless of gender, social class, ethnic group, or religious affiliation to attend school, Morocco has firmly asserted itself as a nation willing and eager to participate in the rapidly globalizing world. Education is, without a doubt, one of the most obvious ways for a nation to increase its participation in international organizations; as a larger percentage of the populous receives education, the status of a country increases rapidly in the eyes of global leaders.

Contemporary Issues in Education

Now, 55 years after independence, Morocco faces a fairly different set of issues when it comes to education. Education in Morocco is free and compulsory through primary school (age 15). Nevertheless, many children, particularly girls in rural areas, do not attend school, and most of those who do drop out after elementary school. The country's literacy rate reveals sharp gaps in education, both in terms of gender and location; while countrywide literacy rates are estimated at 39.6% among women and 65.7% among men, the female literacy rate in rural areas is estimated only at 10%.[12] According to Dr. Lahcen Haddad, a member

of the Committee for the Promotion of Girls' Schooling in Rural Areas and President of Bejaad for Sustainable Development, the main obstacle facing Morocco in its efforts to achieve universal education is accessibility. Because a large proportion of the population lives in rural communities which do not necessarily have schools, the education rate is very low among these people.[6] Children sometimes have to walk miles in order to reach a secondary school, as most villages have primary schools, but a group of villages will usually 'share' a secondary school to supplement the lack of available teachers and funding. Furthermore, Dr. Haddad says oftentimes families cannot pay to send all of their children to boarding schools in order to continue their education. In this situation, male children are almost always given preference over female children. To combat this, organizations such as the Committee for the Promotion of Girls' Schooling in Rural Areas have begun to crop up all over Morocco. These organizations offer rural girls the opportunity to live in dormitories for free and study at the secondary level. But what happens to the girls who are not lucky enough to attend school or become literate? Are they relegated to live rural lives, following in the uneducated footsteps of the ancestors? In learning more about Moroccan society through personal experiences in rural and urban communities, the conclusion was reached that the women who live these rural, uneducated lives are living lives that are equally as valuable and meaningful as their educated, urban counterparts.

Challenges Specific to Rural Populations with Regards to Education: Observational Research in Feriyat

As was mentioned earlier, significant portions of Moroccans live in rural communities. Generally speaking, rural communities are much more 'traditional' than their urban counterparts, with gender roles being much more polarized. Women bear the brunt of cooking, cleaning, fetching water, and generally ensuring that their household runs smoothly. Men tend to the livestock and were observed to have an enormous amount of leisure time compared to women. A very small number, if any, of the adult women in Feriyat, the rural village where a portion of the observations were conducted, were literate. However, most of the female children in this community were enrolled in the local school, illustrating the shift in attitudes towards and the availability of education.

These five women, who were all as different in looks as in personality, were the kindest, most beautiful women I have ever had the pleasure of meeting. None of them had attended school and all were illiterate, but living with them that helped me realize that education does not always come in the classroom. Rayhana, the eighteen-year-old wife of one of my brothers, was seven months pregnant with her first child. Despite being so young, so newly wedded, and so very pregnant, Rayhana was the first of my three "sisters-in-law" to truly welcome me into her household. She decorated me with beautiful henna, asking questions about my life and, when my limited Darija ran out, speaking to me as if I could understand simply because she did not want me to feel isolated in this strange new home. Rayhana had been married into a polygamist household, that is, she had two mothers-in-law. Both of these women, whom I called Mama Wahed and Mama Jooj, were incredible human beings who possessed enormous hearts capable of loving any and every one that came into their home. They were around 55 years old and had born many children: in my home, five sons remained and several of the other families in the village were borne of their kin. I wrote a short story that showed that education does not only imply formalized schooling, and that women from very different backgrounds in Morocco are able to live in an inexplicably beautiful manner regardless of whether or not they have gone to school.

Wabi-Sabi, or, The Act of Finding Beauty in Inherent Imperfection

Khadija, so it was said, was born on a cloudless night when the moon was so bright that one could see as if it were day. Her birth was miraculous, so it was said, because despite the cloudless sky, rain pounded the earth and the wind raced through the air, creating rivers that swept away chickens and threatened to flood even the loftiest of compounds. Perhaps owing to her extraordinary beginning, Khadija possessed an innate ability to shed light on beauty in the most dire of circumstances. Or perhaps, it was her ability to do this that allowed her to be born in the midst of a wild storm. Despite her incredible creation, Khadija was not destined to live an extraordinary life, at least not in any tangible manner. Her life was to be spent raising and caring for a sprawling family in the rural Moroccan countryside, toiling away to create a paradise for her loved ones.

The lazy buzz of flies in the mid afternoon sun lulled Khadija into a soothing rhythm as she wound row upon row of colorful yarn into intricate patterns, slowly creating a beautiful rug. Sitting on her low stool in the cool comfort of one of the many small rooms in her compound, Khadija half listened to the sounds of the Moroccan hillside and half hummed a song she'd never heard as she waited for her family to assemble en masse for tea.

"Dada Dada Dada!" A miserable wail suddenly pierced through Khadija's calm. Seconds later, a sticky toddler stumbled into the weaving room, tugging on Khadija's pajamas, a look of pure ecstasy at the sight of his grandmother erasing the misery of moments before. It was not just baby Abdhakim who was filled with happiness whenever he was in the presence of Khadija, but everyone who encountered her.

Khadjia could always solve the most difficult of problems. When she was sixteen, she became the second wife of Hassan, a man in the village next to where she had grown up. Hassan's first wife, Fatima, was a beautiful, fiercely proud woman, and was furious that her husband had deigned to take a second wife. Fatima, of course, could not fully express her anger to Hassan, but held no reservations in ensuring that Khadija knew she was not welcome in Fatima's home. Fatima refused to help Khadija in fetching water, causing Khadija to spill much of her precious cargo whenever she returned home. Her young arms were too weak to lift the heavy jugs from the family's donkey. Khadija bore these cruelties with a smile, never complaining about her burden and answering in only the sweetest tones when Fatima addressed her. At first, this caused Fatima to become colder and angrier. But slowly, Khadija's shy smile and laughing eyes melted Fatima's heart, and she relented in her anger.

Khadija, of course, had recognized Fatima's hatred for her and was not unaffected by it. She was acutely lonely for the first months of her marriage, living in this strange village with an unwelcoming woman and large, gruff husband. Khadija's mother had always taught her that if she could laugh instead of weep, smile instead of frown, and hug instead of hit, Allah's kindness would beam down upon her. And so this is what Khadija did for several years until, one day, finally, Fatima relented.

"It is not the fault of Khadija that Hassan took her to be his wife. It could not hurt to show this girl some kindness," Fatima thought. And so, day-by-day, Fatima allowed Khadija to become a member of the family and to share in the love that enveloped their home.

Khadija and Fatima grew old together under the hot sun of the African sky,

fetching water, cooking bread, and birthing many, many children. The two women grew inseparable, to the point that it seemed impossible to imagine a time when they had been anything less than sisters. Their daughters grew up and moved on to have families of their own, some of their sons moved abroad to seek their fortunes, but the two women were never alone. Slowly their remaining sons married women, who, like Khadija had done so many years before, made treks from villages near and far to appear at their compound, eyes wide with nerves and hands clutching their belongings. Fatima realized that Khadija was most adept at welcoming these frightened, young women into their homes and serves them their first tea.

The first child to bring a new wife into Khadija's home was Abdwahed, her second son and most beloved (though this was, of course, a secret). When Abdwahed's young wife, Miriam, appeared in Khadija's home, the elderly woman's heart swelled with empathy as she recalled her first time stepping through the low doorway into the dusty compound. Miriam wore a similar look of fear and anxiety as Khadija poured her a glass of impossibly sweet tea. Khadija told Miriam all about her new home, trying to prevent the foreboding silence from creeping into the cracks of their conversation. Miriam sat, her eyes like orbs, as she did her utmost to listen to the words of this kindly woman and blink back the tears that constantly threatened to fall in her tea. Gradually, Miriam's pounding heart slowed its pace and her tears dried before they could even fall.

As the years passed, new wives entered Khadija's home and soon there were babies and children running around her family's compound. Their musical voices carrying through the still summer nights. Khadija too grew older as the seasons changed, yet her ability to soothe those around her remained. Even during the harshest of winters, when it seemed the sun would never emerge from its lair and when the food was scarce and the wells frozen, Khadija refused to let her family go hungry. She meticulously portioned every morsel of food at each meal, ensuring that all the members of her continually growing family were always well fed and left mealtimes with smiles upon their worn faces.

Khadija taught generations of girls how to stretch food during the winter, how to weave fantastically beautiful rugs and where, precisely, to touch a cow in order to coax her into giving milk. Her granddaughters were able to attend the new elementary school, proudly showing Khadija their painstakingly written alphabet while she clapped in delight, her joy in sharing in their knowledge more than if she herself had suddenly learned to read. The many years under the unforgiving sun and stinging dust had taken its toll on Khadija's body, yet her spirit went unmarred.

Khadija's gift lay not in any ability to toil away at a desk or in leading a country, but in teaching those around her how to love one another, and the steps necessary in order to carry out their lives in a peaceful, wonderful, beautiful way. In order to do all of this, Khadija always said, you must share what Allah has given you, and only then will the world become beautiful.

While this story depicts a woman whose life falls far outside of the UNMDGs, it is meant to show that living a life outside of these lofty goals is by no means less meaningful or less "good" than living a life within the UNMDGs. Despite Khadija's lack of formal education, she passed on what are perhaps the most important human qualities to her children and those around her, teaching them to be kind, sharing, and patient.

Final Conclusions

It would be ignorant and cruel to say that the women in Feriyat are better off without formal education or to say that their lives are filled with a simplicity that is unavailable in the developed world. These women lead intellectually and physically challenging lives, in a way that is unimaginable to someone from the western world. Would their lives be different if they had been able to attend school? Would Mama Wahed and Mama Jooj have entered into a polygamous marriage as very young women? Would they still be living in Feriyat, weaving rugs and baking loaves upon loaves of bread? Would they still be laughing with their sons, engaging in animated conversations with each other and appreciating the mere presence of their family members in the same way? Most likely, the answer is no. In comparison with their urban counterparts, the women in the village are far more welcoming and possess a unique and innate sense of sharing.

After a thorough examination of Feriyat through the lens of the UNMDGs and after spending considerable time with the El Amm family, a conclusion has been reached: it is not reasonable to speculate how a rural community would be changed if the UNMDGs were accomplished. Furthermore, trying to decide whether or not a community would be "better off" after having achieved the UNMDGs is seemingly impossible. Of course, there is no question that any community would be better off without HIV/AIDS, infectious disease, malnutrition, and poverty. However, altering the employment, education, and "global connectedness" status of any community, rural or not, would have an enormous impact upon societal functions. One of the most common effects of an increase in education in a community is an increase in the number of people who choose to leave their homes after finishing education. Because education is known to open students' minds to new ideas, cultures, and places, it stands to reason that after finishing a full course of schooling a student would choose to live elsewhere. This can be positive, if the hypothetical is escaping a community that is rife with poverty or violence or this can be negative because it contributes to a loss of culture. With each person that moves away, their cultural traditions are diminished as they change to accommodate the cultures of the new community. That being said, universal education should remain a global priority: the positive externalities of educating even a small portion of any community are numerous, and the opportunities made available to educated persons are virtually endless. In light of the struggle to achieve universal education, Morocco has done a wonderful job in implementing new strategies to make up for so-called "lost time" during its colonization. Morocco, as a whole, has done a great deal in furthering girls' primary and secondary education. Various political and social programs have resulted in a shift in cultural norms, particularly within the last fifty years that encourage girls to attend school and to seek work outside of the domestic sphere. While there is still a long way to go before Morocco can be categorized as a country with universal education and equality in education, the recent efforts of the Moroccan government and various NGOs should inspire hope in the eyes of education-activists. Only time will tell how the efforts of the global community to create a system of universal education will impact global society and social constructs. If progress continues to be made at its current rate, it would appear the future holds great things for universal education and gender equality.

References

[1](2010) "We Can End Poverty 2015." *A Gateway to the UN System's Work on the MDGs.* <UN.org> (11/29/2011)
[2](2011) "Technical Notes: Technical Note 4. Calculating the Multidimensional Poverty Index." *Human Development Report 2011.* UNDP. 173.
[3](2011) "Morocco Country Profile: Human Development Indicators." <www.Undp.org> (12/06/2011)
[4](2010) "Jersey Cow Facts." *Woodside Farm Creamery.* <http://woodsidefarmcreamery.com> (02/24/2013)
[5](2011) "Technical Notes. Technical Note 3: Calculating the Gender Inequality Index". *Human Development*

Reports. UNDP. 171.
[6](2009) "CIA-The World Factbook: Morocco." Central Intelligence Agency. <www.cia.gov> (01/17/2011)
[7](2010) United Nations International Children's Fund. "At a glance: Morocco." <www.UNICEF.org> (10/31/2011)
[8]Siham A. (2010) "Moroccan prostitutes focus of controversial AIDS education effort." <www.Magharebia.org> (10/05/2011)
[9]Degore, B. (2002) "The Modernization of Education: A Case Study of Tunisia and Morocco." *The European Legacy*,Vol. 7. Issue 5. Pg. 579-596.
[10](2010) *Morocco Education System Overview*. <education.stateuniversity.com> (12/15/2011)
[11]Lewis, W.H. (1960) "Rural Administration in Morocco." *Middle East Journal*, Vol. 14. Issue 1. Pg. 45-60.
[12](2010) "Background Note Morocco." United States Department of State. <www.USDP.gov> (11/23/2011)

The Impacts of Westernization through Short-Term Volunteer Teaching in Ayutthaya, Thailand: An Observational Study

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Analyzing the manner in which developing nations are incorporating western ideas into their social, economic, and educational development is integral in understanding the consequences of "globalization/ westernization" in developing nations. In emerging Southeast Asia, the incorporation of western values into a traditional system has greatly increased in the last several decades. Therefore, it is an ideal region for observational research on globalization in the volunteer teaching industry.

While it has been frequently occupied by various militaries throughout its history, Thailand is the only country in the region that has retained autonomous rule. Because of this, Thailand's experience with globalization/westernization is fairly distinct. The globalization of Thailand has largely been driven by a strong tourism as Southeast Asia, and Thailand in particular, is one of the most popular backpacker destinations in the world.[1]

Thai Culture: The Struggle to Maintain Tradition in a Global System

Ayutthaya was the capitol of the Kingdom of Siam until 1767 and is still often considered the cultural capitol of Thailand. Located two hours by train outside of Bangkok, Ayutthaya has a constant stream of tourists who patronize the many ruins and more than 500 temples within the city. With this constant flow of foreign nationals comes an inevitable exchange of ideas between tourists and inhabitants.

The presence of tourists and foreign business people is not new to Ayutthaya. In its peak in the 18th century, Ayutthaya was a haven for a large portion of the world's sea merchants. Indians, Malays, Vietnamese, Japanese, Persian, and later Dutch, Portuguese, Spanish, and French traders made their port in Ayutthaya.[2] The presence of these traders has created many sub-groups within Ayutthaya, as strong Chinese and Japanese influences can be seen in the architecture, religious sites, and food. Because so many non-Thai traders came to Ayutthaya to sell their wares, the Thai people and government have had to adapt accordingly to the presence of non-traditional beliefs. In the 17th century a large number of Muslim merchants had settled in Ayutthaya so King Narai (r.1656-1688) built a mosque to show that his government and country welcomed the Muslim merchants and respected their faith.[3] This shows that the Thai kingdom has a history of "openness" when faced with the necessity to adapt, a willingness that is more than crucial in the globalization of an ancient culture.

This readiness to adapt can further be seen in the recent increase in Foreign Direct Investment (FDI) in Thailand and, surprisingly in Ayutthaya. Several Chinese and Japanese factories have businesses in Central Thailand and have branches in the greater Ayutthaya area.[4] With an increase in FDI; the region has seen a shift in employment opportunities, especially for the middle class. This in turn has caused the government to design educational reforms meant to better prepare students for non-traditional jobs. While these businesses may be Eastern in nature, their presence suggests a greater level of connectivity between Thailand and other Asian countries. Furthermore, the move of these companies into Thailand will likely provide job opportunities that may necessitate some sort of standardized education, which is where a generic "western" education could come into play.

A culture steeped in tradition, the idea of introducing a western education is often met with resistance from Thai people. "In the past…the lives of Thai people revolved around the temple…[but] ever since [Thailand] opened its doors to the western powers, the Buddhist condition has gradually deteriorated. Our ruling elites were sent to study abroad instead of to the local temples… relegating the temple to the backseat."[5] With western cultural influences come western ideals, some of which are thought to be rather incompatible with a culture rooted in Buddhism. According to Sulak Sivaraksa, one of Thailand's leading Buddhist scholars, "technology and modernity uproot and destroy the traditional way of living and the traditional conception of beauty and goodness. …All this is being done in the name of 'being civilized' or western civilization."[6] Buddhism does not hold the same place in modern Thai culture as it once did in the traditional culture. However, it is still seen as an integral part of the education system and society. For example, the older student body of Pratomwittahyakom School visited Wat Pranhomyong. During this time at Wat Pranhomyong, students were instructed by monks in the many different ways of praying, meditating, and the core social values of Buddhism. The monks used a range of media to instruct the children, utilizing mainly a lecture style for prayer instruction, interactive sessions for meditative instruction, and a variety of videos downloaded from the internet to illustrate the Dos and Don'ts of Buddhist culture. The usage of non-traditional media to convey very traditional Thai values epitomizes the changing status of Thai culture within the education system.

While Ayutthaya is a relatively heterogeneous population (approximately one third are Muslim, a very high concentration for its region), Thailand as a whole is still relatively homogeneous in religious association. 90% of Thailand nationals identify with Theravada Buddhism, which is the traditional and more conservative form of Buddhism.[6] Buddhism has influenced Thai culture and education in many ways, including the strong belief in the importance of literacy and the emphasis of learning through experience. It has also lead to the building and maintenance of expansive libraries and to the idea of learning from all fields of knowledge.[7] Buddhism played a fundamental role in the development of Thailand as a state and culture, a fact which is still observable in educational values.

The History of Western Ideals in Thailand: The Pibul Era

Returning once more to Thailand's history of autonomous rule, it is important to examine the manner in which Thailand has introduced western ideals into its society. Unlike their Malaysian, Indonesian, or Vietnamese counterparts, the Thai people never had a western societal framework brought on by colonization. This has resulted in the Thai government having to internally set up a system of globalization, which, according to some, was highly detrimental for Thai society.

Chao-Tzeng Yawnghwe, of the University of British Colombia wrote that "Pibul was very much influenced by ultra-nationalistic, authoritarian European regimes and Japanese militarism… He strove to emulate…Mussolini and Hitler. Thus inspired by fascism, he introduced measures to instill a nationalistic, militaristic spirit in the Thai people and to socialize them in the ways of modern nationhood… Pibul changed the name of the country from Siam to…Thailand in English. He also decreed a set of "cultural mandates", which compelled Thais to learn the national anthem…and dress in western garb, among other things. He employed the mass media and educational system to popularize a new history, [which] was filled with prideful ethno-national rhetoric, tales of great saviour-kings, ancient empires, and glorious wars… It deliberately emulated contemporary western history books which aimed to provide the nation with an organic-historical link to its "golden past"".[8] Unlike many developing nations, Thailand has had to internally force westernization through political policy instead of through colonization.

History of the Thai Education System

The easiest way to indoctrinate a society with a new ideology is through the education system; if young children grow up learning a certain set of ideas, they will act in accordance with those principles. Indoctrination of a countries youth allows ideas to become the norm of a society. When Thailand laid out its ambitions to create a more global youth, they identified the educational system as one of the most important targets. This was seen in 1999 during the World Conference on Education for All in Jomtien, Thailand, where leaders from 155 states from around the world agreed that education was a basic fundamental right and necessary to develop.[9] The government has passed many minor and several major reforms in order to ease the transition of a totally Thai education system to one that is capable of using western material and teaching styles.[10]

Throughout Thailand's long history of education, there have been several periods of extreme change within the system. In Buddhism, the concept of education is very important. Literacy is thought to enable one to better understand and analyze the world, which furthers one's ability to understand how to distance oneself from the mundane and eventually achieve Nirvana. Thailand has had at least some form of formal education for several thousand years and the education system has changed based on the introduction of new ideas or even on the whim of a leader. The first modern reform for education came in 1921, when King Rama V enacted a compulsory primary education law that made some primary education mandatory for all children. Previously, the formal school system consisted of four years of lower-primary, three years of upper-primary, three years lower-secondary, and two years of upper-secondary, or three years for the vocational stream. It was colloquially called a 4:3:3:2 (or 3) formal school system with four years compulsory for all geographical areas and seven years compulsory for some large communities. In this system, it was found by many researchers that half of the fourth-grade leavers did not graduate to the fifth grade and in addition, a third of those fourth-graders lapsed back into illiteracy after three years. New reform changed the 4:3:3:2(3) school system to the 6:3:3 system with six years compulsory for all Thai children.

Prior to this law, only students living in the metropolitan areas were legally required to attend school. For the next several decades, the percentage of students enrolled in schools increased at a steady rate with the implementation of minor education reforms, until 1999 when another series of major reforms were set into motion. A sudden, dramatic increase was not seen partially due to the shortage of trained teaching personnel. The 1999 reforms dictated several things: firstly, that the standard lecture/rote memorization system of learning was no longer ideal and that a more dynamic and analytical form of teaching would be used in the school system; secondly, the education system was also decentralized (from being controlled entirely by the Ministry of Education (MOE)) to allow communities to have more autonomy in their educative decisions; under these reforms, teachers are now required to undergo more rigorous training in order to obtain and maintain their positions; and, finally, a more stringent internal and external evaluation system was developed by the MOE.[11] In implementing these reforms, the Thai government sought to bring the Thai education system up to the same standards as its western counterparts; no longer would Thai students learn by rote memorization. Instead, an actual understanding of the "why" aspect would become an integral part of the education system.

The actual presence of these reforms in Prattomwittahyakom School was less than obvious. While it is not possible for this research to include a comparative field analysis of the pre and post-1999 reforms, it is worthy to note that at least within Prattomwittahyakom School, the teaching style continues to be based almost entirely off of rote memorization. With that being said, and with a short background of the history of the region and general education reforms within Thailand, an analysis of Ayutthaya's school system and its efforts to modernize can begin.

The Education System and Westernization: Challenges with Short-Term Teaching Volunteers in Ayutthaya

As the process of globalization has greatly accelerated within the last several decades, the Thai school system has determined that the need to modify its educational system in accordance with global expectations is of great importance. This movement has resulted in many schools undergoing major reforms to incorporate a global curriculum into their existing school system. However, some other schools have chosen to adhere to their traditional values and incorporate only small amounts of global material in their curriculum. The struggle between globalized culture and Thai culture can be found time and again in the education sector. This dichotomy between the two cultures has manifested itself in the "widespread recognition that the current [education] system is inefficient and ineffective at meeting the demands of the emerging era… [but] the values and assumptions underlying these 'modern' educational practices run counter to traditional cultural norms of Thai Society".[12] In Pratomwittahyakom School, this dichotomy has manifested itself into the importation of both native and non-native English-speaking teaching volunteers in an effort to bring an element of the West to students' education without overhauling the entire system. There are many advantages and disadvantages to this system of teacher-importation. Two of the most obvious are that Thai students benefit through the opportunity to learn from Westerners of different nationalities and backgrounds, but then are conversely

subjected to the many different "dialects" of English that are heard between American, German, British, Australian, and Icelandic teachers. During research, volunteers from all of the aforementioned regions as well as France, Mexico, Ecuador, and Canada. The subject of the different accents of English upon English acquisition in English language learners has been studied for a number of years. In one such study done on English language learner accent preference, 52% identified American English as the easiest form of English to understand.[13]

This may be because American English (when compared with English spoken with British, Chinese, and Mexican accents) is spoken at 147 words per minute, as is Chinese English, while British English is spoken at 160 wpm and Mexican English is spoken at 180 wpm. This conclusion was evident in the communication between various volunteers at Pratomwittahyakom school; teachers and students alike were virtually incapable of understanding English spoken by British volunteers purely because of their accents. On multiple occasions, a British volunteer would ask a question and, instead of attempting to respond, the person in question would immediately default to an American volunteer to repeat the question. Thus suggesting that it was the accent, and not the content, that caused Thai persons such difficulty in comprehension. In Ayutthaya, there is an ever-changing range of accents, from European to North and South American, making it even more difficult for students to understand how to pronounce many English words and even how to identify certain objects. For instance, in Britain and Australia the word Honeydew refers to what is called Cantaloupe in America. The word Honeydew, in America, refers to a melon that is called Winter Melon elsewhere. While this may seem like a trivial matter, these minute differences in the English language exacerbate the difficulty of learning English from foreign nationals.

Many of the detrimental aspects of short-term volunteer teachers observed in Ayutthaya were also found in a study on volunteer teaching conducted in the Ningxia province of China. In both the Ayutthaya and the Ningxia province, the short-term aspect of the teachers "failed to solve the fundamental problem of teacher shortage [and]…disrupted the normal teaching schedule."[14] Because teaching contracts in the aforementioned study are usually around one year long, the challenges seen by schools in Ayutthaya are greatly exacerbated. Volunteers in Ayutthaya are usually present between one and eight weeks, but occasionally up to twelve or more. During the period of study in Ayutthaya, the school semester was in full swing and the constant introduction of new volunteers to the schools was a continual disruption for both permanent faculty and students. Each volunteer is essentially dropped into his/her respective school without any guidance from the organization or host school, and it is up to the individual volunteer to create a lesson plan each day. This is clearly less than ideal; volunteers whose teaching experience ranges from none to being retired school teachers are left to their own devices to design and implement daily lessons for children of all ages. Without the continuity of a long-term curriculum, students' English education is extremely choppy and disorganized, which has little benefit for the students. Furthermore, because so many of the teaching positions are often occupied by western volunteers, the need to find permanent teachers does not seem as prudent as it truly is. The schools do not seem to be understaffed, when in reality there is an extreme shortage of hired teachers. At Pratomwittahyakom School, for instance, there were seven full time teachers and between one and three volunteers depending on the week. The use of short-term volunteer teachers by Ayutthaya's school system is an extremely unsustainable manner in which to teach English. Furthermore, it brings about negative economic consequences for Thai teachers. That is, that Westerners *pay* to teach in the schools instead of being hired. Also, the flow of volunteers is inconsistent and subject to major increases and decreases depending on the season. The high tourist season in Thailand is from November until mid April, and with the influx of tourists comes an increase in volunteer teachers. Because the Central Thai school year starts in May and goes until February, with a month long break for the flood season in mid-September through mid-October, the arrival of volunteer teachers rarely coincides with the beginning of a school year. This uneven distribution of teachers causes even more disruption to students' education and teachers' yearly activities.

Role of the Volunteer and Effects Upon Students

It is entirely up to the volunteer to design and implement teaching plans, and the level of planning for this depends exclusively on the dedication of each volunteer. There was no guidance from the volunteer organization or from the school on the classes' English abilities. No advice was given on what to teach or the most effective manner for English instruction. Besides the obviously frustrating set of circumstances for each volunteer, this "laissez faire" system is detrimental to the Thai students' English acquisition. Because there was no coherent lesson plan available for volunteers, each volunteer created his or her own curriculum on a daily basis. Throughout the time spent researching for this project, there was never a coherent flow of teaching, mainly due to the informal nature of the instruction as well as the school's ever-changing placement of each volunteer.

A volunteer's placement varied on a daily basis between classrooms and age groups, depending on the availability of the actual teachers. It appeared that the role of each teacher at Pratomwittahyakom School was undefined. Whenever a volunteer was sent to a classroom, the class was always unsupervised. One could go hours without walking by a supervised classroom. In these "free" classrooms, students either sat in their desks quietly, played with whatever materials were available, or talked to each other; teachers could often be found snacking outside or chatting in the staff lounge. It should be noted that, due to the researcher's lack of comprehension of Thai, it is possible that the teachers were planning lessons or discussing topics relevant to the school year. However, due to the body language and facial expressions of the teachers during these periods, this seems unlikely.

In a news article written by a teaching volunteer in Israel, he noted that the school was always "noisy, with kids running around everywhere, and no one listening to anyone else. While the teachers do not seem fazed by this, it seems impossible to get anything done."[15] This observation is entirely applicable to the Ayutthaya school system-- not only were teachers not fazed by the chaotic nature of their students, but they seemed blissfully unaware of what was going on or, at least, were not perturbed by the lack of formalized learning. At Pratomwittahyakom School, the majority of the teachers could usually be found sitting under a tree and talking during school hours, while the Head Teacher (principal) was nearly always doing paperwork in the teacher's lounge. Unlike their western counterparts, principals in Thailand are responsible not only for paperwork and the daily goings-on at their school, but they are also responsible for teaching one or two classes per day.

A daily conversation in Prattomwittahyakom School occurred between Miss Sudha (the English teacher) and various volunteers consisting of: "Head Teacher busy, you teach." It was

never made clear *why* so many classrooms were unattended, or what the day-to-day duties of the teachers actually were. Upon entering unattended classrooms, students sometimes appeared to be occupied: on one such occasion, students were busily copying a scientific diagram of the human digestive system. A teacher informed the researcher that this was "Health" class, and promptly left the room to attend to other matters. The secondary classes in particular were a most concerning environment. When teaching in the secondary classes, there was a visible divide between students who cared about school and those who did not. This became evident in their handwriting and their ability to read, write, and speak English. This observation is not meant to be unique to Thai schools; this scene could be played out in any school in the world. It was of note mainly because of the gross discrepancies in English fluency between students. Some students are able to read and write English and understand the concepts of punctuation, plural versus singular, and understood that spaces are a necessity between words. Others simply do not have this understanding, and their handwriting is barely legible. While this issue cannot be blamed upon the short-term volunteers, their presence does not help the matter. As was already mentioned, volunteers teach alone or with other volunteers, but never with the assistance of a Thai teacher. The language barrier between volunteer and students seemed far greater in the secondary classrooms (due to the type of activities that were presented) and the material far more integral to English comprehension. Explaining the concept of spaces between words is nearly impossible without a minimal comprehension of a common language. The discrepancy between students' English abilities speaks to the need to have permanent teachers who are fluent in English and Thai instead of importing native/non-native volunteer teachers, as many of the issues found during transliteration cannot be communicated without a basic understanding of Thai. In the context of the 1999 education reforms, it is obvious that there is much work to be done regarding teaching style in Pratomwittahyakom School.

Volunteers and the Potential Inconvenience for All

During the period of research, many volunteers came and went without making too many ripples, so to speak. Volunteering attracts a wide variety of people with an equally broad set of goals, there is no assurance for the host-school that their appointed volunteer will be useful, and nothing can be done if the volunteer ends up being a nuisance and overall inconvenience for teachers, staff, students, and other volunteers alike. Even in the cases of volunteers who made every effort *not* to make a fuss, it was clear that their presence was a tax on the school system and staff. Volunteers could not go anywhere unattended to by at least one student or teacher, and could not do anything (such as go to the bathroom) without being asked if everything was okay. Food and beverages were constantly being supplied to the volunteers, and the schools' cooks happily accommodated any and all of the volunteers dietary restrictions (such as vegetarianism), and often made entirely separate meals for the volunteers based upon these restrictions. This, to a certain extent, is part of Thai culture; volunteers are treated with a great deal of respect and thus their happiness is very important to the school. However, it was the continual disruption of the staff's daily activities that warrants note as teachers would consistently accompany volunteers anywhere they went and were known to pop into classrooms just to ensure that the volunteers were not thirsty or hungry. This was an inconvenience for staff and students. The aforementioned negativities of volunteer teachers are completely aside from any of the harmful psychological effects volunteer teachers could potentially have upon students and staff. If westerners are consistently seen as the only legitimate English teachers in Ayutthaya's schools, this could decrease students' motivation to learn English and consider teaching as a profession. Would this not also discourage Thai teachers from learning and then practicing their English with each other or any volunteers present? The observations made during this research are that the answer to these questions is overwhelmingly: Yes. Older students have become so accustomed to seeing westerners as their only role model for speaking English that they appear to have lost any desire to learn the subject and fail to realize why it could be useful in the future. This could be due, in part, to their English books. These books were made in Thailand to teach British English at an extremely advanced level that lacked any cohesion between lessons. One section in particular was extremely inconsistent; in one lesson, students were learning to identify objects in the classroom, and in the next they were supposed to conjugate and practice contracting various verbs. After a month of working with the secondary school students, it became painfully obvious that their grasp of the English language was little better than that of the students in the lower stages of the primary school. Teachers appeared to be terrified to speak English to the volunteers, and would immediately default to Ms. Sudha for any question the volunteer posed to a Thai teacher. Some of this can be attributed to the teachers' lack of knowledge of the English language; several staff members knew nothing beyond basic greetings, while the Head Teacher/Principal did not appear to know any English whatsoever.

Anecdotal Observations of the Relationship between Thai and Western Culture

Despite the lack of an obviously western curriculum in Pratomwittahyakom School, there are many examples of a great awareness of the western world. In the fifth week of this study, students made posters to decorate the various classrooms, and in any poster featuring a person (to represent an action, for instance) the drawing was always of a Caucasian person, and the activity was usually a non-traditionally Thai one, such as Baseball or Ballet. Primary students were given the afternoon off of regular classes in order to make these posters, and were more than happy to curl up on the ground and color away for several unsupervised hours.

Several other observations are that the new school building features western toilets instead of the normal squat toilet found across Thailand. All of the students' English workbooks are made in Thailand to teach British English (and are subsequently filled with activities that revolve around British monuments and history). Students wear uniforms that could be found in any western school, complete with tall socks and sneakers. While research was based at Pratomwittahyakom School, observations were also made in Koh Samet, Koh Samui, Chiang Mai, and Bangkok. In Chiang Mai, where there is a high concentration of hill tribes, mandatory, formalized education might as well not exist; among indigenous people formalized education is a sticky topic. When visiting the hill tribes, it seemed that there was no formal building for education and that all children were assisting their parents in their duties as opposed to attending school. There is the possibility that this was done solely for the enjoyment of the tourists (that is, that when tourists were present the tribes people abandoned their usual duties to participate in more aesthetically pleasing tasks such as embroidery), but with the constant flow of tourists this hardly seems practical. In Bangkok, the education system is comparable to any major metropolitan city in the western world: busloads of uniformed students attend International Schools

(which often times use the International Baccalaureate curriculum) where only trained, hired, and paid teachers are in charge of educating the students. In Island communities, such as Koh Samet and Koh Samui, a dramatically greater number of school-aged children were seemingly not enrolled in any sort of school; children could constantly be seen selling goods on the beach or in touristy spots as well as helping their (assumed) parents with their food/trinket stalls in market areas. Because the Thai islands are infamous for their beauty (assisted by Alex Garland's The Beach infecting a global generation with the travel bug), travelers flock from around the globe to soak up the sun and salt in the Gulf of Thailand. This has resulted in several of the islands, mainly Koh Samui, Koh Phangan, and Koh Samet (which is on the eastern side of the Gulf), becoming Thailand's version of the Hawaiian Islands. Because the culture between Central, South, and North Thailand are all so drastically different, being able to see the island culture allowed this research to gain stronger footing in the basic concept of Thai culture and how the western world fits into this. For instance, take Koh Samui: a beautiful island with several pristine white sand beaches, (most notably Chaweng Beach) which are buzzing with tourists, and is thought to be Southeast Asia's most popular island getaway. However, once one leaves the beach-side lounges available for rent, the ratio of Thai people to westerners resumes its "normal" balance, and, the further one ventures from the tourist hotspots, there are drastically more Thai people than western. The culture of the Thai islands is, like many island states or nations, very laid back. The food takes a long time to cook and is full of fresh, spicy flavor; children run around assisting their parents regardless of the day of the week or time of day; and even the way in which western culture has altered the traditions is different than anywhere else in Thailand. Yoga studios, many of which are owned and operated by westerners, cater to western and Thai people alike, as well as the presence of more "laid-back" western music such as reggae, as opposed to the thundering bass-lines of Dutch "House" music that can be heard throughout Central Thailand. Many of these observations about Thai and western culture were made at the end of the volunteer project and outside of the school environment, and thus provided a different examination of these two cultures than was made at the beginning of the project.

Conclusions

From this study it is clear that Thailand is at a crucial point in its move towards becoming a global giant. The government recognizes the internal changes that are necessary to educate generations of intelligent, "global" adults, but is struggling to actualize these hopes within the school system. This is due, in part, to the history of education in Thailand; without teachers educated in the western manner, it is nearly impossible to produce students who are educated in a western style. From what was gleaned from speaking with other volunteers and through observations at Prattomwitthayakom School, it appeared that, at least for Ayutthaya, the goal of an inquisitive, western style school system is many decades in the future. The observations made in Ayutthaya have led to a rather negative view of the short-term volunteer teaching industry; short-term volunteers are, for a plethora of reasons already discussed, a greater cost to the schools than they are a benefit. While volunteers often come away with a much greater understanding of the world they inhabit and how their cultures differ from the rest of the world, the host-country is left with an economy dependent on tourism and school systems dependent on volunteers. In the context of Thailand as a whole, however, Ayutthaya's experience with western education seems to be relatively balanced between Bangkok's rigidly International approach to education and the loose approach of the island and more rural regions. This is indicative of Thailand's position in the globalizing world; struggling to develop a strong westernized generation while desiring to maintain its cultural roots by allowing indigenous people to continue practicing their traditional way of life.

References

[1]Studebaker, J. (2013) USA Today. <http://traveltips.usatoday.com> (2/22/2013)

[2](1987) "Thailand: A Country Study". U.S. Library of Congress. <http://countrystudies.us/thailand> (06/20/2011)

[3]Ishii, Y. (1994) "Thai Muslims and the Royal Patronage of Religion." *Law and Society Review*, Vol 28. Issue 3. Pg. 453-460.

[4]Ashayagachat, A. (2005) "Trade pacts spurring FDI: Thailand third in Asia on multinationals' list." Bangkok Post Thailand

[5]Sivaraksa, S. (2005) "Engaged Buddhism In A Globalizing World". *Conflict, Culture, Change*. Pg. 115-118.

[6]Lester, R. (1973) Theravada Buddhism in Southeast Asia. The University of Michigan Press. Pg. 83-86.

[7]Guruge, A. (1983) "Contributions of Buddhism to Education." *Contribution of Buddhism to World Civilization and Culture*. Atlantic Highlands: Humanities Press Inc. Pg. 109-110.

[8]Yawnghwe, C. (1997) "The Politics of Authoritarianism: The State and Political Soldiers in Burma, Indonesia and Thailand." The University of British Columbia. Pg. 210.

[9]Brazier, C. (2011) "The State of the World's Children 1999: Education." UNICEF. <http://www.unicef.org> (10/15/2011)

[10]Sippanondha, K. (1984) "Planning and Implementation of the Primary Education Reform in Thailand." *Prospects: Quarterly Review of Education*, Vol. 14. Issue 4.

[11]Kaewdeng, R. (2001) "Opening Address to The First International Forum on Education." <http://www.worldedreform.com> (06/25/2011)

[12]Hallinger, P. and Pornkasem, K. (2000) "Educational Change in Thailand: opening a window onto leadership as a cultural process." *School Leadership & Management*, Vol. 20.2. Pg. 189-205.

[13]Scales, J., Wennerstrom, A., Richard, D. and Wu, S. (2006) "Language Learners' Perceptions of Accent." *TESOL Quarterly*, Vol. 4. Issue 4. Pg. 715-738, 727.

[14]Zhen, L. (2008) "Passion: Burning in the West." *Chinese Education and Society*, Vol. 41. Issue 4. Pg. 13-22, 18.

[15]Dickinson, J. (2011) "Jewish Advocate." *Ethnic News Watch*, Vol. 22.

Does race matter in international beauty pageants? A quantitative analysis of Miss World

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Abstract:

Most research on race and beauty pageants uses qualitative research methods and focuses on national beauty pageants. For this reason it is unclear whether there are broad patterns of racial inequalities in international beauty pageants. My study addresses this issue by using a quantitative approach to examine whether race affects success rates in international beauty pageants. This study examines countries that have competed in the Miss World pageant from 1951 to 2011 and examine each country's probability of winning and being in the semi-finals. The analysis shows that race matters. White countries have a much higher chance of winning and being in the semi-finals than non-white nations. Furthermore, white nations have been over-represented as winners and semi-finalists while non-white nations have been under-represented in both those categories. The patterns of success for white nations and lack of success for non-white nations demonstrates that beauty is not neutral and that global beauty pageants can be seen as a reflection of racial hierarchies and a reaffirmation of the ideology of white beauty.

Introduction

Browsing through the headshots of the 2011 contestants on the websites of the Miss World and the Miss Universe pageants, the women's beauty was indisputable. Each one was endowed with big, round eyes, straight hair, narrow nose, and an attractive smile. I then decided to visit the photo gallery of Miss Universe 2011, the African winner Leila Lopes. I clicked through images of the photo album that featured her homecoming visit to Angola. In the photographs where her body was juxtaposed with her countrymen, what became apparent was that Ms. Lopes was a shade or two lighter than the others. Ms. Lopes' body and the bodies of the other contestants demonstrate that in order to be selected to compete in international beauty pageants one must embody white phenotypic characteristics[1] such as straight hair, light skin tone and, a narrow nose.[1]

It is important to analyze how beauty is defined and what kind of beauty is valorized in forms of popular culture such as beauty pageants. Popular culture shapes notions of feminine beauty for women across the globe[2], and if white phenotypes are privileged and valorized, this could affect the self-image of women who do not embody these characteristics. For instance, the pressure to subscribe to the ideal of white beauty could lead women to lighten their skin with harmful chemicals and may also negatively affect their self-concept.[2]

Studying Race in Beauty Pageants

Although race is a socially constructed concept, the concept of race and racism persists[3], and in order to study racial inequalities, race needs to be operationalized. There are many different ways to study race. For this analysis, the categories "white" and "non-white" are used to study racial inequalities, and this categorization draws on Telles' (2009) study that notes that the white and non-white distinction is a conceptually clear racial divide.[4] The categories of white and non-white are applied to countries, rather than to the bodies, of the Miss World beauty pageant contestants themselves because not all 61 years of images and videos of these contestants were available. However, information on the countries that participated and succeeded in the 61 years of the Miss World pageant was readily available. In order to categorize countries as white and non-white, I viewed the images and videos of pageant contestants that were available and coded the countries that have consistently sent women with Western phenotypes, such as white skin, to the competition as white. This includes all the European countries, Australia, Canada, and the United States. While many of these countries that are coded as white have a portion of their population that does not identify as white, the pageant contestants from these countries do not reflect this racial diversity.

While the concept of race embodies more than simple phenotypes, in beauty pageants where the body is visually displayed, physical appearance is a noticeable way of signifying race.[5] Moreover, the general public that view beauty pageants also understand race and racial (in)equality in terms of skin colour.6 Furthermore, as previously mentioned, most of the pageant contestants possess Westernized facial features such as straight hair, narrow nose and large eyes; thus skin colour remains one of the key ways to differentiate the women in the international pageants.

As with all methodologies for studying race, there are limitations to the one employed in this analysis. Race is a complex concept and the method used in this study cannot fully capture its nuances.

National Pageants and Race

Studies have shown that many non-white countries send representatives that look white to the global beauty pageants in order to increase their chances of winning.[2,7,8,9,10] King-O'Riain noted "both India and Venezuela have recently sent taller, more Western-looking women forward to international pageants because they want a queen that will win on the global stage."[8] While King-O'Riain noted that there is a practice where Western-looking women are preferred, Runkle and Barnes' studies show that the process of favouring Western-looking women is a very active process.[7,10]

Table 1: Studies on Beauty Pageants

Author	Methodology	Pageant Studied	Time Period Studied
Lawson and Ross (2010)	Quantitative analysis	Miss Universe	1952-2008
Oza (2001)	Ethnography	Miss India	1996
Parameswaran (2004)	Media analysis	Miss India	1998-2000
Russel (2006)	Media analysis	Miss India	1996
Barnes (1994)	Historical analysis & media analysis	Miss Jamaica	1950s-1980s
Callahan (1998)	Historical analysis & media analysis	Miss Thailand	1934-1990s
Edmonson (2003)	Historical analysis	Pageants in the Caribbean	1999
Mattson and Pettersson (2007)	Media analysis	Miss Sweden	1949-1960
Runkle (2004)	Ethnography	Miss India	2003
Shissler (2004)	Media analysis	Miss Turkey	1929-1932
Wilk (1995)	Ethnography	Miss Belize	1990s
King-O'Riain (2008)	Literature review	Multiple	---
Banet-Weiser (1999)	Ethnography	Miss America	---
Balogun (2012)	Ethnography	Miss Nigeria	2009-2010

Runkle's ethnographic study on the month-long training boot camp prior to the Miss India pageant reveals the salience of skin colour in the lives of these beauty queen aspirants. Each girl was on some type of medication to lighten her skin colour. Runkle demonstrates that light skin is not only preferred, it is created. The dermatologist administering these skin treatments justified the process by citing the benefits of light skin in beauty pageants – "we still lighten their skin here because it gives the girls extra confidence when they go abroad [to international pageants]."[10]

Barnes' study on Miss Jamaica reveals the preference for a light-skinned Miss Jamaica by demonstrating the active selection of a white beauty queen. Although 90% of Jamaicans identify as black, white women have been favoured as beauty queens. In the 1980s, the Miss Jamaica contest was turned over to private sponsorship and the focus shifted to selecting beauty queens that would have the best chance of winning the international pageants and providing financial rewards. It was during these years that white women were selected to win Miss Jamaica.[7]

In national pageants, there is a clear preference for whiteness or lightness. By continuously selecting white or light-skinned women as winners in national pageants, "white aesthetic standards do not appear more attractive – they become the norm."[11]

International Pageants and Race

Many studies on beauty pageants have focused on national pageants,[7,11,13,14] and while some of these studies have shown that there is a favouring of lighter-skinned contestants on the national stage, none of the studies addressed the issue of skin colour and its implications for racial inequities in international beauty pageants. Research has shown that whiteness influences the success rates in national pageants,[8,15] but there is a lack of research on whether whiteness also plays a significant role in determining success on the global stage.

Although there is a lack of research on international pageants, studies on national pageants, such as India, the United States, Jamaica, and Belize, provide evidence that race plays a role in global pageants. Osuri points out that "dark-skinned winners are still fewer in comparison to light or white-skinned contestants."[15] King-O'Riain suggests that the "continued valorization of 'whiteness' or 'lightness' and European beauty standards seem to be impacting the Miss World and Miss Universe pageants even with an increase in the proportion of women of colour named as queens."[8] While King-O'Riain does not elaborate on what this means (how and in what way do European beauty standards impact global pageants?), she does suggest that whiteness plays a role in global pageants. Banet-Weiser also argues that "although non-European nations and heritages are clearly supported in terms of their presence on stage, the structural and ideological basis of the pageant remains firmly embedded in US and Western European values and histories."[16] Both King-O'Riain and Banet-Weiser suggests that whiteness has an advantage in beauty pageants since the pageants are so steeped in white privilege. Non-white women may embody white aesthetics, but they are still at a disadvantage compared to white women. These studies suggest that race plays an important role in international

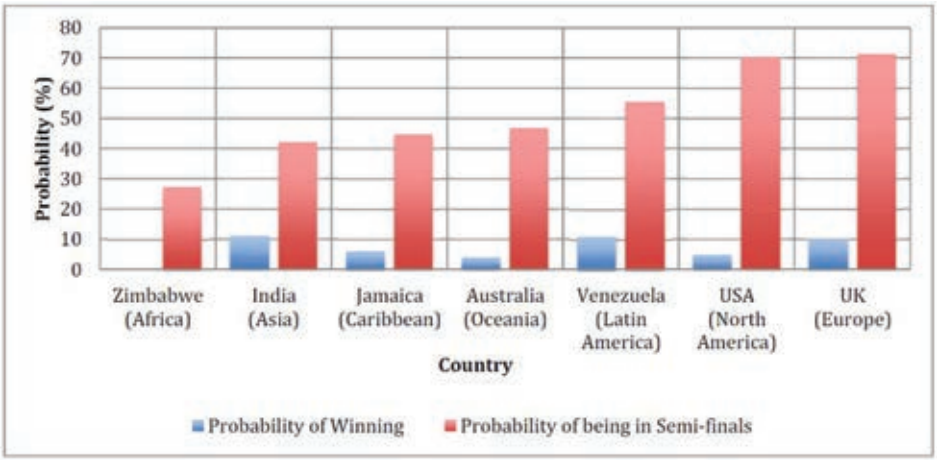

Figure 1: Countries with the highest success rate from each continent.

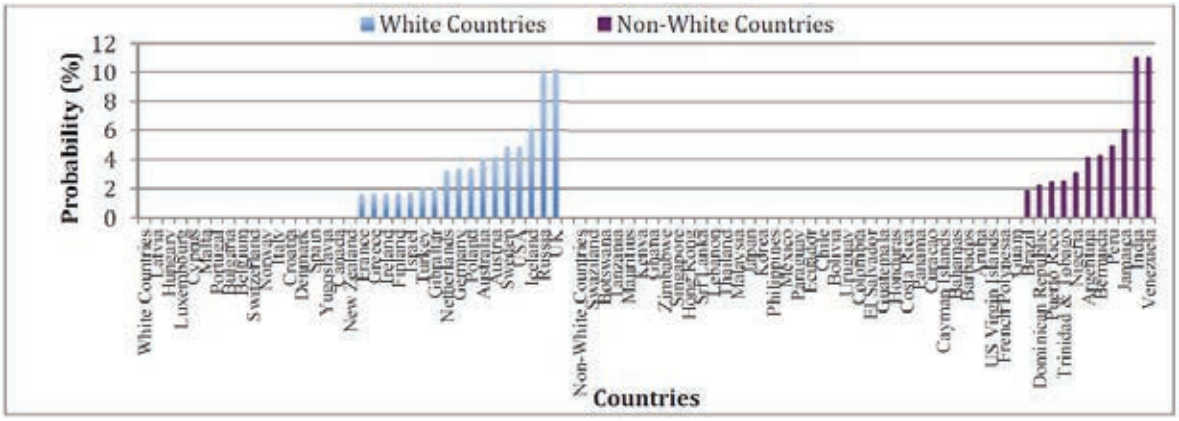

Figure 2: Probability of winning for each country.

Table 2: Miss World Winning Countries and Winning Probabilities

Country	Probability of Winning (%)
France*[W]	1.64
Greece*[W]	1.69
Ireland*[W]	1.69
Finland*[W]	1.72
Israel*[W]	1.75
Brazil	1.92
Turkey*[W]	2.00
Gibraltar*[W]	2.04
Dominican Republic	2.27
Puerto Rico	2.50
Trinidad & Tobago	2.56
Nigeria	3.13
Netherlands*[W]	3.28
Germany*[W]	3.39
Poland*[W]	3.45
Australia*[W]	4.08
Argentina	4.17
Austria*[W]	4.17
Bermuda	4.35
Sweden*[W]	4.92
USA*W	4.92
Peru	5.00
Iceland*[W]	6.12
Jamaica	6.12
Russia*[W]	10.00
UK*[W]	10.20
India	11.11
Venezuela	11.11

Note: Countries with (*W) indicates white countries

beauty pageants and they open up the space for questions. Is there a privileging of white women over non-white women as winners and semi-finalists in global beauty pageants? How much does whiteness affect the winning outcomes? What will this tell us about the type of feminine beauty that is produced, valorized, and circulated on the global stage?

Why Study International Pageants?

International beauty pageants are a form of what Malkki calls the "family of nations," which "constitutes the differences between national units in a way that is dehistoricizing, depoliticizing, and ultimately homogenizing of differences."[17] This homogenizing of differences is found in the judging of the pageant. Each girl is assigned a score for each round and the scores are awarded based on the merits of each girl, which invokes an "idealized conception of equality."[16] By studying and critiquing the practice of privileging whiteness in these international pageants, we can dismantle the "romance of internationalism"[17] that frames these spectacles and recognize the racial inequities in global beauty pageants.

Miss World and Miss Universe are public spectacles and they beam images of smiling beauty queens around the globe. Images of the contestants are easily accessed, downloaded, or purchased from the Internet. However, circulating along with these images of smiling beauty queens is a particular message about feminine beauty – a message where whiteness is equated with beauty. The mass media and forms of popular culture such as the Miss World and Miss Universe pageants shape notions of feminine beauty.[2] By continuously displaying images of light-skinned women on the global stage and by privileging European aesthetics, international beauty pageants problematically entrench the ideal of white beauty[11] and reaffirm existing racial hierarchies.

The ideal of white beauty could affect how women who do not embody these characteristics view themselves. Many have turned to skin bleaching and cosmetic surgery to achieve this ideal, which poses health problems.[2] Moreover, research has shown that beauty is associated with privilege and resources.[18] If Western aesthetics are what is considered beautiful, then those with these physical traits will have access to more resources than those without.

Quantitative Approach

Most studies on beauty pageants are qualitative studies (Table 1). To date, only one study[19] has employed a quantitative method to studying beauty pageants. However, this study looks at the role of a nations' market liberalism on the success rates of the contestants and does not address issues regarding the racial inequities inherent in these beauty pageants.

The qualitative studies use methods of ethnography, historical analysis, and media analysis to understand and explore issues surrounding national pageants. While qualitative studies contribute excellent knowledge to the body of literature on beauty pageants, the qualitative studies on beauty pageants tend to be restricted by time period. The fourth column in Table 1 shows that most of these studies often do not cover long time periods. A quantitative approach can provide a way of looking at the long history of the international beauty pageants (61 years of Miss World and 60 years of Miss Universe), giving us a clearer picture of the patterns of inequities in these pageants. Qualitative studies have indicated there is a general preference for light or white-skinned women in international pageants, but have not elaborated on this trend. A quantitative study can provide empirical evidence for this trend and can show us exactly how often white skin has been favoured over dark skin throughout the history of international beauty pageants. All the qualitative studies

have only considered the winners of beauty pageants and have neglected the significance of the semi-finalists. A quantitative approach can examine beauty pageants through the bodies of the winners and the bodies of the semi-finalists.

Method

This study examines the countries that have succeeded in the Miss World pageant from its inception in 1951 to a recently held pageant in 2011. Data was gathered on the competing nations, the winning countries, and the semi-finalists through the Miss World website, pageant fan-sites, as well as through viewing video clips of the pageant uploaded by fans. In the analysis only the countries that competed at least 20 times throughout the 61 years of the Miss World pageant were considered.

I used 81 competing countries in my analysis whose competing years range from 20 to 61. They also vary tremendously in the amount of successes in the pageant (0-6 wins and 0-43 semi-finalists). For a list of the 81 countries see Appendix A.

I coded the 81 countries into seven different continents – Europe, Africa, Asia, North America, Latin America, Caribbean and Oceania. Latin America includes Central America, South America, and Mexico. Central America and Mexico are geographically part of North America, but are culturally more similar to South America. I combined Central America, Mexico and South America to recognize the similar histories and cultural traditions between these areas. The Caribbean is geographically part of North America, but some parts can be considered part of Latin America. I coded the Caribbean as a separate continent because of its distinct traditions and experiences with beauty pageants. Geographically, Turkey straddles both Asia and Europe. After viewing several photos of Turkish contestants in the Miss World and Miss Universe pageant, I coded Turkey as European. After viewing photos of Israel's contestants in the Miss World and Miss Universe pageant, I also coded Israel as European. For a list of countries coded into continents see Appendix B. I also coded the 81 countries as white countries and non-white countries. White countries include all the European countries and North American countries as well as Australia and New Zealand. Out of 81 competing countries, 34 countries are white countries and 47 countries are non-white countries. White countries make up 42% of the competing nations and non-white countries constitute 58% of the competing nations. For the list of white and non-white countries see Appendix C.

For this analysis, I looked at each country's probability of winning and reaching the semi-finals. The probabilities of winning were calculated by dividing the number of times each country has won by the number of times they competed in the Miss World contest. The probability of being in the semi-finals was calculated by dividing the number of times each country has been represented in the semi-finals by the number of times they competed in the Miss World contest.

Data

Different continents have varying levels of success in the Miss World pageant (Figure 1). Figure 1 provides us with the probability of winning and of being in the semi-finals for the most successful nation from each of the seven continents. Africa has been the least successful in both winning and being selected for the semi-finals, while Europe has been one of the most successful continents. Zimbabwe has a much lower probability (27.27%) of being in the semi-finals than UK (71.43%). While each country's probability of winning the Miss World title is quite low, certain countries have a higher probability of winning than others. India, Venezuela, and the United Kingdom have the highest success rate, while Zimbabwe has yet to win the Miss World title. A black country like Zimbabwe has a much lower chance of being selected for the semi-finals and capturing the crown compared to white countries such as the United States and the United Kingdom, or brown countries like Venezuela and India.

India and Venezuela have higher rates of winning than some white countries. However, these countries' rates of being in the semi-finals do not display their advantage over non-white countries. Since winning is a highly visible act, perhaps the crowning of two non-white countries suggests that tokenism plays a role in international beauty pageants.

Many white countries have had the opportunity of winning the Miss World contest, but a large proportion of non-white countries have yet to win the Miss World competition (Figure 2). The graph also shows that compared to white countries, a much larger proportion of non-white countries have not been able to win the pageant.

Tables 2 and 3 examine the information presented in Figure 2 in more detail. Table 2 lists all the countries that have won the Miss World competition from its inception in 1951 to 2011. Throughout the 61 years of the Miss World competition, 28 different countries have successfully captured the Miss World title. Out of these 28 countries, 17 of the winning countries are white (60.7%) and 11 of the countries are non-white (39.3%). This means that even though white countries make up 42% of the competing nations in the Miss World competition, they constitute 60.7% of the winning countries. White countries are over-represented as winners of the Miss World pageant, while non-white countries are under-represented.

Table 3 lists the 53 countries that have yet to win the Miss World title. Out of these 53 countries, 17 (32.1%) are white countries, while 36 (67.9%) are non-white countries.

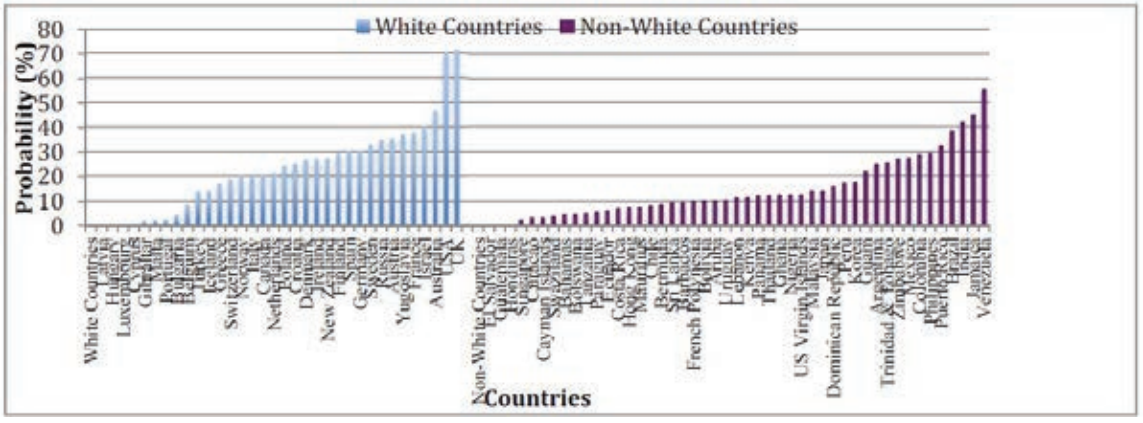

Figure 3: Probability of being in semi-finals for each country.

Table 3: Countries That Have Not Yet Won Miss World

Country	Probability of Winning (%)
Tanzania	0.00
French Polynesia	0.00
Croatia*W	0.00
Latvia*W	0.00
Botswana	0.00
Barbados	0.00
Hungary*W	0.00
Zimbabwe	0.00
Bulgaria*W	0.00
Ghana	0.00
US Virgin Islands	0.00
Swaziland	0.00
Luxembourg*W	0.00
El Salvador	0.00
Kenya	0.00
Mauritius	0.00
Guam	0.00
Yugoslavia*W	0.00
Guatemala	0.00
Curacao	0.00
Cayman Islands	0.00
Panama	0.00
Honduras	0.00
Paraguay	0.00
Chile	0.00
Portugal*W	0.00
Uruguay	0.00
Singapore	0.00
Bolivia	0.00
Aruba	0.00
Hong Kong	0.00
Thailand	0.00
Sri Lanka	0.00
Malaysia	0.00
Switzerland*W	0.00
Bahamas	0.00
Costa Rica	0.00
Malta*W	0.00
Lebanon	0.00
Philippines	0.00
Colombia	0.00
Spain*W	0.00
Mexico	0.00
Cyprus*W	0.00
Ecuador	0.00
Norway*W	0.00
Korea	0.00
New Zealand*W	0.00
Denmark*W	0.00
Canada*W	0.00
Italy*W	0.00
Japan	0.00
Belgium*W	0.00

Note: Countries with (*W) indicates white countries

Table 4: Top 30 Countries with Highest Probability of Being Selected for Semi-Finals

Country	Probability of being in Semi-finals (%)
Netherlands*W	21.31
Guam	22.22
Poland*W	24.19
Croatia*W	25.00
Argentina	25.00
Trinidad & Tobago	25.64
Denmark*W	26.92
Ireland*W	27.12
Zimbabwe	27.27
New Zealand*W	27.45
Mexico	27.66
Colombia	28.89
Finland*W	29.31
Philippines	29.55
Spain*W	30.44
Germany*W	30.51
Puerto Rico	32.50
Sweden*W	32.79
Russia*W	35.00
Austria*W	35.42
Yugoslavia*W	37.04
France*W	37.71
Brazil	38.46
Israel*W	40.35
India	42.22
Jamaica	44.90
Australia*W	46.94
Venezuela	55.56
USA*W	70.49
UK*W	71.43

Note: Countries with (*W) indicates white countries

Table 5: Bottom 30 Countries with Lowest Probability of Being Selected for Semi-Finals

Country	Probability of being in Semi-finals (%)
Latvia*W	0.00
Hungary*W	0.00
Luxembourg*W	0.00
El Salvador	0.00
Guatemala	0.00
Honduras	0.00
Cyprus*W	0.00
Gibraltar*W	2.04
Malta*W	2.27
Singapore	2.56
Portugal*W	2.63
Curacao	3.23
Cayman Islands	3.23
Swaziland	4.00
Bulgaria*W	4.35
Bahamas	4.65
Botswana	4.76
Tanzania	5.00
Paraguay	5.71
Ecuador	6.25
Costa Rica	6.98
Hong Kong	7.32
Mauritius	7.41
Chile	8.11
Belgium*W	8.62
Bermuda	8.70
Barbados	9.52
Sri Lanka	9.52
French Polynesia	10.00
Bolivia	10.26

Note: Countries with (*W) indicates white countries

Although non-white countries form 58% of the competing nations, they constitute 67.9% of the countries that have not been able to succeed in winning the crown, thus are under-represented.

Success in beauty pageants can also be measured by looking at the semi-finalists in the pageants. Figure 3 presents the probabilities of white countries and non-white countries being in the semi-finals. The graph reveals that most white countries have a fairly good chance of being selected for the semi-final round. The majority of white countries have a success rate of 20% to 35%. The probability of being in the semi-finals for non-white countries, on the other hand, are spread across a wide range and the majority of non-white countries have less than a 20% chance of being in the semi-finals. The non-white country with the highest probability of being in the semi-finals (Venezuela 55.56%) has a much lower success rate when compared to the United Kingdom's 71.43% and USA's 70.49%. Compared to white nations, non-white countries have a much lower probability of moving forward into the semi-final round. This is more clearly illustrated by considering the average success rate for each group. The average probability of being selected for the semi-finals for white countries is 23.36%, while for non-white nations this probability decreases to 14.9%.

Tables 4 and 5 look at Figure 3 in more detail by examining the countries with the highest and lowest probabilities of being

in the semi-finals. Table 4 looks at the 30 countries with the highest probability of being in the semi-finals. Out of these 30 countries, 18 are white nations (60%) and 12 are non-white nations (40%). White countries make up 42% of the competing nations and yet constitute 60% of the countries with a high probability of successfully moving forward into the semi-final round. White countries are over-represented as semi-finalists, while non-white countries are under-represented. Table 5 considers the 30 countries with the lowest probability of being in the semi-finals. Out of these 30 countries, nine are white countries (30%) and 21 are non-white countries (70%). Non-white countries make up 58% of the competing nations and yet represent 70% of the least likely countries to move into the semi-finals.

Conclusion

Studies have shown that skin colour matters in national pageants, and lighter-skinned women are consistently preferred. This preference for white aesthetics affects the types of contestants we see competing in the international pageants and it also lends evidence to racial inequities based on colour.[2] This paper extends the analysis of skin colour and beauty pageants by considering whether race affects success rates of countries competing in the international pageants.

The analysis showed that race does matter. Race affects the probability of being selected as a semi-finalist and of being selected as Miss World. The data showed that white countries have a much higher chance of winning and reaching the semi-finals than non-white nations. White nations have also been over-represented as winners and semi-finalists while non-white nations have been under-represented. African countries have the lowest success rate in winning and reaching the semi-finals while Western countries such as the United States and the United Kingdom have had the most successes in the Miss World beauty pageant.

Racial inequalities operate on two levels in these international pageants. Firstly, non-white contestants selected to compete on the global stage embody Western aesthetic norms because they are lighter in skin colour and have more Westernized features, such as straight hair, narrow nose, and large eyes, than women in their home countries. Furthermore, once on the global stage, non-white women have a much lower success rate in these global pageants compared to white women. The consistent patterns of success for white nations and lack of success for non-white nations demonstrates that beauty is not objective or neutral – it is raced. Global beauty pageants like the Miss World competition reflects racial hierarchies and reaffirms the ideology of white beauty. Furthermore, this pattern also suggests that the occasional success of non-white nations is not due to a genuine valorization of non-white beauty, but rather it is a way to display diversity while preserving the privilege of whiteness that underlies these spectacles.

References

[1]Keith, V.M. (2009) "A Colorstruck World: Skin Tone, Achievement, and Self-Esteem among African American Women." *In Shades of Difference: Why Skin Color Matters.* Glenn, E.N., ed(s). Stanford University Press. Pg 25-39.

[2]Glenn, E.N. (2008) "Yearning for Lightness: Transnational Circuits in the Marketing and Consumption of Skin Lighteners." *Gender & Society.* 22.3. Pg 281-302.

[3]Winant, H. (2006) "Race and Racism: Towards a Global Future." *Ethnic and Racial Studies.* 29.5. Pg 986-1003.

[4]Telles, T. (2009) "The Social Consequences of Skin Color in Brazil." *In Shades of Difference: Why Skin Color Matters.* Glenn, E.N., ed(s). Stanford University Press. Pg 9-24.

[5]Glenn, E.N. (2009) "The Significance of Skin Color: Transnational Divergences and Convergences." *In Shades of Difference: Why Skin Color Matters.* Glenn, E.N., ed(s). Stanford University Press. Pg 7-8.

[6]Gore, A. (2012) "'White as Snow' Miss France Beauty Queen gets Caught in Racism Row and Catfight with Previous Winner." *Daily Mail.* Daily Mail. http://www.dailymail.co.uk/news/article-2248200/White-snow-Miss-France-beauty-queen-gets-caught-racism-row-catfight-previous-winner.html/. (Accessed 12/14/2012).

[7]Barnes, N.B. (1994) "Face of the Nation: Race, Nationalisms and Identities in Jamaican Beauty Pageants." *The Massachusetts Review.* 35.3. Pg 471-492.

[8]King-O'Riain, R.C. (2008) "Making the Perfect Queen: The Cultural Production of Identities in Beauty Pageants." *Sociology Compass.* 2.1. Pg 74-83.

[9]Leung, F., Lam,S. and Sze, S. (2001) "Cultural Expectations of Thinness in Chinese Women." *Eating Disorders.* 9.4. Pg 339-350.

[10]Runkle, S. (2004) "Making "Miss India": Constructing Gender, Power and the Nation." *South Asian Popular Culture.* 2.2. Pg 145-159.

[11]Kang, J.M. (1997) "Deconstructing the Ideology of White Aesthetics." *Michigan Journal of Race & Law.* 2. Pg 283-357.

[12]Callahan, W.A. (1998) "The Ideology of Miss Thailand in National, Consumerist, and Transnational Spaces." *Alternatives: Global, Local, Political.* 23.1. Pg 29-61.

[13]Parameswaran, R. (2004) "Global Queens, National Celebrities: Tales of Feminine Triumph in Post-Liberalization India." *Critical Studies in Media Communication.* 21.4. Pg 346-370.

[14]Wilk, R. (1995) "The Local and the Global in the Political Economy of Beauty: From Miss Belize to Miss World." *Review of International Political Economy.* 2.1. Pg 117-134.

[15]Osuri, G. (2008) "Ash-coloured Whiteness: The Transfiguration of Aishwarya Rai." *South Asian Popular Culture.* 6.2. Pg 109-123.

[16]Banet-Weiser, S. (1999) *The Most Beautiful Girl in the World: Beauty Pageants and National Identity.* University of California Press.

[17]Malkki, L. (1994) "Citizens of Humanity: Internationalism and the Imagined Community of Nations." *Diaspora.* 3.1. Pg 41-68.

[18]Anderson, T.L., Grunert, C., Katz, A., and Lovascio, S. (2010) "Aesthetic Capital: A Research Review on Beauty Perks and Penalties." *Sociology Compass.* 4.8. Pg 564-575.

[19]Lawson, R. and Ross, J. (2010) "Economic Freedom and Beauty Pageant Success in the World." *Journal de Economistes et des Etudes Humaines.* 16.1. Pg 2.

(Appendices on next page)

Appendix A

Table 1: Countries Used in Analysis

Country	Probability of Winning	Probability of being in Semi-finals	Total Wins	Total Semi-finalists	Number of Times Competed
Tanzania	0	5	0	1	20
French Polynesia	0	10	0	2	20
Croatia	0	25	0	5	20
Russia	10	35	2	7	20
Latvia	0	0	0	0	21
Botswana	0	4.762	0	1	21
Barbados	0	9.524	0	2	21
Hungary	0	0	0	0	22
Zimbabwe	0	27.273	0	6	22
Bulgaria	0	4.348	0	1	23
Bermuda	4.3478	8.696	1	2	23
Ghana	0	12.5	0	3	24
US Virgin Islands	0	12.5	0	3	24
Swaziland	0	4	0	1	25
Luxembourg	0	0	0	0	26
El Salvador	0	0	0	0	26
Kenya	0	11.538	0	3	26
Mauritius	0	7.407	0	2	27
Guam	0	22.222	0	6	27
Yugoslavia	0	37.037	0	10	27
Poland	3.4483	24.138	1	7	29
Guatemala	0	0	0	0	31
Curacao	0	3.226	0	1	31
Cayman Islands	0	3.226	0	1	31
Nigeria	3.125	12.5	1	4	32
Panama	0	12.121	0	4	33
Honduras	0	0	0	0	34
Paraguay	0	5.714	0	2	35
Chile	0	8.108	0	3	37
Portugal	0	2.632	0	1	38
Uruguay	0	10.526	0	4	38
Singapore	0	2.564	0	1	39
Bolivia	0	10.256	0	4	39
Aruba	0	10.256	0	4	39
Trinidad & Tobago	2.5641	25.641	1	10	39
Peru	5	17.5	2	7	40
Puerto Rico	2.5	32.5	1	13	40
Hong Kong	0	7.317	0	3	41
Thailand	0	12.195	0	5	41
Sri Lanka	0	9.524	0	4	42
Malaysia	0	14.286	0	6	42
Switzerland	0	19.048	0	8	42
Bahamas	0	4.651	0	2	43
Costa Rica	0	6.977	0	3	43
Malta	0	2.273	0	1	44
Lebanon	0	11.364	0	5	44
Dominican Republic	2.2727	15.909	1	7	44
Philippines	0	29.545	0	13	44
Colombia	0	28.889	0	13	45
India	11.1111	42.222	5	19	45
Spain	0	30.435	0	14	46
Mexico	0	27.66	0	13	47
Cyprus	0	0	0	0	48
Ecuador	0	6.25	0	3	48
Argentina	4.1667	25	2	12	48
Austria	4.1667	35.417	2	17	48
Gibraltar	2.0408	2.041	1	1	49
Iceland	6.1224	14.286	3	7	49
Jamaica	6.1224	44.898	3	22	49
Australia	4.0816	46.939	2	23	49
UK	10.2041	71.429	5	35	49
Turkey	2	14	1	7	50
Norway	0	20	0	10	50
Korea	0	17.647	0	9	51
New Zealand	0	27.451	0	14	51
Denmark	0	26.923	0	14	52
Brazil	1.9231	38.462	1	20	52
Canada	0	20.755	0	11	53
Venezuela	11.1111	55.556	6	30	54
Italy	0	20	0	11	55
Japan	0	14.286	0	8	56
Israel	1.7544	40.351	1	23	57
Belgium	0	8.621	0	5	58
Finland	1.7241	29.31	1	17	58
Greece	1.6949	16.949	1	10	59
Ireland	1.6949	27.119	1	16	59
Germany	3.3898	30.508	2	18	59
Netherlands	3.2787	21.311	2	13	61
Sweden	4.918	32.787	3	20	61
France	1.6393	37.705	1	23	61
USA	4.918	70.492	3	43	61

Appendix B

Table 3: Countries Coded According to Continent

Europe	Africa	Asia	North America	Latin America[a]	Caribbean[b]	Oceania
Croatia	Tanzania	Singapore	Canada	El Salvador	Barbados	French Polynesia
Russia	Botswana	Hong Kong	USA	Guatemala	Bermuda	Guam
Latvia	Zimbabwe	Thailand		Panama	US Virgin Islands	Australia
Hungary	Ghana	Sri Lanka		Honduras	Curacao	New Zealand
Bulgaria	Swaziland	Malaysia		Paraguay	Cayman Islands	
Luxembourg	Kenya	Lebanon		Chile	Aruba	
Yugoslavia	Mauritius	Philippines		Uruguay	Trinidad & Tobago	
Poland	Nigeria	India		Bolivia	Puerto Rico	
Portugal		Korea		Peru	Bahamas	
Switzerland		Japan		Costa Rica	Dominican Republic	
Malta				Colombia		
Spain				Mexico	Jamaica	
Cyprus				Ecuador		
Austria				Argentina		
Gibraltar				Brazil		
Iceland				Venezuela		
UK						
Turkey[c]						
Norway						
Denmark						
Italy						
Israel[d]						
Belgium						
Finland						
Greece						
Ireland						
Germany						
Netherlands						
Sweden						
France						

Notes:

[a] Latin America includes Central America, South America and Mexico. Central America and Mexico are geographically part of North America, but is culturally more similar to South America. I combined Central America and South America to recognize the similar histories and cultural traditions between these two areas.

[b] The Caribbean is geographically part of North America while some parts can be considered part of Latin America. I coded the Caribbean as a separate continent because of its unique and distinct traditions and experiences with beauty pageants.

[c] Geographically Turkey straddles both Asia and Europe. After viewing several years of Turkish contestants in Miss World and Miss Universe I coded Turkey as European

[d] After viewing photos of Israel's contestants in the Miss World and Miss Universe pageant, I coded Israel as European

Appendix C

Table 3: List of Countries Coded as White and Non-White

White Countries	Non-White Countries
Croatia	Tanzania
Russia	French Polynesia
Latvia	Botswana
Hungary	Barbados
Bulgaria	Zimbabwe
Luxembourg	Bermuda
Yugoslavia	Ghana
Poland	US Virgin Islands
Portugal	Swaziland
Switzerland	El Salvador
Malta	Kenya
Spain	Mauritius
Cyprus	Guam
Austria	Guatemala
Gibraltar	Curacao
Iceland	Cayman Islands
Australia	Nigeria
UK	Panama
Turkey	Honduras
Norway	Paraguay
New Zealand	Chile
Denmark	Uruguay
Canada	Singapore
Italy	Bolivia
Israel	Aruba
Belgium	Trinidad & Tobago
Finland	Peru
Greece	Puerto Rico
Ireland	Hong Kong
Germany	Thailand
Netherlands	Sri Lanka
Sweden	Malaysia
France	Bahamas
USA	Costa Rica
	Lebanon
	Dominican Republic
	Philippines
	Colombia
	India
	Mexico
	Ecuador
	Argentina
	Jamaica
	Korea
	Venezuela
	Brazil
	Japan

The Crippling Effects of Labeling on the Public School System

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The constituents of our nation seem to expect the school system to solve all the nation's problems concerning inequality; however, more often the policies of our school systems have perpetuated these inequalities. How to '-save America-' using our schools has been a polarizing topic through the years. In current years, No Child Left Behind has continued this tradition of polarization, casting doubts in the minds of many citizens as to the future of our public education system. By analyzing the theories of Paul Violas, Mike Rose, and J.D Anderson, one sees the negative repercussions of cultural and socio-economical prejudice present in our schools. One can also use their research to theorize a better way of incorporating all students into the curriculum. Furthermore, through the study of tracking; special education; and No Child Left Behind one can theorize the effects this kind of prejudice is having on our public schools.

Paul Violas, a researcher of the American education system, explains to his readers in "Manual Training," how the development of vocational education was co-opted by corporations to create a stronger work force. He theorizes that despite positive progressive intentions during the beginning of the curriculum development for manual training, the backers of these programs were commonly industrialists who felt the intellectual side of the curriculum was cumbersome. The original idea behind manual training was to add to the classic curriculum to create a better-rounded student explaining that, "education should do more than train the mind or the intellect."[1] Unfortunately, support turned against this form of training when industrialists did not see direct gain from the new curriculum. Instead, this new form of education began to take on a more strictly industrial track. In fact, students began being labeled based on what social expectations deemed suitable for them. If a student came from a lower income or working class family, that student was more likely to be placed in an industrial program devoid of classical education. Violas' use of history causes his readers to reflect on more modern tracking systems. Do we still expect certain students will only reach a certain level? Do we still train our students to adhere to the wants of future employers? Violas explains that as the system developed, the concern about "'-higher learning-'" was that this form of education was leaving workers unhappy and bored. The school systems were not there to educate future factory workers to be unhappy with their jobs. Violas explains, "As industrial tasks continued to subdivide into increasingly minute and simple operations, worker boredom and alienation became more acute. Hence, the educational problem posed by most industrial laborers involved personality adjustment, habit formation, and value conditioning."[3] This reform in the schools undermined the earlier American ideal of universal education for all. Violas explains that "this justification was found in the Substitution of "equal educational opportunity" for the older ideal of "equal education."[1] With the continuation of arguments that the schools ought to ensure students' later success in life, the tradition of inequality in American schools was cemented.

Violas' critique of these early policies and his understanding of the development of an American mind-set towards class distinction, helps modern readers to analyze the current school system's policies. Although most "'-tracking-'" programs have been removed from the public school systems, there are distinct levels of programs that separate students. Although one can claim that the use of placement testing is fairer to students and evaluates their ability on an individual basis, it is hard to ignore the fact that different educational opportunities are still available to students based on their environment. Regionally, students enrolled in suburban schools are given more opportunities for club activities, mentorship programs, and advanced placement classes; whereas in other regions, behavioral problems have become a justification for placement into special education classrooms. As a nation, we have learned to better disguise the inequality in our schools; however, class inequality is still present.

The next researcher that brought inequality to the forefront of the debate on education in America is Mike Rose. Rose used his personal experiences to highlight the problems with labeling students, whether it is direct or indirect. Rose explains the misfortune he experienced as a child when he was accidently placed in a low track. The lower track he was placed in was referred to as the vocational track. The shocking realities he reveals are those of incompetent teachers, less than desirable lesson plans, and violence within the classroom. He also explains the myriad of students within the classroom and their disillusionment with their future. A heart-wrenching moment in Rose's tale comes when a student declares a desire to just be average. Rose does not relate more to the readers about his former classmate, the reader is left to infer that the student succeeded in remaining unnoticed. Rose explains the difficulty of being a student in a lower track, "If you're a working-class kid in the vocational track, the options you'll have to deal with this will be constrained in certain ways: you're defined by your school as "slow"; you're placed in a curriculum that isn't designed to liberate you but to occupy you, or, if you're lucky, train you, though the training is for work the society does not esteem."[2] Rose was able to get out of the lower track but found it hard to move up after years of being behind. Luckily for him, he managed to find mentors that inspired him to rise out of the negative situation he had been placed in. Rose used these experiences to criticize the education system and recognized that too often the labels we are place on children cause them to fail.

In Rose's article "Our Schools Our Children," he analyzes the problems he encounters dealing with students at UCLA. Rose watches as students are labeled "at risk" and begin to subscribe to the social expectations placed on them. As he observes an English class filled with "at risk" students, Rose notes the wide array of knowledge these students possess, and a knowledge brought out by a teacher that refuses to toss these children aside; however, many students are not fortunate enough to have teachers with this kind of sentiment. These students walk into the classroom disengaged because they expect to be passed over. Rose explains that these students "know more than the tests reveal but haven't been taught

how to weave the knowledge into coherent patterns."[3] Rose reveals a very poignant analysis of the problem in American schools. He explains that expectations of past success are inaccurate, and that the diversity in our schools is a challenge that ought to be embraced. Instead of looking to strict guidelines for success based on rigorous ideals of past academic standards, the nation ought to look at ways of channeling students' knowledge into a more "coherent pattern." Unfortunately, the kind of "'-back-to-basics-'" education Rose discusses as consistently flawed stems from the exact kind of logic used to justify the increasing amount of standardized testing used in NCLB. Rose's analysis of the problems in the public schools is still relevant today as we continue to ignore the cry of the individual mind.

J.D Anderson gives his readers a thorough understanding of the inequalities present in the development of a "'-free-'" education system for emancipated slaves and their ancestors. In his chapter, "Common Schools for Black Children, A Second Crusade," Anderson reflects on the hard work and dedication of the black community as they worked to create schools for their children. His work reveals how the members of the community put private funding and work into these schools. During the time period before the Great Depression, African American communities endured double taxation in order to guarantee the running of these new schools. At a certain point during the depression, "their financial resources had been drained thoroughly by the process of double taxation."[4] Due to this, many of these schools were unable to keep up with proper maintenance, causing outsiders to view African American communities as neglectful. Anderson explains how these kinds of sentiments caused a negative stereotype to develop that claimed African Americans did not value education. Similar to the stereotyping of the classes that Violas and Rose note, Anderson reveals to readers how these stigmas cause poor outcomes, and how these stereotypes are repeatedly perpetuated by inequalities in the school system. For instance, during a lecture in 2004, Anderson discusses that in the times prior to World War 2, African American schools did not extend to high school. He explains how this neglected fact reveals the injustice behind statistics showing low enrollment in college for African Americans. With high school as a requirement for college, and high school not open to African Americans, how is a student expected to enroll in college? Although we have remedied some of the more glaring inequalities between blacks and whites in American schools, it is impossible to ignore the effects of decades of injustice.

In another work, Anderson takes note of cultural differences in America alongside the difficulties these differences created in attempting to create a universal curriculum and history for America. Just as he exposed in his work on common schools, cultural history is a huge part of the American dynamic. Not every immigrant or student has come from a background with opportunities. As Anderson explains in his article, "Can Public Schools Save America?", "The nation's past is characterized by different and conflicting American Dreams, not by surveys of what we want in the present."[5] In this article Anderson brings to light harsh realities about American views on racial superiority in former decades. He explains how this affected the current educational system and led to many issues of inequality within the nation. He reminds his audiences that ignoring cultural differences is not healthy for the students themselves. Although using the differences to decide how the students will succeed is wrong, it is equally wrong to ignore the different cultures these students came from. Culture is a huge component of who we are and how we perceive the world around us, to pretend that every student is in fact created with a similar background would result in repression of the minority. In addition, Anderson brings up the economic inequality present in our current school system and how that undermines the success of many students: "Throughout American history professional educators and their political allies have looked to public schools to create a homogeneous people while maintaining schools that differed sharply along race, ethnic, and class lines"[5] (Anderson 8). How can we as a nation teach our children a universal set of values when our schools fail to reflect universal equality? Anderson highlights the extreme poverty gap in America. He explains that culture plays a huge part in the development of a people and those years of economic repression cannot simply be ignored in policy.

All three of these researchers concluded that the individual being labeled or lost is a large factor in children becoming disillusioned with schools. One can draw a conclusion from studying these three that a focus on the student's culture and personal goals can help the student to succeed. Similar to how Rose sees the problem, we need to find a way, as a nation, of channeling the knowledge of students into applicable sources. Unfortunately, as shown through Anderson, a homogenized system does not seem to be the key. Furthermore, as Violas reflected, vocational education cannot help students if it limits their opportunities. When the curriculum begins to label students American students are left with the classrooms of Rose's past. When we focus on labels we place students in tracks towards failure; although we claim we are placing them on career tracks.

Tracking is a placement program in use in American public schools. *A Dictionary of Sociology* defines tracking as follows: "an organizational device used in some schools by which students are divided into separate tracks according to supposed ability," the entry goes on to state, "Tracking is an extremely controversial practice and many believe that it promotes inequalities, with students in lower tracks suffering from a less challenging academic environment."[6] Although modern educational practices have sought to do away with standard tracking programs, placement continues to be a factor in our children's education. In most states some form of placement tests are administered in elementary school when a child is deemed worthy of advanced placement. In turn, students are also flagged in elementary school for special education. Once placed in one of these two programs the future of the student's education is altered. For special education students, more opportunities are available for teacher assistance, extra time on tests, and various other program incentives geared towards ensuring the student is able to succeed in a general education classroom. However, the label of special education also carries with it a lower rate of graduation and a negative feeling of being "'-at risk-'". With the label of "'-gifted-'" a student is given more challenging classroom activities and a label that promotes a positive self-image. As these students age and continue along the track the opportunities available begin to differ dramatically. It is as though the school system has taken two siblings and told one that he is destined to succeed and the other that he will always need help to make it. Although the school system uses different language to justify it, tracking is still apparent in today's schools and is a clear example of the inequality in our nation. For instance, inner city schools tend towards a much higher rate of special education students and a lower rate of available AP classes; while suburban schools reveal a higher rate of gifted programs and a lower rate of special education students. Furthermore, in many of these suburban schools the students most frequently given a special education label are students with African American or Hispanic heritage.[7]

If special education is a new form of tracking, how do we as a nation support students with learning disabilities without undermining their success? An answer to some of the problems surrounding the special education programs comes from Joel McNally in his article, "A Ghetto Within A Ghetto." He brings to light the overrepresentation of black students in special education programs and the problems associated with it, "racial disparity makes it less likely that black students receive high school diplomas, less likely they will be employed after leaving school, and more likely they will end up in the criminal justice system."[7] McNally also reveals that an increase in African American student enrollment in special education is directly correlated with the additional categories of emotional/behavioral disturbance to the special education grouping. These categories allow teachers to place students who are disruptive in the classroom into special education. Frequently, clashes in cultural understanding play a part in a teacher's inability to control a student, not the student's tendency towards a disability. Although there are supposed to be checks to ensure students are not unjustly placed into special education, continually, the schools are too overwhelmed with referrals to properly follow these procedures and special education classrooms become overwhelmed by "'-trouble-'" students. McNally does not blame the teachers for this problem but reflects on the fact that no other option is available for them. He quotes Daniel Losen as saying, "Teachers can be trained to think about it and actually change their practices. You know, bias doesn't necessarily make you an evil person. It just makes you a member of society"[7] McNally's analysis of the special education program problems reveals a similar conclusion to Violas, Rose, and Anderson: it is the failure to understand the needs of the individual student and the culture they come from that causes negative repercussions for the students in those neglected cultures.

The name No Child Left Behind insinuates a commitment toward equality in American public schools; however, in reality the policy has failed to create equality in the education system. In fact, in many cases NCLB has actually served to undermine poverty stricken schools and provide justification for further discrimination against the nation's poor. Anyon and Greene argue that, "for more education to lead to better jobs, there have to be jobs available."[8] They explain that NCLB has been used to justify the prevention of job creation programs in the country. That the nation claims the promotion of education will help pull families out of poverty. Anyon and Green's article, "No Child Left Behind as an Anti-Poverty Measure," uses statistical evidence to back-up that the education system cannot operate on its own as a means of preventing poverty. They explain that the jobs available to graduating students are often within poverty-wage rates and that further education has failed to prove financially advantageous to many women and minorities. They explain that the increase in standardized testing has directly benefited corporate America, "Schools that fail to raise test scores, for example, give way ultimately to vouchers in the market model, but first to a variety of expensive, pre-packaged curricula, testing, and tutoring programs. As a result, companies have already accrued billions of dollars of profit."[8] Under NCLB, if a school is labeled as failing students are allowed to transfer to a passing school. In fact, funding is actually removed from the school in order to provide this opportunity to families. However, in many inner city and impoverished communities the constituents are unaware and unable to use these funds for opportunities for their students. Failing schools need financial support in order to move towards success, NCLB punishes these schools by removing funding, but ends up punishing the students.

If a person is looking to find examples of inequalities in schools, our current educational policy of NCLB is a perfect example. The reliance on standardized testing forces all students to prove their knowledge in the same way; and yet, there is no way to create the same learning environment for every child. The added pressure this program has placed on our already spread thin inner city schools sets many of these schools up for failure. The fact is, these schools no longer have the time to invest in programs of cultural education that could prevent teachers from misunderstanding their students and placing them in special education. The only way that NCLB is succeeding in equaling the education opportunities nation-wide is by lowering our schools chances for success.

Researchers Violas, Rose, and Anderson allow readers a look into American educational history as a window into understanding some of the current problems in our educational system. Inequality due to class and race has been a major proponent throughout our history and cannot be ignored. We cannot expect all students to learn in the same way and we cannot force students into specific career tracks based on our expectations of them. The key to success in American schools is opportunity. As our history reminds us our universal education was a founding principle of our nation. Mann explains:

> The Pilgrim Fathers amid all their privations and dangers conceived the magnificent idea, not only of a universal, but of a free education for the whole people. To find the time and the means to reduce this grand conception to practice, they stinted themselves, amid all their poverty, to a still scantier pittance; amid all their toils, they imposed upon themselves still more burdensome labors; and amid all their perils, they braved still greater dangers. [9]

Although this goal is a difficult one to maintain, by focusing on the individual and the cultures in America today we may be able to elevate all students to a higher level of success and reach some of the idealistic goals of our founders.

References

[1]Violas, Paul C. (1978.) "Manual Training, The Training of the Urban Working Class." *The Training of the urban working class: a history of twentieth century American education*. Rand McNally. Pg 125-153.

[2]Rose, M. (1990) "I Just Want to be Average." *Lives on the Boundary*. Penguin Press.

[3]Rose, M. (1990) "Our Schools Our Children" *Lives on the Boundary*. Penguin Press.

[4]Anderson, J.D. (2005.) "Can Public Schools Save America?" *Culture, Race, Academic Achievement, and the American Dream, State of the Discourse*. Du Bois Review.

[5]Anderson J.D. (1988) "Common Schools for Black Children, The Second Crusade 1900-1935" *The Education of Blacks in the South 1860-1935*. The University of North Carolina Press.

[6]Lawson, Tony. (2001) *Dictionary of Sociology*. The Bath Press.

[7]McNally, J. (2003) "A Ghetto Within a Ghetto: African-American students are over-represented in special education programs." Rethinking Schools 17.3

[8]Anyon, J. and Green, K. (2007) "No Child Left Behind as an Anti-Poverty Measure." *Teacher Education Quarterly* 34.2. Pg 157-162.

[9]Mann, H. (1846) "Tenth Annual Report of the Secretary of the Massachusetts State Board of Education." Massachusetts State Board of Education.

Sovereignty and Underdevelopment in China: The 1842 Treaty of Nanjing and the Unequal Treaties

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Abstract:

China's current position in global affairs as a fierce international competitor and growing trade and military superpower has raised many concerns in the West about Chinese government agencies and corporations playing by a different set of rules and gaining an unfair advantage. It has not always been this way. In fact, Anglo-Chinese negotiations at Nanjing in 1842 to conclude the first "Opium War" initiated a century of Western exploitation of China's land and population. The Treaty of Nanjing set several precedents for 100 years of Unequal Treaties with numerous European (and American) powers, which effectively stripped away China's sovereignty and forced its underdevelopment. While Chinese officials at the time were not fully aware of what the implications of the Treaty would be – due to unfair strategies used by their British counterparts – they did understand that their country was being wronged. As time went on, the devastating impacts became more apparent and Chinese authors and leaders began to resist. As Unequal Treaty discourse became ingrained in Chinese mentality, a sense of victimization and resentment developed against Western powers – to such an extent that such feelings may be residual today.

Since its inception, the Middle Kingdom towered above its neighbors, dominating economically and militarily, conducting relations strictly on Chinese terms. However, the arrival of Europeans to Asia posed a serious challenge to the long-established Tributary System. For several hundred years the Qing Empire was able to limit Western influence to one port city at Canton, but by the beginning of the nineteenth century the tables had started to turn.[1] Less than one hundred years later, China had been stripped of its sovereignty, national pride, and all-but subjected under imperial power. Forced to relinquish various concessions and rights to the Western powers, China fell painfully from the top of East Asian relations to the bottom of the global system. The Unequal Treaties, most importantly the Treaty of Nanjing of 1842, initiated an era of underdevelopment and quasi-imperialism in China, perpetuated by Western privilege-seeking, which manipulated and exploited a Chinese government ignorant of the ramifications of what they were accepting. A brief history of the events leading up to the drafting of the Treaty of Nanjing, a summary of the terms of the treaty, and its precedential role – considering several other Sino-Western treaties signed up to the Convention of Beijing, 1860 – provide an informative discussion of the treaty. Exploring in more detail the most important provisions of the Treaty of Nanjing and the subsequent Unequal Treaties don insight as to their respective effects on China's sovereignty and development. Chinese perspectives on the treaties' implications both at the time of their drafting and signing, as well as the evolving perception of the treaties' consequences were especially revealing in regard to China's (under)development and sovereignty in the century after the Treaty of Nanjing.

The Treaty of Nanjing brought peace, concluding the 1839-1842 Anglo-Chinese War; understanding the causes, events and results of this "Opium War" shed some light on the composition of the treaty and its impacts. From the Chinese point of view, forced and illegal importation of foreign opium was poisoning the population, and as of 1829, the balance of payments reversed and China's silver surplus began to drain out of the country.[2, 3, 4] The British, while appreciating the reversal of their trade deficit, denied that the war was fought for drug-related purposes. Rather, Britain perceived the war as one fought for recognition of national equality with China, for free trade and the abolition of the Cohong merchants' monopoly on trade at Canton, and for the application of "more civilized" British law over nationals living or trading in China.[3] With tensions on both sides peaking in the late-1830s, the smallest of incidents could have incited conflict.

Such sparks were neither few nor far between. The 1833-1834 abolition of the British East India Company's monopoly on the China trade flooded the port at Canton with new merchants and traders from England; the resultant competition overwhelmed the rigid Cohong system, increasing tensions while augmenting the influx of opium and dispersing its entry.[5] Another strain on relations followed the murder of a Chinese man by drunken British sailors in Canton. Qing officials insisted that the sailors be tried in Chinese courts, but the British claimed the right to prosecute their own nationals – both sides refused to yield, stimulating the pressures of war.[5] The breaking point came with the dispatch of Lin Zexu, the "incorruptible official," to Canton to oversee foreign trade in 1838.[5] Commissioner Lin's mission was specifically concerned with controlling foreign trade, yet he took the initiative to put down opium sales and consumption as well, widely viewed as disastrous for China's finances.[2] As such, Lin confiscated 20,283 chests of opium from British storehouses in March 1839, completely destroyed the contents and offered no reimbursement to foreign merchants.[3] With that act, Lin banned British merchants from the port of Canton. They retreated to Macao, a Portuguese possession at the time, where the British navy rendezvoused and began a series of naval assaults. The British dominated the war, due in large part to their superior military might and naval expertise; the Chinese were

forced to succumb to negotiations almost immediately. Fighting and negotiations continued simultaneously, but no substantive results were produced until the British capture of Nanjing, the southern capital, in the summer of 1842.[1, 2, 3,5]

On 29 August 1842, a Treaty of Perpetual Peace and Friendship, or the Treaty of Nanjing, was concluded between Britain and China at Nanjing. The Treaty was signed on board HMS *Cornwallis*, by British negotiator Sir Henry Pottinger and Chinese High Officials Ch'i-ying and I-li-poo, marking the end of China's relative inclusion as Britain used their victory in the Opium War as "leverage to pry it open".[7,8] Written in English and only crudely translated into Chinese, the Treaty ended the Anglo-Chinese hostilities with a peace between the two nations specified in Article I, the cession of Hong Kong Island to the British by Article III, payment for the destroyed opium as well as a twenty-one million dollar war indemnity to the order of the Queen as stated in Articles IV and VII. Also included in the terms of the treaty were Articles VIII and IX, which demanded all British subjects and Chinese collaborators to be released to the British authorities, Article V's abolition of the Cohong system at Canton, and the opening of four additional ports at Ningpo, Shanghai, Fuzhou, and Amoy, to British merchants for trade under fair and regular tariffs to be agreed upon by British authorities (Articles II, V, and X respectively). Collectively, these five access ports became known as "treaty ports." Finally, Article XI demanded that communication between China and Britain, regardless of the level of the official, take place on "footing of perfect equality." Should all of the above demands receive compliance, Article XII promised the withdrawal of British troops and the formal ending of the war.[9] The treaty's true significance existed not in any of its thirteen articles; rather it was the precedential nature of the Treaty of Nanjing that marked the beginning of a century of unequal, "gunpoint treaties".[3,8] Such treaties were not only unfair, but also unethical – written and negotiated in English or other Western languages – creating a grave misunderstanding of the potential impacts among Chinese negotiators.

The supplementary Treaty of the Bogue between Britain and China followed the Treaty of Nanjing just over a year later on 8 October 1843. Signed by Pottinger for the British and Ki Ying on behalf of the Qing Emperor, the supplementary treaty extended and specified tariff of export and import duties, as well as the general regulations on trade.[10] Additionally, the Treaty of the Bogue reinforced Britain's exclusive access to the five treaty ports opened by the Treaty of Nanjing. Legal extension of extraterritoriality to foreigners also originates with this treaty. Paramount within the Treaty of the Bogue was the most-favoured-nation clause (MFN), which effectively helped pry the door to China ever-wider open.[10]

The United States (US) practiced "hitchhiking imperialism" in China, quickly following the British precedent with an unequal treaty of its own.[8] Caleb Cushing was sent to China to negotiate terms of a trade agreement with a Qing government eager to play the Western powers against each other. On 3 July 1844 the Treaty of Wanghia was signed, largely regarding terms of trade and residence in the treaty ports opened by the British negotiations at Nanjing. It also built extensively on the foundations of extraterritoriality established by the Treaty of the Bogue. As with the British treaties, communications were to be on the basis of equality; unlike the British, however, the Treaty of Wanghia officially banned American merchants from selling opium and from trading outside of the treaty ports. The China-USA agreement also included a most-favoured-nation clause, automatically granting the Americans any rights ceded by China to another nation.[11] The French quickly followed suit later in July 1844, signing the Treaty of Whompao with China. The French treaty was very similar to the Anglo-American versions, further extending extraterritoriality and treaty port concessions, while also granting privileges to Roman Catholic missionaries to travel in China and preach Christianity. By way of the most-favoured-nation clauses in the British and American treaties, missionary privileges were further extended to Protestant and Baptist missions, as well as other Christian creeds.[12]

The 1856-1860 Anglo-French Arrow War against the Chinese was concluded officially at the Convention of Beijing, where the US, France, and Britain had forced the Chinese to accept the Treaties of Tianjin, written in June 1858. By this time, Belgium, Sweden, Norway, Prussia, Portugal, Italy, and Russia had signed similar Unequal Treaties. The Tianjin treaties were brought into force at the 1860 Convention of Beijing and reaffirmed the earlier treaties, adding or abrogating provisions of the previous agreements. The Treaties of Tianjin forced the opening of ten additional ports, including four along the Yangtze River deep into inland China. Treaty rights and concessions were extended to each of the new ports, while also establishing permanent Western legations at Beijing. In addition, foreigners were granted permissions to travel throughout China; inland tariff dues were further decreased to 2.5% *ad valorem*; another large indemnity was demanded of the Qing government; and missionaries were guaranteed free movement and the right to own property outside of the treaty ports. Moreover, the British claimed the Kowloon Peninsula, opposite Hong Kong Island, as a colonial possession, on which to base their trade and military operations.[5]

Each subsequent unequal treaty effectively eroded the sovereignty of China, reduced reciprocity, restricted its economic autonomy, and forced the Qing to conduct Sino-Western relations according to foreign terms.[13] The most-favoured-nation clause, the opening of China, extraterritoriality, settlement rights, and the equality of communications provisions were especially devastating to the traditional Chinese government and social structure.

Of all the articles and concessions granted in the Unequal Treaties, initiated at Nanjing, most-favoured-nation statuses had the most wide-ranging, detrimental effects on China's sovereignty. Captured in Article VIII of the Treaty of the Bogue:

> The Emperor of China having been graciously pleased to grant, to all foreign Countries, whose Subjects, or Citizens, have hitherto traded at Canton the privilege of resorting for purposes of Trade to the other, four Ports of Fuchow, Amoy, Ningpo and Shanghai, on the same terms as the English, it is further agreed, that should the Emperor hereafter, from any cause whatever, be pleased to grant, additional privileges or immunities to any of the subjects or Citizens of such Foreign Countries, the same privileges and immunities will be extended to and enjoyed by British Subjects.[10]

Every subsequent treaty with a Western power included some imitation of this clause, thereby fostering an automatic, unconscious cooperation between the West to pry China open, and maintain equality of opportunity in the China trade.[12] Equality in China's relations was not an imposition of the West, however, as the Tributary System existed for millennia before the Opium Wars based on non-discrimination. What changed under the Western imposed the most-favoured-nation system was China's relative position in international relations – from the top of its Tribute System, where

all others were subordinate, to the bottom of the global system, exploited by all who pleased.[12] Though most-favoured-nation status was originally sought for commercial equality among the Western powers, it eventually "embodied a limitless doctrine of equality of opportunity capable of expansion in many directions," effectively amplifying the perverse effects of the treaty port concessions, extraterritoriality, and the like.[12]

In addition to the MFN clauses, which stripped away Chinese sovereignty piece by piece, the opening of China and the foreign control over tariff rates and terms of trade effectively "deprived China of control over its own economy".[8] With the Treaty of Nanjing, British merchants broke free from the confines of the Canton system, which was wrought with corruption and the restrictions of the Cohong traders. Chinese officials granted access to trade and settle in Amoy, Fuzhou, Ningpo, and Shanghai in addition to Canton, mistakenly assuming this would dilute the foreign trade rather than intensify it. This ignorance of the economic functioning of the free market world emerging in Europe also led the Qing government to relinquish their tariff autonomy.[2,3,5] Tariffs were fixed by British official consent, only to be modified with approval from the Crown. By 1860 ten additional ports were open to foreign trade, and foreign consuls and trade officers were firmly in control of the customs duties and tariffs levied on Western imports to the treaty ports.[1,4,5] Foreign merchants and their Chinese counterparts were able to manipulate legal loopholes, ignoring China's sovereign rights, and on some occasions, evading taxes altogether.[8]

It became difficult, if not impossible, for China to protect nascent industries and young Chinese companies against foreign trade, especially after the 1895 Treaty of Shimonoseki with Japan, which expanded foreign rights in treaty ports to include the construction of manufacturing companies. By virtue of most-favoured-nation status, such a privilege was granted to all foreign powers with that had signed an Unequal Treaty with China; foreign direct investment spiked, further endangering China's domestic manufacturing economy.[4,8] Conversely, the stimulus provided by foreign direct investment and the Western trade created tremendous growth in the treaty ports, suggesting that China's integration into the capitalist world market was not entirely negative, and that its underdevelopment was only relative.[4,14] While such arguments have some validity, the development in treaty ports was grossly unbalanced with the underdevelopment of the rest of the country – siphoning capital away from local markets and domestic firms into the treaty ports significantly hindered modernization processes in China.[4]

Extraterritoriality, defined as "the right of jurisdiction by foreign consuls over their nationals," proved devastating to China's position as a sovereign state in the global system.[5] Because of the Unequal Treaties, China was hardly sovereign in its own territory, as Western citizens were protected while in China, yet the lack of reciprocity gave China no such right to safeguard their own nationals.[8] Extraterritoriality was a long process in the making for Europeans (and Americans) in Asia; various events occurred in the history of Sino-Western relations leading foreign powers in China to demand rights for their nationals to be tried under a consul and not in Chinese courts, which were considered cruel, clandestine, and repugnant.[15,16] Jurisdiction over nationals in treaty ports also applied to those traveling through inland China once such movement was allowed; Chinese officials were often pressured by foreign governments to turn criminals of Western nationality over to foreign consuls, or even to let Christian missionaries deal with Chinese citizens who had converted to Western religions.[17] Essentially, China had been deprived of sovereign legal jurisdiction within its own borders – any wrong move might lead to Western intervention, more conflict, or further unequal treaties.

As subsequent treaties were signed, extraterritorial rights were extended from peoples to concessions granted as neighborhoods in which foreigners could live. Entire districts of the treaty ports fell under the jurisdiction of Western powers – such settlements became self-governing, establishing independent police forces, municipal governments, and administrations.[6] The concessions forced by the foreign powers, especially by the Treaties of Tianjin and beyond, provided Westerners rights to rent, and eventually own, properties in treaty ports. They erected buildings and churches on leased territories, with little regard for Chinese concern with *feng-shui*.[5] Protected by extraterritoriality, foreign zones in the treaty ports became havens and places of refuge for foreign merchants, their Chinese counterparts, and their possessions – tax exemptions from their national governments and shelter from Chinese corporate and criminal laws made these regions very attractive. In reality, concessions and settlements, combined with the automatic extension of extraterritoriality and Western legal traditions and systems of trade by most-favoured-nation clauses, embodied an exploitative quasi-colonialism, rendering the Qing government powerless to control their own territories.[1,4]

Related to the settlement rights granted under concessions to the West, was the permission of foreigners to learn Chinese, travel inland, and preach Christianity. All of China was opened to proselytizing missions and travel, producing significant cultural disruption to match the economic disruption resultant from foreign commerce penetrating the interior.[5] Missionaries saw China as a vast, populous, and idolatrous territory, which provoked excitement among Christian sects seeking converts. The missionary drive reinforced economic expansion, while economic and legal expansion beginning at Nanjing, supplemented religious rights and privileges of Western missionaries.[4] The impact of Christianity should not be overestimated, as the number of converts remained quite low; however, the disruptive effects of Catholic and Protestant missions, and foreign travel inland were readily observable.[17]

For as long as Britain had been in contact with China, and communications between officials were sent and received, the degrading language used by Chinese in reference to foreigners was not much appreciated.[1] By the 1830s, as the tides turned in Britain's favour, they actively sought amelioration of the humiliating treatment practiced by the Chinese.[12] The term 'I' (barbarian), with which Chinese officials referred to all foreigners, regardless of status, was most despised. From Nanjing onwards, however, such derogatory terminology in the Qing vernacular was forcibly diluted and officially banished from official communications. The principle of diplomatic equality enshrined the worth of Western standards of diplomacy, shattering remnants of the old Tributary system, which saw China at its apex.[8] Regrettably for China, equality was broadly interpreted by the foreign powers to mean Sino-inferiority and dominance of Western methods, traditions, and norms. More unfortunate was the utter lack of understanding on behalf of the Qing officials about exactly how Western diplomatic relations worked – this led to further exploitation. Treaties were most often written in Western languages and converted crudely into Chinese – meanings were regularly lost in translation, with the document in the Western language held as law.[5] The advantage in diplomatic relations quickly swung from the Chinese imperial

court, to parity, then to the governments of the foreign powers who used such devious methods to manipulate Chinese negotiators further transgressing China's sovereignty and abetting its underdevelopment.

The detrimental effects of the most-favoured-nation statuses, treaty ports and trade exploitation, extraterritoriality, settlement and inland travel rights, and Western-imposed standards of international relations are clearly visible in hindsight as they continued to build with each successive treaty. Still, the original reception of the Treaty of Nanjing was that of relief. This was best represented by Chang Hsi's *Fu-i-jih-chi* (The Pacification of the Barbarians), in which Chang, an advisor to I-li-poo, recalls the negotiations and signing of the Treaty. Chang's diary reveals that while Chinese officials were primarily concerned with appeasing the superior English navy, they also had minimal knowledge of foreign affairs and international law, exacerbating the problems of their hasty submission.[3] I and Ch'i hardly looked at the terms of the treaty, instead rejoicing at the prospect of the British withdrawing – even if they did read the proposed terms carefully, they had been given authority from the imperial court to act as the circumstances required and make whatever concessions necessary in the name of peace.[3,7]

Moreover, the Qing did not fully understand the significance of the Treaty of Nanjing either intrinsically or as a precedent. To them it was simply an extension of concessions they had already made to the Kokland people of Central Asia to the maritime frontier, as a way of diluting Western influence and pressure.[1] The ramifications of such an important event were oversimplified and underestimated both in the Qing imperial court, and by Chinese high officials charged with negotiating its terms. The Treaty of Nanjing was seen as only a minor setback in the grand scheme of Chinese relations; Westerners had been subdued, held to the shores of China's great empire, and opium remained illegal.[5] Additionally, the treaty failed to ease tensions in Canton and all self-respecting Chinese retained an "implacable hatred" of the British.[2]

It was only as subsequent treaties were signed, building from the legal foundation set by Nanjing that the Chinese began to realize the severity of their negative impact. By the 1870s it was increasingly common to find demands for *gongping* (China's treatment as an equal), *ziding* (autonomy), and *zhoquan* (sovereignty).[13] *E'wai quanli* (extraterritoriality) – a term invented in 1883 – was gradually more refuted, especially because the Chinese argued that no such laws existed between European nations.[13] China's humiliation and inferiority was resented more and more from the Convention of Beijing onwards. Hatred of the West was augmented by the growing economic strains of constantly paying indemnities and loans, without the tariff autonomy necessary to earn revenues. Although domestic factors also had a major impact on the underdevelopment of China, "imperialist-colonialist behavior of the West (including Japan) was, on balance, inimical to Chinese development".[4]

The weak Chinese economy and state was partially held together by self-strengthening reforms and a strong, centralized authority – which collapsed with the death of the Empress Dowager in 1908.[14] Imperial Qing China destabilized and collapsed. From the ruins rose competing nationalist and communist movements, both seeking to exploit *bupingding tiaoyue* (Unequal Treaty) rhetoric to reap emotional and political support.[13] First expressed by Sun-Yatsen, a founding father of Republican China, in 1924, he stated: "All Unequal Treaties … foreign concessions, consular jurisdiction, foreign management of customs service, and all foreign political rights on China's soil, are detrimental to China's sovereignty. They all ought to be abolished so as to leave the way open for new treaties based on the spirit of bilateral equality and mutual respect for sovereignty".[18] Unequal Treaty discourse in the early twentieth century followed three paths. The moral discourse was used in attempts to persuade foreign powers to recognize their unfairness and resultant injurious effects in China. Legalistic discourse also sought to reason with the West on the basis of the illegitimacy of the Unequal Treaties. Finally, rhetorical discourse was aimed at generating a strong emotional response from ordinary Chinese citizens against Western imperialism.[13] Through Unequal Treaty discourse, national humiliation and China' victimization at the hands of the West became integrated into the Chinese-ness of the masses in the early-1900s.[13]

The Treaty of Nanjing, 1842, represented the beginning of the century long system of unequal treaties in East Asian history. The intentional vagueness in which it was written served to enable the West to extort more and more rights away from the Qing, contributing to the centuries-old dynasty's decline.[13] In the process of events, the villainous intent of the West was apparent – imposing, by force if necessary, anything and everything they could have wanted onto the Chinese. Ultimately, The Treaty of Nanjing set an important precedent of exploitation in East Asian international law, followed for nearly one hundred years as the basis for diplomatic relations between Asia and the West. The Treaty of Nanjing set an important precedent in international law for foreign dealings with the Chinese Empire. Followed for nearly a century as the basis for diplomatic relations between Asia and the West, the Unequal Treaty model served to exploit immense opportunities in China by ripping away Chinese sovereignty and ensuring underdevelopment in the Middle Kingdom.

References

[1]Waley-Cohen, J. (1999) *The Sextants of Beijing*. W.W. Norton & Co.
[2]Waley, A. (1958) *The Opium War through Chinese Eyes*. George Allen and Unwin Brothers Ltd.
[3]Teng, S. (1944) *Chang Hsi and the Treaty of Nanking*, 1842 Chicago University Press.
[4]Lippit, V.D. (1978) "The Development of Underdevelopment in China." *Modern China* 4.3 Symposium on China's Economic History.
[5]Borthwick, M. (2007) *Pacific Century: The Emergence of the Modern Pacific Asia*, 3rd edition. Westview Press
[6]Bloch, K. (1939) "The Basic Conflict over Foreign Concessions in China." *Far Eastern Survey*. 8.10.
[7]Fairbank, J.K. (1940) "Chinese Diplomacy and the Treaty of Nanking, 1842." *The Journal of Modern History*. 12. Pg 1-30.
[8]Herring, G.C. (2008) *From Colony to Superpower: U.S. Foreign Relations since 1776*. Oxford University Press.
[9]UCLA Center for East Asia Studies. "Treaty of Nanjing (Nanking), 1842." *East Asia Studies Documents*.
[10]"The Treaty of the Bogue, 1843." *Bilateral Documents – 19th Century*.
[11]"The Treaty of Wanghia, 1844," *Bilateral Documents – 19th Century*
[12]Prichard, E.H. (1942) "The Origins of the Most-Favoured-Nation and The Open Door Policies in China." *The Far Eastern Quarterly*.
[13]Wang, D. (2003) "The Discourse of Unequal Treaties in Modern China." *Pacific Affairs*. 76.3. Pg. 401
[14]Bergere, M.C. (1984) "On the Historical Origins of Chinese Underdevelopment." *Theory and Society*. 13.3. Pg. 327-337
[15]Swisher, E. (1951) "Extraterritoriality and the Wabash Case." *The American Journal of International Law*. 45.3. Pg. 564-571
[16]Wright, Q. (1943) "The End of Extraterritoriality in China." *The American Journal of International Law*. 37.2. Pg. 286-289.
[17]Lee, J.T.H. (2000). "The Lord in Heaven versus Jesus Christ: Christian Sectarian Violence in Late-Nineteenth Century South China." *Positions: East Asia Culture Critique* 8.1. Pg.77-99.
[18]Xiaoyi, Q. ed., (1989) *Guofu quanji [The complete collection of the founding father's writings]. Vol 2*. Jindai zhongguo chubanshe. Pg. 138.

On Ice

By Nicholas Kirkland
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It was over 100 degrees out and Mickey turned ten that day. He pouted like a little brat because he thought I forgot, and maybe I did. We left the old folks home and there in the sweltering parking lot I made him promise me. This was one of the few times I bothered to look him in the eye, because it was important. I knelt down and took his head in my hands and pressed hard against his skull.

"You listen to me," I said, "You listening, Mickey?"

Mickey tried nodding, but he couldn't move his head.

"If I ever end up like gramma in there, I want you to promise me something. You promise me. You drive me out to the middle of nowhere and you leave me there and you drive away. Got that?"

"You're hurting me."

"I said promise me, Mickey." And I breathed my cigarette breath onto his face.

"I promise."

I released him and I didn't look him in the eye after that.

It was a moment of weakness and I said, "Good boy."

I saw him smile all big in the corner of my eye.

*

Thirty-five years later, Michael drives his father out to the desert for a second time. His car is not like the beat-up truck his father used to have. He keeps the car clean and his feet don't have to crush piles of cigarette cartons when he takes a seat. The car blasts cold air onto cold leather and he worries about his father in the passenger seat, smiling out at the passing orange horizon. Michael half-expects his father to split apart somehow and leak drool and tar all over the upholstery.

He drags up old memories and tells his father about them. He tells his father about the divorce with Sarah and how he has felt clumsy and disconnected since then.

My daughters never call me since they went off to college, Michael says. *But I think they call Sarah.*

His father points at the mountains and says, *Well, it must've taken a lot of work to dig those up.*

Michael glances at his father's face. The skin is relaxed off his skull where it used to be hard and yellow with nicotine and rage.

Those are mountains, Dad, Michael says.

He pulls off the road to a gas station, the last one on the edge to complete isolation. He tells his father to wait and sucks in the Nevada heat on his way into the gas station. Inside, everything is red plastic and fluorescent lighting. At the slushie machine, he fills a cup with cherry slush.

Sugar and water, that's all it is, Michael thinks. *Do you know what that does to teeth?*

Michael is a dentist. His closet is stiff with neutral colors and he drinks health shakes and flosses regularly. He tries to keep his mind and body from rotting; he trains for marathons and just last week he mastered drilling thin blocks of wood and turning them into polished wood pens. In the coming week, he will look for a new hobby to keep him occupied. He doesn't drink alcohol. He doesn't watch television.

The same kid from last time slouches behind the counter, thumbing through a magazine about neon green crotch rockets. Michael places the slushie on the counter and waits for the kid to ring it up. He wants to smack the kid, tell him to stop sucking down sodas and go jogging, go find a real job. Michael tells himself he's a nice guy, he's really a very nice guy. He pays for the slushie. The cup beads with water when he leaves the building.

In the car, he hands his father the slushie. His father looks happy.

*

The day I made Mickey promise, I took Mickey to see his gramma at the old folks home. I drove him there and the kid tucked into himself as if scared to touch anything. I didn't think I could raise such a shrimp. But accidents happen and condoms split and it feels so good for a moment. Then you end up with a kid you don't want and a woman gone sour.

I slammed the truck into park near a low building made of yellow stucco.

"You're going to meet your gramma," I told Mickey.

"We aren't going to the movies?"

"We aren't going to the movies what."

"We aren't going to the movies, sir?"

"No."

I told him to move, get out of the truck. I hated this place. I hated this place because it wasn't a nice place; I couldn't afford a nice place. This place was run by ugly little women who wheeled old folks in front of windows and let them rot in the sunshine. They fed the old folks mush. Holidays warranted grocery store pies. Every so often someone arranges some Luau bingo and I went to one of those and, Christ, no wonder those people weren't getting better. Momma'd grown worse, though I didn't tell Mickey because what did it matter? Kids don't need to know that, kids should just be kids and stay out of the way.

The sweaty receptionist recognized me and let us into the "community room." The walls were faggot pink and had drawings of boats and beaches framed in corners. Mickey scrunched his face up and I knew he smelled this place, a dry perfume dressed up so you don't recognize it as urine. I made my way to the semi-circle of couches and rocking chairs and Mickey followed, out of my direct sight. Old folks in rocking chairs sat staring at a TV – an endless parade of Lodge members and war veterans. The TV was still a black-and-white set, for God's sake. I knelt at the fungus yellow couch where Momma sat alone, so fat and deflated you'd swear she was a failed cartoon. I pulled Mickey over and pushed on his shoulder to make him kneel with me.

"Momma," I said, "it's me. This here's your grandson, Momma. His name's Mickey." I hit the back of his head. "Where's your manners?"

"Nice to meet you, ma'am," he said. Momma looked at us, pleased as punch, and I couldn't help but think of the woman she used to be. Through the blue veins along her arms and folds in her neck, it got hard to recognize her as the only good thing in my life. It also got a whole lot easier in a shitty sort of way. She used to make bowls of tapioca and she sent me to my room when Papa came home with his fists tight.

"Well," she said. She didn't understand, and that isn't the worst thing about this kind of old age. The worst is that I could see her frowning a little, like she knew something

was wrong, like there was a stain on her shirt she hadn't noticed but everyone else had.

"You spend time with your gramma," I told Mickey. "You talk to her, okay?"

"Yes, sir."

And then I stood up to go have a smoke and I passed a husk draped in a bathrobe, muttering about Krauts and mud. I hoped Mickey would hear; it would toughen him up. I smoked outside and frowned about Mickey's mother. She left a month ago. She said I drink too much, but she was just the same as me. I told her to take the boy with her, but she didn't. Same as I would have done.

I didn't want to go back to Momma. It didn't seem to matter what I said to her, the words made no difference and she understood nothing anyways. Why bother? But that kind of thinking got me guilty. I was a lot of crummy things, but I wasn't the guy who'd give up on his mother. I finished my last cigarette and went back inside.

Mickey stood next to Momma like he'd been caught with a boner. "I don't know what to do, sir."

A new smell hit me and I looked at Momma, poor Momma. She looked embarrassed too, her chin dipped down into her neck. She shit the couch and, boy, I started yelling for someone to come get her clean. I started cussing, just a string of words that didn't make sense after a while. The old folks watched me yell, their heads turning slow to watch me. Dull yellowed eyes.

*

Michael drives his father out to the desert for a third time and tells him:

A long time ago, Eskimos would set their old folks out onto ice floes when times were hard and food ran out. The old folks couldn't contribute, so they were put on ice or thrown in the sea or starved or buried alive in the snow and in the cold. Sometimes, an entire village would pack up and move while the old folks slept. Just imagine waking up to that cold. You'd look around and the people you love are gone and you can't recognize anything, the world around you is flat and white and the only color you can find is in the turquoise roots at the edge of the sea. You're shaking and maybe it's the age. The color of your skin goes brown to red until that fades too. You think about the family that left you here. The day before, had your daughters avoided your gaze? How much of a burden were you? You can't think coherently, but you feel these things, some part of you still does. You lie on your back and stare at the sky before you sleep. You try to remember a time when you felt bigger. You turn onto your stomach and crush your face into the snow and ice. Your teeth scream and your marrow thickens and turns blue and you don't feel anything anymore.

*

A lady in purple scrubs came by to lead Momma away and clean her. She told me to stop yelling.

"I'll clean the couch," I told her, clenching my teeth. "She's my mother."

The lady looked at me wearily. She was all limbs and wispy black hairs. "Don't bother. We'll just throw it out."

"Let me," I said. I gestured to Mickey to take one end of the couch. "She's my mother."

The lady shrugged and led Momma away and I watched them go before I took the other end of the couch.

"We'll take it through the front," I told Mickey. "Ready?"

When he nodded, I counted down from three and we lifted the couch and it turned out heavier than I thought. We gripped the bottom of the couch. Stray nails bit my palms and some genius managed to get staple ends sticking out down there too. Mickey trembled and struggled to keep his end steady but he kept his face straight and didn't say anything. I didn't tell him good job because I wanted him strong. I let him walk forward while I walked backwards, glaring over my shoulder to see the way. I kicked open the glass doors behind me and we wriggled the couch through the doorway, getting close to the smell. And then we swayed out into the Nevada sun and Mickey was trembling again, red as sin. I barked orders at him and we started a faster pace out here, around the corner of the old folks home and in sight of the simmering parking lot where I would make Mickey promise me.

Nearly fifty feet away from the dumpsters, Mickey dropped his end of the couch, cracking wood against pavement. He gasped and jumped back and some piece tumbled off.

The heat got me. The heat boiled my brain.

*

Michael drives his father into the desert for a fourth time and tells him:

Do you remember when I was seven? You and Mom had a party and you sent me to bed early. But I was excited and I couldn't sleep because of my balloon. Mom brought me to the dentist earlier that day and I was scared they'd drill into my jaw. But the dentist was kind, he had big sideburns and a crooked nose. And then he gave me cherry toothpaste and tied a balloon to my wrist. It was red with fat white letters on it. Do you remember that? I stared up at the balloon, watched it bob against the ceiling. I was so happy and proud, I got up to show you. You and Mom and your friends greeted me in the living room with shouts, you were happy to see me, you were drunk. You picked me up and sat me on your lap and laughed smoke. You took your cigarette and pressed it against the balloon and you and Mom and everyone laughed and laughed. Remember?

His father smiles uncertainly. *Oh*, he says, *well.*

So Michael turns up the air conditioning and tells his father again about the divorce, how bad it had been. He and Sarah ended up hating each other after the kids left for college and pulled away that last buffer between them.

We were too different, he tells his father, *or too similar. She told me I was stuck in the past and I told her it doesn't work that way, the past sticks to you, it changes as you change. When your neighbor called to tell me how you were and I hadn't spoken to you in fourteen years, I don't know. Sarah wanted you in a rest home. She told me to just let go. It was sad, she said, but you weren't worth the effort. I lifted my hand to hit her but our marriage ended before that, I guess.*

Michael drives in silence for a while.

Well, his father says. He sips at his slushie.

*

I threw down my end of the couch and grabbed at the armrest and pulled.

"God."

I dragged the couch a couple of feet. And the couch tipped onto its back.

"Damn."

I kicked the bottom of the couch and pounded my fists onto the wood paneling. The nails and those staple ends stabbed my skin.

"Couch."

I was the only one. The woman left, and all that was left was me and the boy and some godawful fear running its fingers down my ribs. I couldn't do anything. I wasn't supposed to have a kid. Momma wasn't supposed to end up a brainless sack. I wasn't supposed to end up like my old man.

I couldn't budge one little couch. I sat on the pavement and stared at it. What little damage I did.

"Sir?" Mickey said. He hadn't moved from the spot where he dropped his end of the couch. He was not so far away.

"Sir?"

I stared at the couch and I thought, I should get Mickey something for his birthday. Doesn't really matter what, I guess, just something. Maybe, I thought, after this, I'll drive him over to a gas station and grab him a slushie or something. I'll get some

cigarettes for me.

"Dad?"

I needed a smoke.

*

Michael drives his father out to the desert and takes a turn off the main road. Rocks make small chirps against the body of his car and his father looks out the window at the rising dust. He pulls up to a hiking trail that isn't used much anymore; there are rocks here but nothing beautiful and no ancient petroglyphs for tourists to touch. He parks the car, unbuckles his father, and takes the slushie from him to fit it in the cup holder.

He goes out toward the rocks and checks the sparse shade for snakes and scorpions. Satisfied that there are none, he returns to the car and helps his father out.

I'm going to leave you here for a bit, Michael says. *For a bit, okay?*

His father says, *Well, okay.*

And Michael leads his father over to the rock his father likes most. Then he leaves his father to sit in the sun and he returns to the car. He has performed this ritual several times. Michael starts the car and drives away. The first time, he couldn't even do that. He had sat in the car for twenty minutes, watching his father watch him. Then he got out and brought his father back.

The second time, he drove away but drove back in a matter of ten minutes. The third time, he forced himself to wait half an hour before returning. He had spent that half-hour parked in the middle of nowhere, his head pressed against the steering wheel and one foot tapping against the brake pedal. He waited an hour last time and this time he could wait two.

Michael parks on the side of the road. He grips the steering wheel and tells himself one more hour, I'll wait here just one more hour. He thinks about his daughters who don't look away from the blue glow of their cell phones. Maybe he would call and see if they would listen to him talk about the past. He would ask about their lives.

One more hour, he tells himself. Then I'll drive back to him. I'll pull up to him and he'll give me that puzzled smile, the one he uses when he's trying to figure out who I am. One more hour, and I'll come back and pull right up to him. I'll get out and open the passenger side door for him. I'll tell him I'm his son and I'll look him in the eye and hold out the melted slushie he didn't finish.

And my father will be the happiest he's ever been to see me.

An Epiphany from One Crazy Time when I Went to the Library for the Books

By Elizabeth Strait
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Finals week of this past fall semester I went to the library. But this time it was different because for once it wasn't in search of a couch to sleep on between classes or a "quiet" place to study, for almost the first time in my college career I actually went there for the books. As I walked past crowded tables with kids furiously writing papers or pulling their hair out over study questions, I realized I was probably the only person who wasn't there for finals.

I was actually there to prepare for *after* finals when I would have an entire month off from school. As a senior on track to graduate in May, I had decided that over break I needed to make the most of what would probably be the very last time in my life that I would have an entire month of with almost zero obligations and decided that "making the most of my time" meant reading a *lot*.

I ended up at the library to find books because I was in search of a solid yoga asana book, one with a good balance of diagrams/pictures as well as clear explanations and tips. But I had a really difficult time deciphering from the descriptions on the library's website which book was exactly what I was looking for. So I decided to go old school and finally follow some advice my capstone professor gave me; find the call number for a book that you think you want (or at least close to what you want), then physically go find it and while you're there investigate the books on the shelf around it.

So that's what I did. And it actually worked out really well! I landed on "The Complete Illustrated Book of Yoga" by Swami Vishnudevananda, which from the plain binding and black and white pictures seemed a little dated but the contents seemed very promising. I checked it out, along with a few other books as well and headed home.

My epiphany didn't really start though until I started actually reading it later. What got me thinking was how horrible the page numbers were; sometimes picture pages accounted for page numbers and sometimes they didn't. Which was really frustrating for me because I was trying to use the index to look up certain pages the inconsistency in numbering was making it extremely difficult.

Worse, the index was poorly formatted as well; it only listed the poses in alphabetical order by their Sanskrit names and not the English translations that I knew only slightly better.

What the hell was the editor thinking? I thought. *When was this published?*

Like a super book nerd, I turned to the front to check out the copyright page.

I barely saw it. It was placed randomly: after title page and adjacent to the introduction and not before the table of contents, odd—I know. It was the smallest front and shortest bit of copyright jargon I'd ever seen. I considered that it may have actually been nothing but a stamp, perhaps to save money on printing…

How old was this? I thought as I closed the book and turned it over, spine up.

I realized that it was bound rather similar to the way that the old CSU bound thesis books looked. It also had the same way-back-when-CSU font spelling out the long version of the university's name across the top of the spine. The authors name and title weren't even there at all, instead the decimal point which indicated its proper location when on the shelves was typed across the bottom to indicate its proper location on the shelf. *A librarian came up with that*, I thought. And then I realized, probably after far too long, that this was not the original binding of this book and that it had to have been rebound by CSU at some point.

A professors told me once that if you ever found books in the library that were clearly rebound like this book must have been, they were some of the books that were saved from the devastating flood in 1997. The flooding of the basement of the CSU library was only a small part of the destruction throughout the town of Fort Collins.

This book was one that was saved, I thought tenderly, probably too tenderly.

How old is it? Was it reprinted later? How many times and how long ago was the last? How could I find out without the original binding? I thought all at once until I went to my default answer, *Internet!*

I found out from Google it had been reprinted at least a few times and there had also been some later summaries and adaptations printed as well.

Wiki told me it was originally published in 1959 but on Amazon the top two results were 1995 and 1960 reprints. The third result had absolutely nothing to do with what I was looking for at all and so I thought, *No other editions? That's probably not a good sign for getting my hands on an original…*

Or so I thought, buried on results page two and a few scrolls down was a book with an almost identical cover to the 1960 reprint that appeared as the first result. Only this edition, to my extreme delight, wasn't the royal blue as the first reprint but a beautiful teal which matched nail-polish, my yoga mat and far too many other things that I own.

I giddily clicked on it and rapidly searched up and down the resulting page for a publication date, but one didn't just jump out at me. I was forced to actually closely read the words on the webpage to try to figure it out instead of just scanning them like I usually do to find what I need from a website. After a bit I found that the line that started with "Publisher…" followed the colon up with the words "Bell, 1st edition." And I sort of wanted to gasp a little bit, I was holding the binding of a book which was less than a decade and a half old and yet it was filled with pages which were more than half a century old.

I started to understand why there were two separate pages from which little "Due Date" cards hung riddled with uneven stamped dates; one of them was adhered to significantly whiter and newer paper, presumably from the rebinding process.

I reminisced a little about the logistics of using card catalogues to keep track of books, a practice that was phased out for me in middle school. The top of each card sternly cautioned me with the explicit words:

"SUBJECT TO RECALL
DUE DATE MAY CHANGE"

Oh right, I thought, *When a student or even a professor went to write a paper about something they didn't simply pop keywords into the sleek, empty box on Google's homepage and find out in seconds what they needed to know with Ctrl+F. They couldn't paste in every question from their study guide and immediately have a link to the answer. They had to go physically find an obscure book that may or may not have the information they were searching for but they would have no choice but to search it through any way just to see if it was there. So if someone checked*

out the book you needed for a couple or even a few weeks then they had to delay writing their paper for that long.

I thought of the timeframe that I usually write my own papers in, *how could they write a paper the night before it was due* that *way?*

Beyond that, I realized, *They were forced to learn about a subject not just barely enough to stretch down five to eight pages of paper like I do. They had to read through pages upon pages of a variety of published books and from all those of researched pages hope to find the information they needed. After all of that research they probably had to actually* cut *all sorts of interesting and relevant researched information so as to get their assigned point across without exceeding the assigned number of pages.*

Somehow, thoughts about the editing flaws in *The Complete Illustrated Book of Yoga* had led me to an entirely unrelated epiphany. The internet provides instant access to an incredible number of resources, it makes it easy to find exactly what you want without "wasting" a ton of your time, and if we're not careful it can make us lazy. I thought about what it would take for me to write every one of my papers the next semester without using the internet at all, and I thought it seemed like an *awful* lot of hard work—and that perhaps I might learn something really valuable that way.

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Morgan Library at Colorado State University
Photo by: Jeffrey Dale

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