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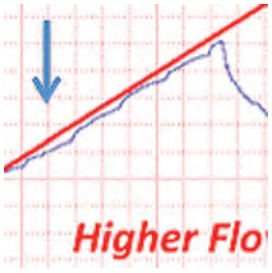

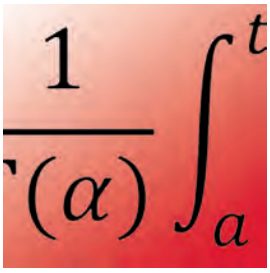

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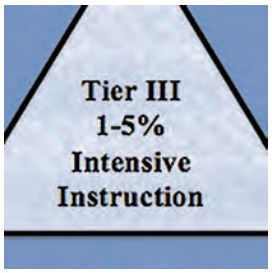


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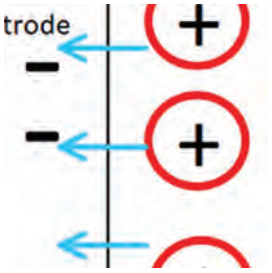

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LETTER FROM THE DIRECTOR

Dear Reader:

I am pleased to present the third volume of the *Proceedings* of the Berry Summer Thesis Institute.

Pedagogically, the work here represents the Program's attempt to introduce some of our top student researchers to the practice of peer review and publication — complete with the consternation created by reviewers' critiques and formatting requirements. It also represents the opening bell of our Berry Summer Thesis Institute students' entrance into the arena of their field as scholars, the latest in a line of researchers stretching through their thesis advisors and back into previous centuries — in some cases back to the ancients. In addition, it represents the fruit of the generosity bestowed upon our Program, first by John W. Berry Sr., and subsequently by his children and Foundation. Please join me in thanking the Berry family, our faculty mentors and our students.

Enjoy!

David W. Darrow, Ph.D.
Director
University Honors Program
University of Dayton

Thanks to a gift from the Berry Family Foundation and the Berry family,
the UHP offered nine rising juniors the opportunity
to participate in the 2015 Berry Summer Thesis Institute.

First initiated in the summer of 2012, the Institute introduces students
with a proven record of academic success and interest in intensive research,
scholarship opportunities and professional development
while earning Honors credits towards their Honors Program diplomas.

Students selected for the Institute were competitively selected for participation
by the University Honors Program review committee.

Each student pursued a 12-week summer thesis research project
under the guidance of a UD faculty mentor.

In coordination with the Center for Social Concern, Campus Ministry
and the Fitz Center for Leadership in Community,
the students also learned about civic engagement and servant leadership
by volunteering with local community partners.

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Disentangling the cis and trans Causes for Diversity: A Transgenesis Approach to Infer the Genomic Sites Responsible for Differences in Gene Expression

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Abstract

Every trait for every organism requires the expression (making functional RNAs or proteins) for a gene or genes. Thus, every gene experiences some form of expression regulation, often which occurs at the step of transcription initiation. This involves *cis*-regulatory element (CRE) sequences located near genes (in "*cis*") being bound by transcription factor proteins coming from genes located at more distant (in "*trans*") genome locations. When a gene's expression differs between two organisms, the genetic differences might be located in a CRE controlling the gene's expression ("*cis*-evolution") or in a sequence for another gene or genes ("*trans*-evolution"). A poorly understood question is how do mutations in CREs cause functional *cis*-evolution? The answer will offer new insights on the genetic basis for normal versus disease states and for the adaptations of organisms to new environments. Unfortunately, few CREs have been identified that are bona fide sites for *cis*-evolution, leaving scientists with little to study. My Berry Summer Thesis Institute research project uses the evolved expression differences for the *tan* and *yellow* genes that contribute to fruit fly pigmentation diversity as a model to implicate or exonerate two CREs as targets of *cis*- and *trans*-evolution. I isolated the pertinent CREs for these genes from two species differing in expression and pigmentation and coupled them to the GFP gene to make reporter transgenes. These transgenes will be integrated into the genomes of the two species by the use of the *piggyBac* transposon system. The CRE activities, as monitored by GFP production in these host species, will indicate whether *cis*- and/or *trans*-evolution occurred. Outcomes implicating *cis*-evolution will validate these CREs as excellent models to study how DNA sequence changes alter transcription initiation. Outcomes implicating *trans*-evolution will steer future studies towards resolving the *trans*-regulatory basis for these genes' expression differences.

Introduction

Diversity of life on this planet can be seen in all places: from out in the wild, the plants at a botanical garden, the animals at the zoo, and to even the numerous subtle distinctions between genetically similar people. The common ancestor to all forms of life diverged and developed over billions of years into a vast diversity of organisms, giving rise to the millions of species on Earth today. Diversity within and between the three different domains of life is the outcome of an evolutionary process, which can be studied through an organism's instruction book: its genome. For animals, these genomes seemingly hold all of the instructions for developing from

a single cell zygote to a complicated adult made up of several to hundreds of different cell types encoded in the letters of DNA sequence. These instructions are for the basic body plan, and the various traits that increase fitness and/or comprise adaptations to ecological niches. Body plan and trait diversity has arisen from the changes, ranging from the extensive to the miniscule, in the information content of genomes. As these changes accrue, they can give rise to a new species. Relevant to humans, mutations, in isolation or small combinations, can have consequences to health and disease. However, genomes are vast, mutations are numerous and most mutations appear to have no positive or negative consequence — they appear to be functionally neutral. So finding the function-altering mutations within a genome is a central goal of genetic investigation, though this remains a daunting challenge for our times.

Discussion

Genomes, Genes and Gene Parts

The "genome" can be considered the complete manual of genetic instructions for an organism. For complex, multicellular organisms, a copy of this manual is found in nearly all cell nuclei. Chromosomes located in each nucleus can be equated to an individual chapter of the manual. Each word in a chapter is a gene that is spelled using the four DNA nucleotides, letters of the genetic language: A, T, C, and G. Genomes are made of double-stranded helix (or helicies) with pairs of complementary nucleotides (Griffiths *et al*. 2011). The number of base pairs, as well as the number of genes, varies among complex organisms. The smallest recorded genome size is 19.56 mega base pairs (Mbp) (the nematode *Pratylenchus coffeae*) and contains ~6,000 genes (Gregory 2015). The largest documented genome is 129,907 Mbp (the marbled lung fish *Protopterus aethiopicus*); however the number of genes is yet to be discovered for this species (Gregory 2015). A human's genome is about 3,423 Mbp and contains an estimated 32,000 genes (Gregory 2015). Since the year 2000, when the first sequencing project for an animal genome was completed (Adams 2000), genomes and gene inventories have been obtained for over a hundred animal species, including: numerous fruit fly species (Richards *et al*. 2005; Garrigan *et al*. 2012; Clark *et al*. 2007), the human genome (Lander *et al*. 2001), and even for the extinct close relative of humans — the Neanderthal (Green *et al*. 2010). What has emerged from comparing genomes is the realization that animals share a similar inventory of genes, which can differ subtly (*e.g*. between different humans) or modestly (between humans and a fruit fly) (Carroll *et al*. 2005; Davidson & Erwin 2006). The inventories of genes seem inadequate to explain the multitude of variations in traits that exist between different organisms, so it has been reasoned that differences in the uses (expression) of these shared genes may explain a greater proportion of this diversity (King & Wilson 1975; Carroll 2008; Carroll 2005; Stern 2000; Stern & Orgogozo 2008; Wray 2007). Studying the typical parts of genes provides some insights on how body plan, trait and species diversity has arisen.

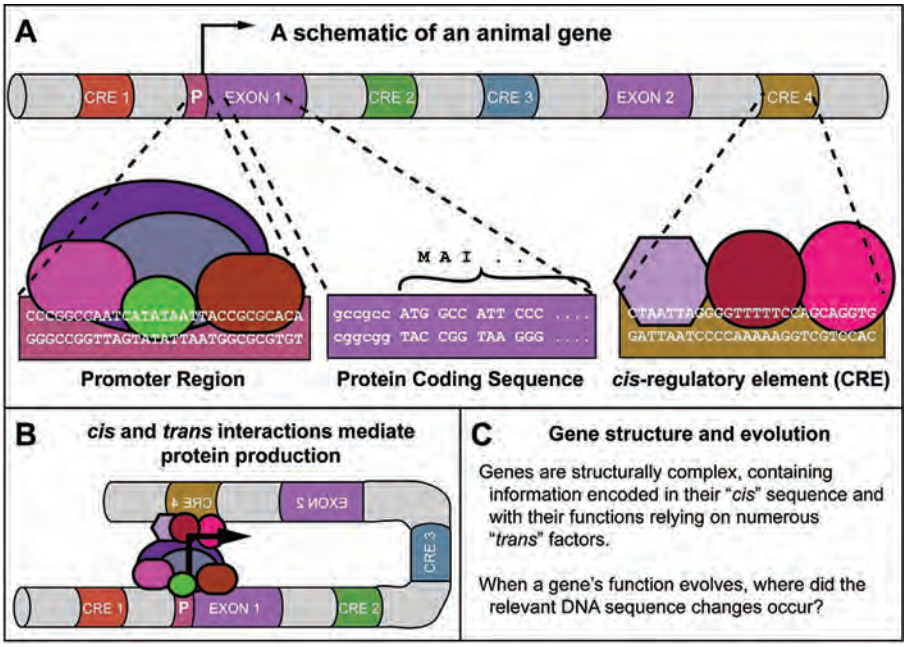

Figure 1. Schematic of a typical animal gene's structure. (A) The RNA or protein product of a gene is encoded within its exon(s) sequence. This information is initially transcribed starting from a promoter (P). The cell type, developmental timing, and level of transcription are directed by the activity of CREs. Most genes have multiple CREs. (B) Transcription involves transcription factor proteins recruiting a CRE to the promoter region to engage the RNA polymerase holoenzyme that will accomplish transcription. (C) Evolution in these CREs ("cis" features) and transcription factor genes ("*trans*" features) are likely to be responsible for evolved differences in gene transcription.

The typical parts of an animal gene are the protein coding sequence, *cis*-regulatory elements (CREs) and a promoter region (*Figure 1A*). The coding sequence for each gene includes the DNA sequence that determines the ultimate RNA sequence for a functional RNA or the amino acid sequence for a translated protein. CRE sequences are DNA sequences that contain the information specifying the expression patterns for genes. These patterns include which cell(s) will express a gene, when the gene will be expressed during an animal's life and in what quantities the functional RNAs or proteins are produced (Arnone & Davidson 1997). CREs are typically located near the coding sequence for the gene(s) they control (located in "*cis*"). Transcription factor proteins bind to CREs and bring them in contact with the promoter region (seemingly by interacting with transcription factor proteins bound to the promoter region) of a gene from where transcription is initiated (Dekker 2008). In contrast to the close proximity of CREs to the genes they regulate, transcription factor proteins are the expression products of genes located in distant chromosome regions or from an altogether different chromosome (located in "*trans*") (*Figure 1B*). Thus, the successful use of genes requires the information encoded in their "*cis*" sequences, which are functionally dependent on numerous "*trans*" factors. Studying gene structure and comparing and contrasting structure between the same gene in different individuals or species can reveal the DNA sequence changes that cause a gene's function and, ultimately, traits to evolve (*Figure 1C*). This comparative approach provides the opportunity to begin to understand why certain genetic differences cause differences in fitness.

Putting Genes to Work: the Regulation of Transcription

In order for genes to be expressed as functional RNAs or proteins, and ultimately to contribute to a trait, many molecular processes must take place. The first essential process is known as transcription. Transcription is the process of making RNA from a DNA template (Griffiths *et al*. 2011), an essential step in gene expression. The regulation of gene expression largely occurs at the step of transcription initiation. Transcription is initiated by an enzyme complex, known as RNA polymerase, binding to the promoter region of the regulated gene. This binding results in the separation (melting) of the DNA double helix strands, translocation of the polymerase complex, and the synthesis of a nascent RNA strand with complementary nucleotide bases to the DNA template strand. The cells in which a gene is transcribed depend upon the activity of a CRE or CREs through their interactions with a combination of transcription factor proteins (*Figure 1B*). These transcription factors bind to specific DNA sequences (so called "binding sites") within a CRE. Thus, these CRE sequences and their binding sites seemingly provide many targets for mutations to alter a gene's function at the level of transcription. While it is suspected that changes in CREs have accounted for a great proportion of animal diversity (Carroll 2008) and human health conditions (Wellcome *et al*. 2007; Sethupathy & Collins 2008), how certain DNA sequence changes alter CRE functions remains poorly understood. One such problem with the study of CREs is finding comparisons for which a given CRE has evolved distinct gene regulatory functions.

cis and *trans* Causes of Diversity

For any given gene whose pattern of expression differs between two individuals or between two different species, the difference may be due to "*cis*-changes" in a CRE (*Figure 2A*) or by "*trans*-changes" with respect to the gene with altered expression (*Figure 2B*) (Wittkopp *et al*. 2004; Wittkopp *et al*. 2003). In recent years, a variety of high throughput techniques have made identifying differences in gene expression routine (Wittkopp *et al*. 2004; Wittkopp & Kalay 2012). However, the observation of a gene expression difference does not reveal whether the underlying cause was a *cis*-change, a *trans*-change,

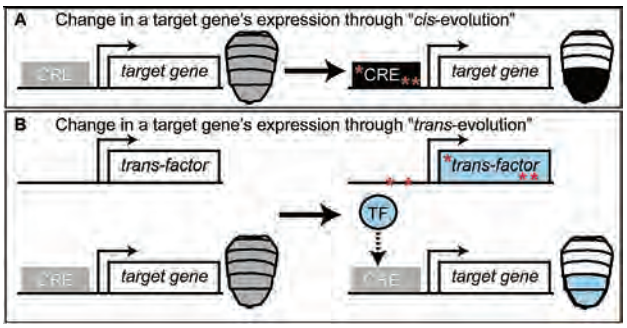

Figure 2. The genomic locations for mutations altering a target gene's expression. (A) Mutational differences in a CRE controlling a gene's expression pattern result in a new, black, pattern of gene expression. (B) Mutational changes in a transcription factor gene result in the Transcription Factor, TF, protein regulating the target gene's expression in a new, blue, pattern. Expression schematics based on the shape of the male fruit fly abdomen. Red asterisks represent function-altering mutations.

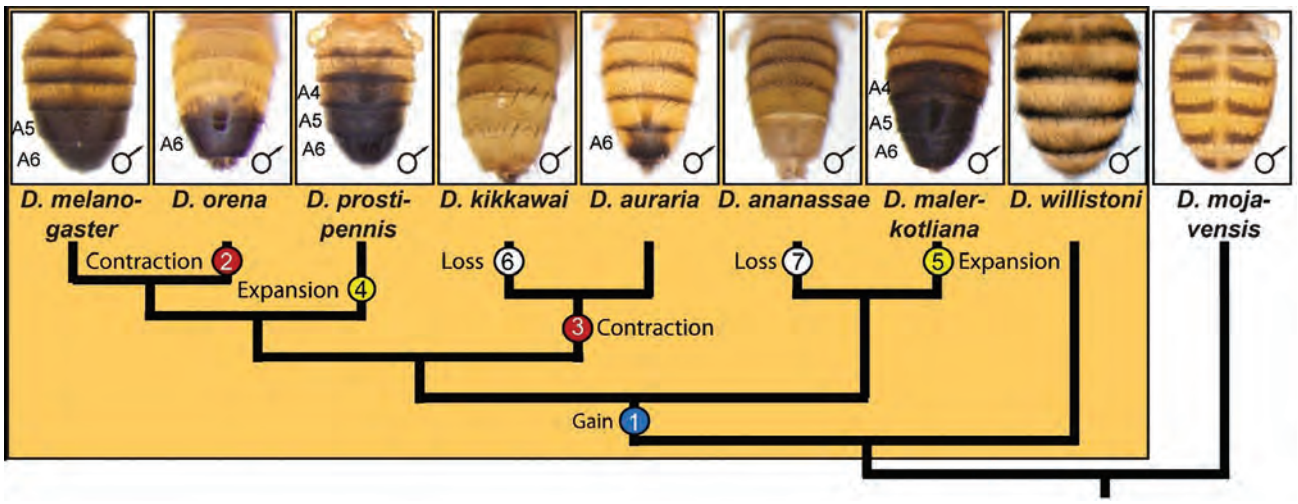

Figure 3. The abdomens of male fruit flies have evolved diverse patterns of pigmentation. Orange background indicates the *Sophophora* subgenus. Figure courtesy of Thomas Williams, 2015.

or both (Wittkopp *et al*. 2003). Furthermore, one gene's *trans*-change can be another gene's *cis*-change — that is, a CRE controlling a transcription factor gene's expression may actually be the site where the function-altering mutation resides that alters the expression of a regulated gene. Making the distinction between *cis* and *trans* changes is critical, as the identification of bona fide cases of *cis*-evolution will provide CREs to investigate in order to elucidate how DNA sequence differences can cause a gene to be expressed differently, a fundamental question of our time.

Fruit Fly Abdomen Pigmentation as a Model Trait to Study Diversity

In order to understand the genetic causes responsible for differences in gene expression, model traits are needed for which different individuals or different species possess the same essential genes to make a trait, but for which a certain gene or genes are expressed in a different pattern. One trait meeting these criteria is the pigmentation pattern on the abdomens of fruit flies from the *Sophophora* subgenus (*Figure 3*). A common feature of fruit flies is the covering of their dorsal abdomen segments by cuticle plates known as tergites. Each of the first six segments (A1-A6) has an overlaying tergite with some form of coloration/pigmentation. For *Drosophila* (*D.*) *melanogaster*, a leading animal model for genetics research, the A5 and A6 segment tergites are completely pigmented in males (*Figure 3*). The evolutionary relationship (phylogeny) between *D. melanogaster* and other *Sophophora* species has been well resolved (*Figure 3*) (Jeong *et al*. 2006; Markow & O'Grady 2006). Based on this phylogeny and tergite pigmentation patterns of the included species, a plausible scenario for color evolution has been put forth where males originally lacked the elaborate A5 and A6 pigmentation (*Figure 3*; as seen for *D. willistoni*, a species from a basally-branching lineage). Pigmentation seemingly was gained in the lineage of *D. melanogaster* after it diverged from that for *D. willistoni*. Following this initial gain, pigmentation has repeatedly been expanded or retracted in tergite number and altogether lost (*Figure 3*).

For *D. melanogaster*, the black tergite pigments require the expression of the genes *tan* and *yellow*, each of which encodes an enzyme involved in making black melanin pigments from tyrosine precursor molecules (Jeong *et al*. 2008; True *et al*. 2005; Wittkopp *et al*. 2002). The expression patterns for these two genes' RNAs can be seen by the technique known as *in situ* hybridization, which causes the epidermal cells possessing their RNAs to become purple in color (*Figure 4*). The strong A5 and A6 patterns of *tan* and *yellow* expression do not occur for the unpigmented species *D. willistoni*, indicating that these genes expression patterns have evolved (Camino *et al*. 2015). There are at least three scenarios by which this RNA presence/absence difference evolved. One is where CREs functionally evolved (*cis*-change) from non-existent ancestral states for the *tan* and *yellow* genes. A second is where equivalent CREs exist in both species, but the presence of a certain transcription factor or factors (*trans*-change) has come to differ between

the two species. Under this latter scenario, the *D. melanogaster* "landscape" of expressed transcription factors allows for *tan* and *yellow* expression to occur in the A5 and A6 segments, whereas the landscape in *D. willistoni* differs to the point that it is incapable of activating *yellow* and *tan* expression. A third scenario is where both *cis*- and *trans*-changes occurred. Distinguishing between these scenarios (*Figure 2*) requires testing the regulatory capabilities for the pertinent CREs in both *D. melanogaster* and *D. willistoni*.

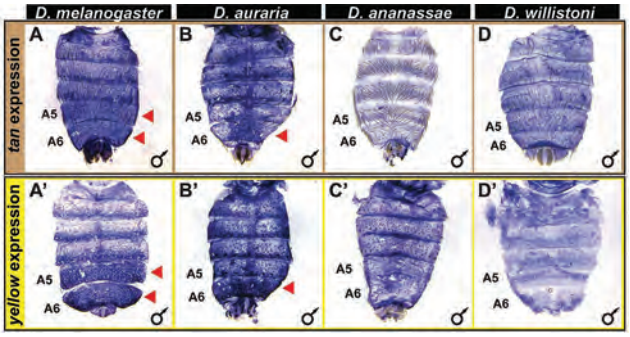

Figure 4. The evolving patterns of tan and yellow expression as seen by in situ hybridization. (A & A') *D. melanogaster tan* and *yellow* are robustly expressed in the male A5 and A6 segments. (B & B') In *D. auraria*, robust expression is limited to the A6 segment where the species is darkly pigmented. (C & C') *D. ananassae* and (D & D') *D. willistoni* lack robust expression. These patterns for *D. auraria* are inferred to be derived losses in expression, and the patterns for *D. willistoni* are thought to represent the ancestral state. The data shown here was kindly provided by Eric Camino and was originally published in Camino *et al.*, "The Evolutionary Origination and Diversification of a Dimorphic Gene Regulatory Network through Parallel Innovations in cis and *trans*" (PLoS Genetics, 2015).

Using Reporter Transgenes to Find Genomic Sites for Pigmentation Diversity

Tan and *yellow* expression in the male A5 and A6 segments (*Figure 4*) are respectively under the control of the CREs known as the *tan* Male Specific Element (or t_MSE) (Jeong *et al.* 2008; Camino *et al.* 2015) and the *yellow* Body Element (or yBE) (Wittkopp *et al.* 2002; Jeong *et al.* 2006). These regulatory activities were discovered using reporter transgenes in transgenic *D. melanogaster* (Rogers & Williams 2011). Here sequences from the vicinity of these genes were separately coupled to a non-native promoter (from the *hsp70* gene of *D. melanogaster*) and the protein coding sequence from an enhanced version of the *A. victoria Green Fluorescent Protein* (EGFP) gene (*Figure 5A*) (Rebeiz & Williams 2011). These reporter transgenes were integrated into the genome of a *D. melanogaster* fly, which can be propagated in transgenic lines of descent. In male transgenic pupae (developmental stage when *yellow* and *tan* are naturally expressed), the CRE activities can be observed by the spatial patterns and amount of EGFP, which can be visualized by its fluorescence through the use of a confocal microscope (*Figure 5B-5E*). Previous research in the Williams lab showed that the t_MSE and yBE sequences from *D. melanogaster* but not *D. willistoni* drive EGFP expression in the male A5 and A6 segments of *D. melanogaster* pupae (compare *Figure 5B and 5D* to *5C and 5E*) (Camino *et al.* 2015). This outcome demonstrated that *cis*-changes have indeed occurred for these two CREs. However, these outcomes cannot rule out the possibility that key *trans*-changes have also occurred. In order to make this determination, these reporter transgenes need to be tested in transgenic *D. willistoni* pupae, a species for which only a few transgenic lines have been created to date (Holtzman *et al.* 2010).

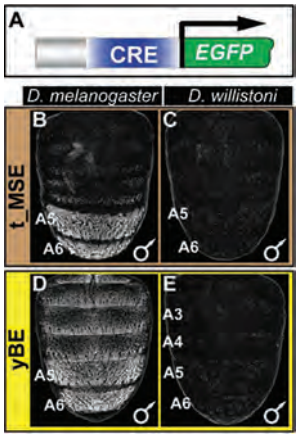

Figure 5. Reporter transgenes as a tool to visualize the activity of a CRE in a host species. (A) Schematic of a reporter transgene, arrow represents the promoter. (B-E) EGFP expression of reporter transgenes in transgenic male *D. melanogaster* pupae. (B and D) Transgenes with CRE sequences from the *D. melanogaster* genome and (C and E) from the *D. willistoni* genome. Data provided by Eric Camino.

A working method for transgenesis was published for *D. melanogaster* in 1982 (Rubin & Spradling 1982), and since this time methods for transgenesis have become increasingly sophisticated for this species (Venken & Bellen 2007). These approaches often exploit the natural *P*-element transposon system identified from *D. melanogaster*, where the *P* transposase (expressed from a vector, so called "helper plasmid") catalyzes the integration of the reporter transgene (which is flanked by well-defined *P*-element terminal repeat sequences) into the host organism's genome (Venken & Bellen 2007; Rubin & Spradling

1982). While *P*-element methods work well for *D. melanogaster*, they have been found to be only 10% as effective in other *Drosophila* species (O'Brochta *et al.* 1991) (so called "non-model" species), and even less effective for insects other than fruit flies (Handler *et al.* 1993). Thus, the use of *P*-elements in transgenesis has been near universally limited to *D. melanogaster*.

Other transposons have been identified that can function in a more broad range of animals (Venken & Bellen 2007), including *piggyBac* which was derived from the moth species *Trichoplusia ni*. Like *P*-elements, *piggyBac* can be used in a binary system that includes injecting host embryos with a cocktail containing a helper plasmid that expresses the *PiggyBac* transposase protein and a reporter transgene vector in which the transgene is flanked by transposon-specific inverted terminal repeat sequences (Horn & Wimmer 2000). Together this system can insert transgene sequences into TTAA sequences (*Figure 6*), which are widespread throughout any animal chromosome.

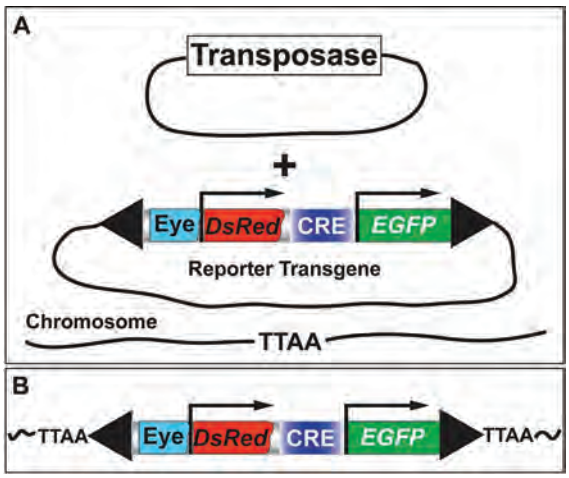

Figure 6. Binary piggyBac transposon system. (A) Fruit fly embryos need to be injected with a vector cocktail containing the *piggyBac* transposase gene and a reporter transgene flanked by inverted terminal repeats. Integration can occur at chromosome sites with a TTAA sequence. (B) An integrated reporter transgene is flanked by duplicated TTAA sequences. Black triangles represent the inverted terminal repeats. Eye refers to the CRE marking transgenic flies by Red Fluorescent Protein expression.

During year one of the Berry Summer Thesis Institute, my first goal was to make *piggyBac* EGFP reporter transgenes for the *D. melanogaster* and *D. auraria* yBE and t_MSE regulatory sequences. My second goal was to create transgenic lines of *D. auraria* for each reporter transgene. This involved culturing *D. auraria* flies and having embryos injected with a cocktail of vectors for the binary system (including a reporter transgene and helper plasmid vector). I successfully completed my first goal, however the second goal remains to be completed. The next step of this thesis project includes crossing the injected progeny with non-injected *D. auraria* flies of the opposite sex in order to obtain a G0 generation. G0 transgenic offspring will be identified by the expression of a Red Fluorescent Protein (DsRed) marker gene in the eyes of transgenic *D. auraria* under the control of an eye-specific CRE (Horn & Wimmer 2000). Male pupae from each transgene line will be analyzed for EGFP expression by the use of a confocal microscope and with a protocol established in the Williams lab (Rogers & Williams 2011). If the EGFP expression patterns match those produced when the reporter transgenes are in the *D. melanogaster* pupae, then I will conclude that the differences in expression are due only to *cis*-changes (*Figure 7*). If either or both of the *D. melanogaster* and *D. auraria* CREs drive different EGFP expression in transgenic *D. auraria* than they did in *D. melanogaster*, then I will conclude that the gene expression differences involved *cis*- and *trans*-changes (*Figure 7*).

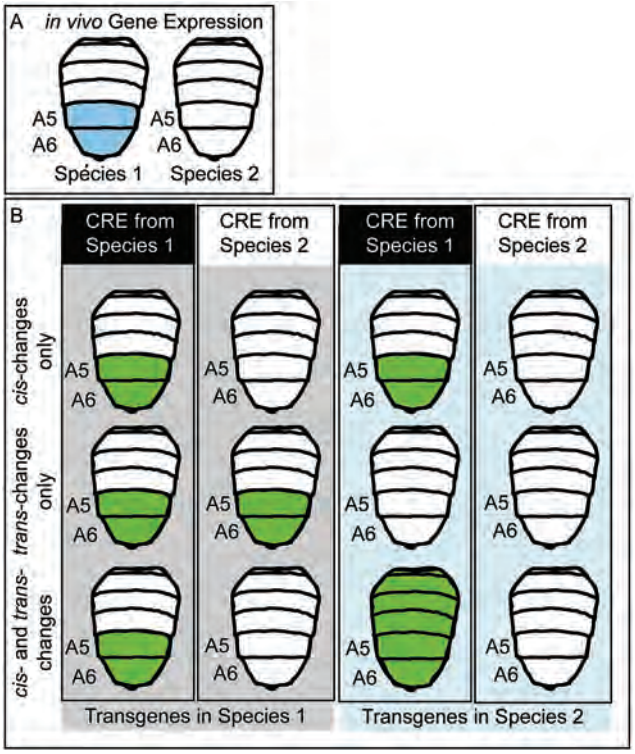

Figure 7. A reciprocal transgenesis assay to determine the *cis*- and *trans*-contributions to a gene expression difference. (A) The *in vivo* expression pattern, blue, for the genes studied here differs between Species 1 and 2. (B) Three possible outcomes can occur for the reciprocal transgenesis experiments with reporter transgenes. These are where the gene expression difference is due to either "*cis*-changes only," "*trans*-changes only" or both "*cis*- and *trans*-changes."

Future directions of this thesis include making *piggyBac* EGFP reporter transgenes for the *D. willistoni* and *D. ananassae* yBE and t_MSE regulatory sequences, and to perform a similar reciprocal transgenesis analysis with these species.

Concluding Remarks

The results from this study will provide conclusive demonstrations for the contributions of *cis*- and *trans*-changes to gains, modifications and losses in the expression of two genes. This might seem like a trivial aspect of fruit flies unworthy of meticulous scientific investigation. However, gains, modifications and losses of gene expression are common themes of diversity for creatures as simple as fruit flies and for those as complex humans. The history of genetics research has time and time again shown that we make rapid progress in understanding animal systems when a phenomenon is first characterized in fruit flies. I anticipate that the same will hold true for the understanding of gene expression and its evolution!

Acknowledgements

The authors would like to thank the Berry Family for their contributions and support. Thanks are owed to Eric Camino and Sumant Grover of the Williams lab for their assistance in teaching me experimental methods and providing me with experimental reagents. J.L. Grilliot is supported by the University of Dayton Honors Program and The Berry Summer Thesis Institute.

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CRISPR CREam for Fruit Flies: Developing a Genome-Editing Approach to Study the cis-Regulatory Elements that Control the Activities of Genes

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Abstract

The *cis*-regulatory elements or CREs are DNA sequences that regulate gene expression, and differences in these sequences are a prominent cause of variation among individuals of a species and between different species. However, these genome components pose a challenge to study in any animal species because their number far exceeds the number of genes in a genome and there is no efficient means to infer a CRE's function from its specific DNA sequence. My thesis attempts to help overcome this challenge by developing an approach to delete CREs from the genome of the fruit fly species *D. melanogaster* and subsequently replace them with an evolutionarily-related CRE variant. I will utilize the CRISPR/Cas9 system of *Streptococcus pyogenes* (a bacterium) as a genome-editing tool to remove particular CRE sequences and then observe how gene expression and development are impacted by the sequence's absence. Then I will insert a CRE variant in the position of the deleted CREs via recombination mediated cassette exchange (RMCE). This will allow for observations to be made about how CRE divergence affects gene expression and development. Success with this approach will provide a powerful genetic capability for advancing the understanding of CRE functions in diverse species, and could possibly be emulated to treat human genetic diseases.

Introduction

Three major open questions in biology ask: What does it take to make a well functioning adult human? How have humans come to differ from other species? And what goes wrong in cases of disease? The answers to each of these questions involve genetics. Before biologists can adequately address these larger questions, they must first address what is still unknown with respect to how genes function. Specifically, biologists seek to understand how genes direct the processes of development, how they change to direct different developmental processes, and how broken genes lead to improper development. While the past one hundred years of genetics research has revealed a great deal about genes and genomes, these three questions remain poorly understood and require new, innovative approaches to bring us closer to answering them.

The human genome contains an estimated 22,333 genes (Pertea and Salzberg, 2010) that collectively build and maintain the human body. Following the formation of the zygote (fertilized egg cell), the process of development leads to the production of an adult with more than several trillion cells, which are broken down into roughly 411 distinct types of cells (*e.g.* rod cell and sebaceous gland cell) (Arendt, 2008; Vickaryous and Hall, 2006). Each of these cell types express (make the function protein or

RNA product) a different cohort of the 22,333 genes through intricate mechanisms of gene expression regulation (Davidson and Erwin, 2006). Often gene regulation occurs at the point of transcription initiation, where sequences known as *cis*-regulatory elements (including activating sequences called enhancers and repressive sequences called silencers) are responsible for initiating or suppressing transcription. In one study it was estimated that the human genome possesses several million of these *cis*-regulatory elements (CREs) to regulate the expression of the 22,333 genes (Kvon *et al.*, 2014).

The massive number of CREs poses several challenges to genetics research. First, we need to understand how the organization of the nucleotide letters "A", "C", "G" and "T" in CREs can encode 411 different cell type-specific expression patterns. Second, we need to understand how mutations create useful evolved forms of CREs (CRE orthologs) between species and how mutant forms between individuals of the same species (CRE alleles) can function poorly and cause disease or elevate disease risk. One way to begin to answer these questions is to study CREs in a simpler organism with fewer cell types, genes and CREs, and that is easy to work with in a laboratory setting.

Discussion

A Fruit Fly Example of Gene Structure, CREs and Transcriptional Regulation

The fruit fly species *Drosophila (D.) melanogaster* has been arguably the most successful model organism for genetic investigation. From this species, chromosome maps were first achieved and the first cohort of genes regulating embryonic development were defined. It also has been a leading model for studying a genome (Adams, 2000) and gene expression regulation (Nègre *et al.*, 2011). One well-studied gene is known as *even-skipped* (*eve*), which contains two exons and five flanking CREs that collectively regulate its seven stripe pattern of expression that occurs during early embryonic development (*Figure 1A*). The exons contain the information to make the Eve protein, and protein production occurs in the stripes of cells under control of the surrounding CREs. One of the best understood CREs in biology is the "stripe 2" enhancer. This DNA sequence contains short DNA sequences that act as binding sites for the repressive transcription factors (Giant and Kruppel) and sites for two activating transcription factors (Bicoid and Hunchback) (*Figure 1B*). These sites make a combinatorial regulatory logic that results in *eve* being expressed in the embryo region between where the repressive factors

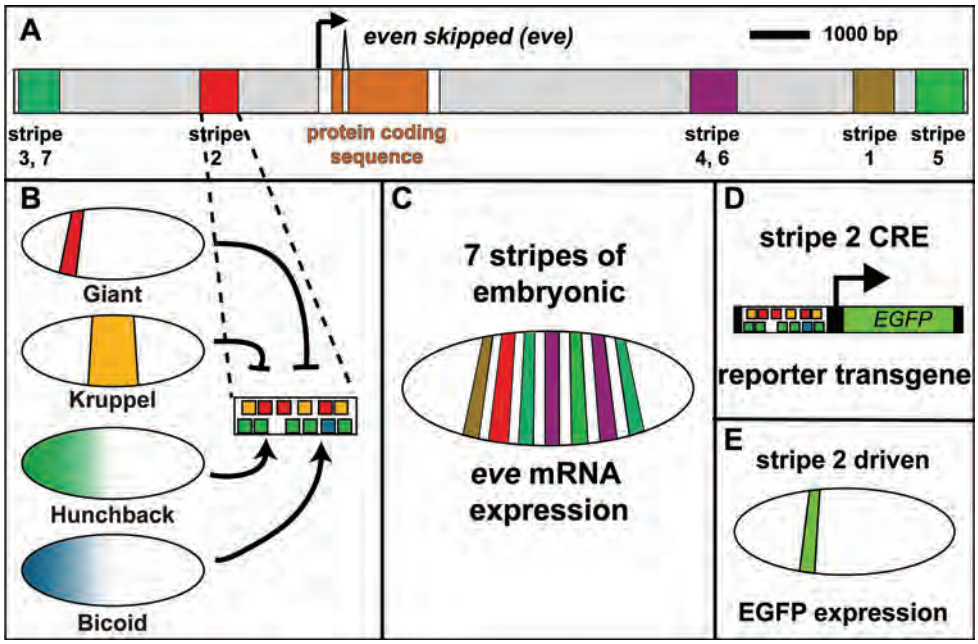

Figure 1. *Even-skipped* as a model for a complex animal gene. (A) Representation of the *eve* gene locus. (B) The stripe 2 enhancer integrates regulatory inputs from at least 4 transcription factors. (C) 7 stripes of *eve* expression are produced across the anterior-posterior axis of the developing *D. melanogaster* embryo. (D) The stripe 2 enhancer can be artificially coupled to the enhanced green fluorescent protein gene and this enhancer (E) is sufficient to activate green fluorescent protein expression in the presumptive stripe 2 domain of a transgenic embryo. Figure courtesy of Alex Hallagan and Thomas Williams, 2015.

are expressed and where the activating factors are expressed (*Figure 1B*) (Arnosti *et al*., 1996; Ludwig *et al*., 1998; Small *et al*., 1992). The stripe 2 enhancer is responsible for only one of 7 stripes of *eve* expression (*Figure 1C*), and the other stripes are established by distinct CRE regulatory logics that include binding sites for activating and repressive transcription factors that place stripes of expression at different embryo positions.

One of the most successful approaches to understand the gene expression-regulating activities of CREs, such as the stripe 2 enhancer, is to couple CREs individually to the protein coding sequence of a gene whose translated protein product or transcribed mRNA are easy to visualize (Rebeiz and Williams, 2011). One such "reporter transgene" is that for the enhanced green fluorescent protein (EGFP). For *eve*, the regulatory activity for the stripe 2 enhancer can be visualized in a transgenic embryo as a stripe of EGFP (*Figure 1D* and *1E*). These reporter transgene assays have been done for hundreds of CREs, revealing that patterned expression is generally encoded as a collection of binding sites for various transcription factor proteins.

However, a generally applicable understanding of CREs remains out of reach for several reasons. First, *D. melanogaster* has an estimated 100,000 CREs (Kvon *et al*., 2014) and this species has over 700 different genes for transcription factors (Pfreundt *et al*., 2010). Thus, the combinations of transcription factors that can exist are vast! Second, CREs evolve at an accelerated rate compared to protein coding sequences (Andolfatto, 2005). Thus, there are a far greater number of CRE alleles and orthologs to consider when trying to understand how individuals and species differ from each other. Third is that reporter transgenes study CRE functions in isolation outside of their normal genome environment. This is significant because a CRE may cooperate with another CRE region that is not included in the reporter transgene. It is possible that a CRE could regulate multiple genes whose expression patterns were not investigated, or that a secondary CRE may exist, a so-called "shadow enhancer," that can compensate for the function of the primary CRE when it is absent or non-functional (Frankel *et al*., 2010; Hong *et al*., 2008). Thus, it is important for studies of CREs to investigate their functions within their normal genomic context to reveal these more complex occurrences.

Understanding the Endogenous Functions of CREs

In order to effectively study CREs, an approach is needed where CREs can be readily deleted (*Figure 2A*) from the genome to see if either the suspected gene's expression pattern or the expression of other neighboring genes is altered. Moreover, to understand evolved variation in CREs, a technique is needed where CRE variants, alleles or orthologs, can be readily inserted into the place of a deleted CRE (*Figure 2B* and *2C*).

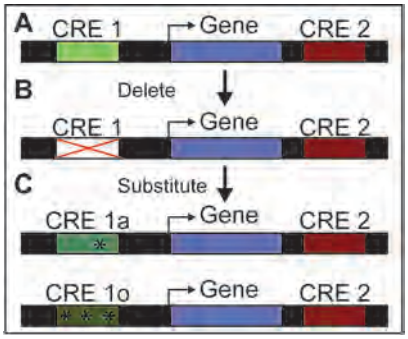

Figure 2. Modifying CRE sequences in their natural gene environment. (A) Transcription of a gene from its promoter (arrow) can occur in multiple settings due to regulation by separate CREs. (B) Exploring the *in vivo* functions for CREs require techniques that can easily remove a CRE sequence. (C) Understanding how mutations affect the *in vivo* functions of a CRE allele (CRE 1a) or an orthologous CRE from another species (CRE 1o) requires a technique that can efficiently replace the endogenous CRE with these CRE variants. Each asterisk represents a mutation event. Figure courtesy of Alex Hallagan and Thomas Williams, 2015.

Inserting alleles and orthologs would allow us to better understand how mutations affect the *in vivo* functions of the CRE. Although genome-editing techniques have existed for years in *D. melanogaster* (Rong and Golic, 2001), they are not scalable by any means to the number of CREs in the genomes and the variant forms that exist within a species and between related species. Resultantly, progress in understanding how genes are regulated remains frustratingly slow in convenient organisms like *D. melanogaster* and progress is even more stagnant in humans. Thus, a major need in genetics research is methods to edit the CRE regions of animal genomes (*Figure 2*).

The CRISPR/Cas Immune System of Bacteria and Archaea

A solution to the problem of genome editing in animals presented itself in the least likely of places: Bacteria and Archaea (Sternberg and Doudna, 2015). Prokaryotic microbes, for example *Streptococcus pyogenes*, live in extremely competitive environments and are frequently attacked by foreign genetic elements. They have adapted to survive this harmful environment through clustered regularly interspaced short palindromic repeats (CRISPR), which forms a unique genetic locus (Barrangou *et al.*, 2007; Horvath and Barrangou, 2010). This adaptive immune system allows microbes to build DNA-encoded immunity by up-taking a short fragment of invader DNA and incorporating it at the leader end of the *CRISPR* locus (*Figure 3A*). Over time, these microbes develop a "history" of past infections and can use these spacer units to recognize the matching virus genome and destroy them. The immune response is first activated by transcribing the *CRISPR* locus into full-length pre-crRNA, which is then processed into small individual crRNAs to form a complex with a tracrRNA. The crRNA possesses a "guide sequence" that matches a complementary DNA sequence in the viral genome (Horvath and Barrangou, 2010). Thus, the RNA complex can then guide the Cas protein, for example Cas9, to this specific sequence in the viral genome with a neighboring protospacer adjacent motif (PAM). From there, Cas9 can then induce a double-stranded (ds) break and efficiently destroy the viral DNA (*Figure 3B*) (Sternberg *et al.*, 2014).

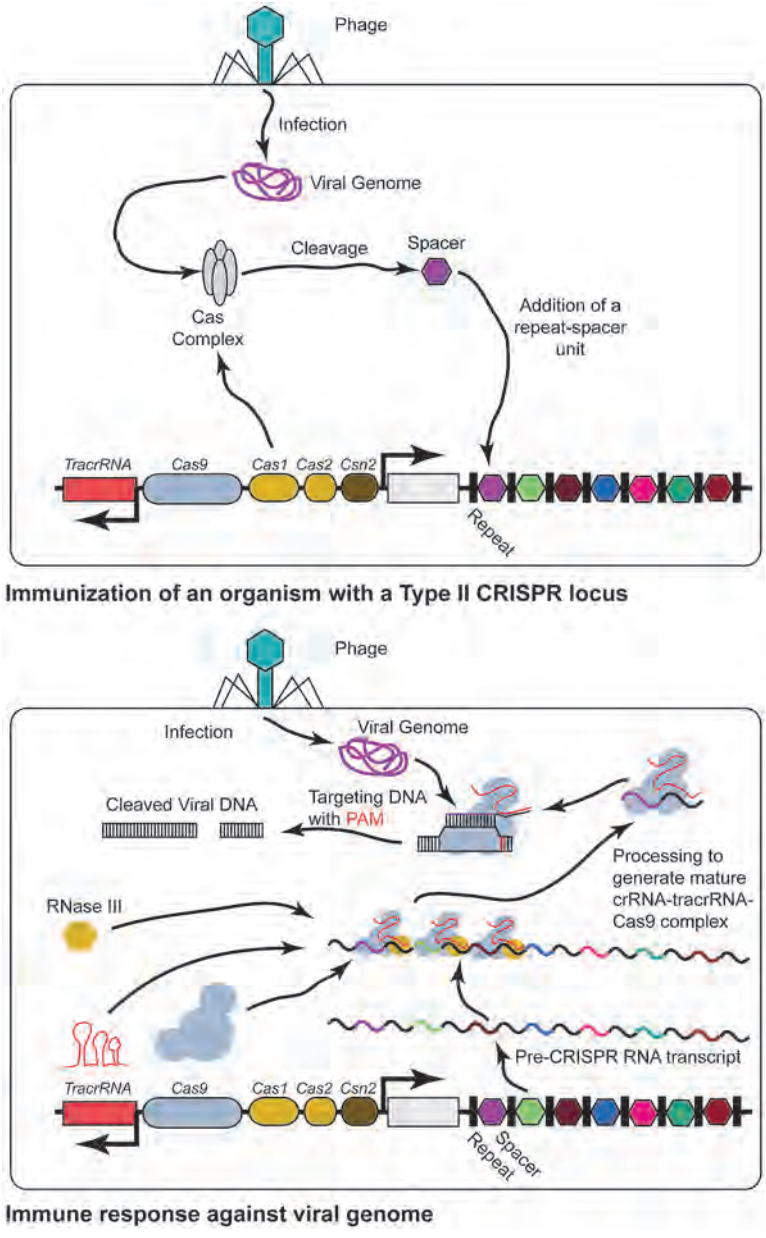

Figure 3. The evolution and function of the Cas9/CRISPR immune system. (A) Upon viral infection, a piece of viral DNA that is situated next to a PAM motif is incorporated as a spacer sequence in the *CRISPR* locus. (B) This spacer can be transcribed and used as a guide to target a double-stranded break to the viral DNA upon any subsequent infection. This figure was inspired by that found in Mali *et al.*, Cas9 as a versatile tool for engineering biology (2013). Figure courtesy of Alex Hallagan and Thomas Williams, 2015.

Applications for CRISPR/Cas9 in Genome Editing

After the discovery of this formerly unknown immune system, scientists found that they could engineer CRISPR/Cas9 to be implemented in organisms who do not naturally possess it (Mali *et al.*, 2013). The RNA-guided Cas9 endonuclease was employed in *D. melanogaster*, for example, to create germline genome modifications (Gratz *et al.*, 2014; S. J. Gratz *et al.*, 2013; Port *et al.*, 2014). The appeal of the CRISPR system in comparison to past genome-editing technology is the ease of creating the guide RNAs and achieving engineered flies within as little time as a month. For efficient use in genome editing, the system has been simplified to two components by combining the tracrRNA and crRNA into a single chiRNA or guideRNA

(Gratz *et al*., 2014). This includes a custom-designed 20 base sequence complementary to the desired target that can then guide Cas9 to induce the ds break at the genome location with the neighboring PAM sequence. Following the breakage event, the DNA can be repaired by the error-prone repair process of non-homologous end joining (NHEJ), which often results in an introduced small insertion or deletion of a few nucleotides. When targeted to a protein-coding sequence, such an indel mutation will shift the reading frame of protein translation and thereby produce a disrupted gene product (S. Gratz *et al*., 2013).

Figure 4. *Drosophila* pigmentation diversity. Adult abdomens from species that exhibit differences in color and spatial pattern of color. Abdomens are viewed from the dorsal surface. Figure courtesy of Alex Hallagan and Thomas Williams, 2015.

These indel mutations, however, are not the most ideal for disrupting CREs, which are not subject to the strict constraints imposed by the 3 nucleotide reading frame of the genetic code. However, a modified approach can allow for a much larger deletion to be induced via CRISPR. Here two chiRNAs can be employed to successfully delete an entire CRE or even gene locus by targeting ds breaks to sequences flanking the CRE or gene region of interest (Gratz *et al*., 2014). This broken chromosome is then attached back together by the use of a novel donor DNA sequence by the process of homologous recombination (HR). This approach to CRISPR utilization can provide a simple, more time-effective way to remove entire CREs from a genome. If this approach can be coupled with a way to integrate CRE alleles or orthologous sequences (collectively referred to as CRE variants), then geneticists would be equipped with a powerful approach to modify the regulatory regions of genomes and advance an understanding of how these sequences function *in vivo*.

Fruit Fly Abdomen Pigmentation as a Model Trait for Gene Expression Studies

D. melanogaster serves as an excellent model organism due to the wide variety of tools available to manipulate the genome, its quick generation time, and low cost to maintain in lab. Moreover, the ethical concerns of manipulating genomes are trivial compared to that which would be encountered for humans. Moreover, this species and its relatives provide an excellent model trait in which to study gene expression regulation in development and its evolution. This trait is the pattern of pigmentation that occurs on the cuticle plates (called tergites) that cover the dorsal segments of the abdomen. The *Drosophila* genus is comprised of well over 200 living species that share a common ancestor that lived approximately 50 million years ago (Markow and O'Grady, 2006). Since these species diverged, tergite pigmentation has evolved a great diversity of spatial and color patterns (*Figure 4)*. Specifically, the pigmentation patterns on the abdomens of *Drosophila* species serve as the ideal model trait to study the complex network of genes that make a simple trait.

The genomes from twelve different *Drosophila* species have been sequenced, and it was observed that a general conservation in the inventory of genes exists (Clark *et al*., 2007; Stark *et al*., 2007). This conservation appears to extend to the genes that encode enzymes (so called pigmentation genes) involved in pigment metabolism (Camino *et al*., 2015; Johnson *et al*., 2015; Ordway *et al*., 2014) and to the genes that encode transcription factor proteins involved in the spatial patterning of pigmentation gene expression (Jeong *et al*., 2006; Williams *et al*.,

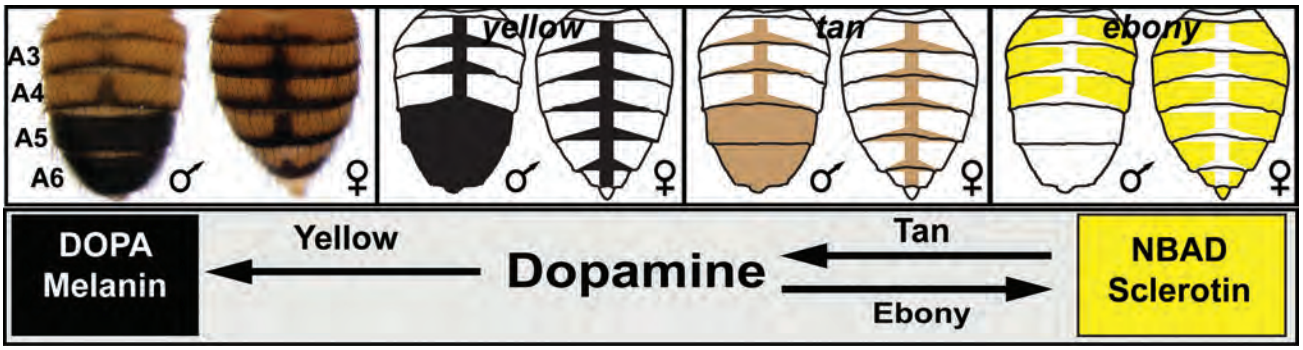

Figure 5. Patterned expression of pigmentation pathway enzymes shapes the ultimate pattern of pigmentation on the *Drosophila melanogaster* abdomen. (A) Pigmentation phenotype for *D. melanogaster*. Depictions of the endogenous patterns of (B) *yellow*, (C) *tan*, and (D) *ebony* expression. (E) The sequence of enzyme activity within a dopamine conversion pathway. Figure courtesy of Alex Hallagan and Thomas Williams, 2015.

2008). Pigmentation in *D. melanogaster* has been well studied for several decades, and many of the key genes have been characterized (Wittkopp *et al.*, 2003). Pigmentation involves the production and modification of dopamine to make derivatives that exhibit specific colors, including black and yellow colors (*Figure 5*). The genes *tan* and *yellow* encode enzymes that are needed to make the black DOPA melanin, whereas the enzyme encoded by the *ebony* gene is needed to make the yellowish NBAD sclerotin (*Figure 5*). Consistent with their function in making pigments, *yellow* and *tan* are expressed in epidermal cells underlying the tergites of the male A5 and A6 abdominal segments (Camino *et al.*, 2015), where as expression of *ebony* occurs in a reciprocal pattern that is pronounced in females (*Figure 5*) (Rebeiz *et al.*, 2009). These gene

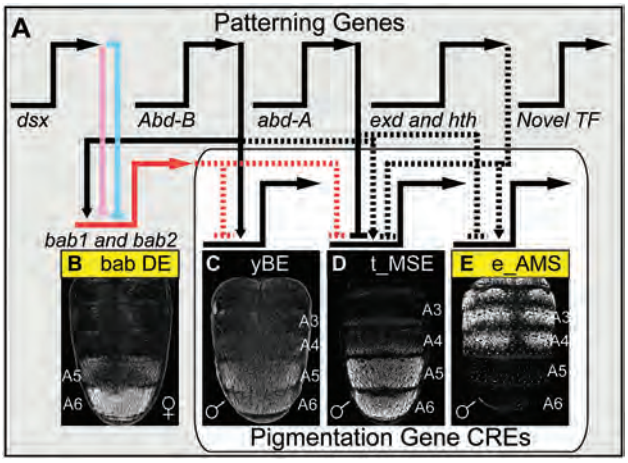

Figure 6. A gene network for pigmentation is structured by transcription factors (TF) interacting with pigmentation gene CREs. (A) A model of the known GRN structure for *D. melanogaster*. (B-D) CREs controlling network gene expression. Solid connectors between genes indicate validated cases of direct TF-CRE binding, and dashed lines are connections that may be direct or indirect. (B-D) EGFP reporter gene expression patterns driven by the *D. melanogaster* CREs. Figure courtesy of Alex Hallagan and Thomas Williams, 2015.

expression patterns have often been found to have been altered in species with divergent patterns of pigmentation (Camino *et al.*, 2015; Jeong *et al.*, 2008, 2006; Johnson *et al.*, 2015; Ordway *et al.*, 2014; Rebeiz *et al.*, 2009). Thus, how these genes are regulated by CREs and how these CREs have functionally evolved makes an excellent experimental model to gain general insights on gene regulation and its evolution.

The *D. melanogaster* CREs controlling the spatial- and sex-limited patterns of expression have been previously characterized in reporter transgene assays. These include the *yellow* Body Element (yBE) (Wittkopp *et al.*, 2002), *tan* Male-Specific Element (t_MSE) (Camino *et al.*, 2015), and the *ebony* AMS (e_AMS) (Rebeiz *et al.*, 2009) (*Figure 6*). Moreover, the network of transcription factors that control these CREs' pattern of regulatory activity continue to become increasingly more resolved. This network includes over 26 transcription factor genes (Rogers *et al.*, 2014), including the duplicate *bab1* and *bab2* (collectively referred to as *bab*) genes that suppress *tan* and *yellow* expression in females. *bab* is expressed exclusively in the female abdomen during the time point when pigmentation is being patterned. This female-limited pattern is controlled by a CRE known as the dimorphic element that is regulated through its possession of binding sites for the transcription factors encoded by the *Abd*-B and *dsx* genes (Williams *et al.*, 2008).

Reporter transgene assays have been useful in identifying the suspected activity of CREs (*Figure 6*), but an efficient technique does not exist to study these CREs in their natural genome location and to understand how variant forms may differ in function. For example, it is still unknown whether these CREs are necessary

for the regulated gene's function *in vivo*. It is conceivable that a shadow enhancer exists that can compensate for such a CREs absence. Thus, my thesis will attempt to confront this problem and devise a strategy using the CRISPR/Cas9 system to investigate how the CRE's sequence corresponds with its *in vivo* function.

CRISPR CREam for Fruit Flies: A Novel Approach

My thesis aims to develop a customized approach to remove CREs known to regulate genes whose encoded proteins function in an enzymatic pathway for pigmentation (*Figure 5*). The CREs of interest include t_MSE, yBE, and the dimorphic element, which are each thought to control the genes *tan*, *yellow* and *bab* (Camino *et al*., 2015; Jeong *et al*., 2006; Rebeiz *et al*., 2009). Following CRE removal, the second thesis aim will be to replace each of these CREs with a variant CRE version from a species or population of flies that has a divergent pigmentation pattern in comparison to that typical for *D. melanogaster*. For *tan* and *yellow*, we will introduce a CRE ortholog from the species *D. auraria* to test whether the orthologous CREs have undergone some functional evolution. For the dimorphic element, I will test allelic forms that were found to differ in their capability to regulate EGFP expression in reporter transgene assays (Rogers *et al*., 2013).

My initial envisioned approach is to use CRISPR/Cas9 to first individually remove these specific CREs by using two chiRNAs to induce ds breaks on each side of the CRE (*Figure 7A* and *7B*). The cellular process of homologous recombination (HR) will then be used to add a dsRed mini-gene in place of the deleted sequence (*Figure 7C*). This mini-gene expresses a red fluorescent protein in the eyes of fruit flies, which can be easily seen and reported in which flies that the CRE was likely to have been deleted. The mini-gene will also be flanked by inverted attP sites from the phage ϕC31 (Groth *et al*., 2004). Following success in CRE removal, the plan will be to swap a variant CRE into the position of the deleted CRE by recombination-mediated cassette exchange (RMCE) (Bateman and Wu, 2008; Bateman *et al*., 2006; Venken *et al*., 2011). This involves the inverted attP sites on the mini-gene undergoing intermolecular recombination with inverted attB site via the ϕC31 integrase enzyme (Thorpe and Smith, 1998). RMCE has been found to occur efficiently in fruit flies. Thus, its use here may support the repeated insertion of CRE variants into the position of the original deleted CRE (*Figure 7E*).

The Broad Utility of CRISPR-CREam in the Future

The easy and efficient genome engineering of CRISPR/Cas9 widens the horizons of research in terms of gene therapy. CRISPR has already shown to be successful in correcting muscular dystrophy in lab mice (Long *et al*., 2014). However, in order for CRISPR to ever be used successfully and safely for therapeutic interventions with genetic diseases, further refinements need to be made through research in species such

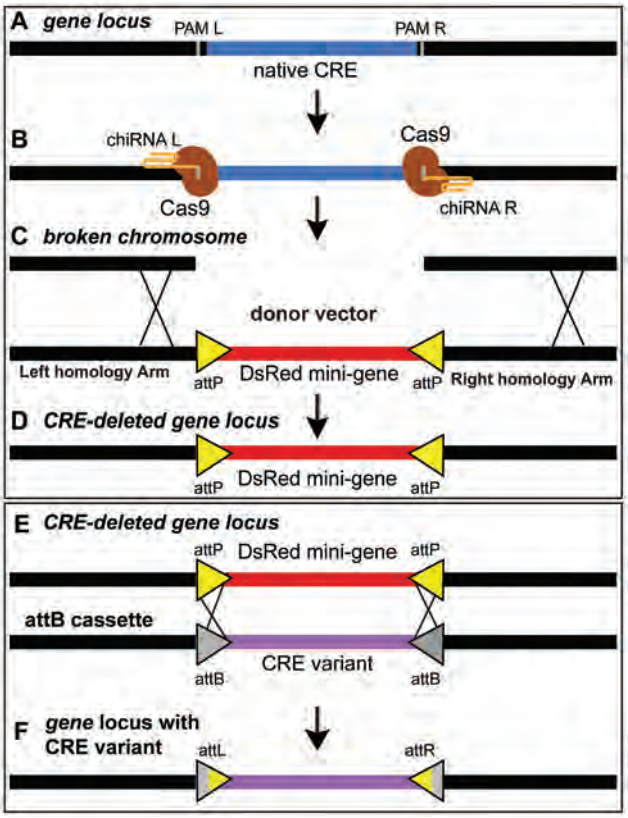

Figure 7. Removing and replacing a CRE in vivo by CRISPR CREam. (A) Representation of a CRE in its natural gene locus. Target sequences with adjacent PAMs occur on the left and right side of this CRE (PAM L and PAM R). (B) chiRNAs will direct the Cas9 enzyme to these target sequences where double stranded breaks will be induced. (C) The gene locus will be repaired by homologous recombination occurring with a donor vector which contains identical sequence to the gene locus (homology arms) on the left and right flank of a cassette with inverted attP sites flanking a DsRed mini-gene. (D) The will result in the inclusion of the mini-gene cassette in the place of the origninal CRE. (E) ϕC31 integrase will catalyze crossover events between the inverted attP and attB sites of the modified gene locus and an attB cassette containing a CRE variant. (F) The end result will be a gene locus where the CRE variant resides in the position of the original CRE. Figure courtesy of Alex Hallagan and Thomas Williams, 2015.

as *D. melanogaster*. Moreover, understanding the genetic language for CRE function requires testing many variant forms in the endogenous genome environment. Success with my thesis will not only clarify and help us understand the role of CREs in the evolving pigmentation network of *Drosophila*, but it will also create a novel genetic approach and molecular tools for use in the broader genetics community. It will allow scientists to investigate CREs or even any genome sequence from seemingly any species. It is truly an exciting time to be involved in genetics research!

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Towards a Fractional Analogue to the Lasota-Opial Theorem for a Nonlinear, Second-Order ODE with a Two-Point BVP

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Introduction

Within the field of ordinary differential equations, there is a set of standard existence and uniqueness results that can be used to prove more interesting and useful results. However, this set of standard theorems within fractional differential equations is much smaller. We intend to expand this set of standard theorems. Specifically, we turn our attention to the Lasota-Opial Theorem, a result for a nonlinear, second-order, ordinary differential equation with an associated two-point boundary value problem. The basic assertion of the theorem is that assuming uniqueness of solutions to the differential equation and its boundary conditions (along with other assumptions) implies the existence of solutions. Our main objective is to build an analogue to Lasota and Opial's result within the framework of fractional calculus.

Riemann-Liouville Derivative

We begin by providing a brief introduction to the inspiration behind fractional calculus and some standard definitions for those who may be unfamiliar with it. For a more complete treatment of this, we refer the reader to [1],[2],[4]. Consider the following example with the classical differential operator.

$$\frac{d^3}{dt^3}(t^{10}) = 10 \cdot 9 \cdot 8t^7$$

In a more suggestive form, we can rewrite the above as

$$\frac{d^3}{dt^3}(t^{10}) = \frac{10!}{(10-3)!}t^{10-3}$$

which is clearly only defined for integers as the factorial function is only defined for integers. However, we can take one step further and rewrite this in terms of the Gamma function as

$$\frac{d^3}{dt^3}(t^{10}) = \frac{\Gamma(10+1)}{\Gamma((10-3)+1)}t^{10-3}$$

Thus given that the Gamma function is well-defined over arbitrary arguments, we derive an ansatz for computing the derivative of order 2.5 as

$$\frac{d^{2.5}}{dt^{2.5}}(t^{10}) = \frac{\Gamma(10+1)}{\Gamma((10-2.5)+1)}t^{10-2.5}$$

Using this example as a guide, it seems reasonable to define the derivative of order α of the function t^{β} for $\alpha < \beta$ as

$$\frac{d^{\alpha}}{dt^{\alpha}}(t^{\beta}) = \frac{\Gamma(\beta+1)}{\Gamma(\beta-\alpha+1)}t^{\beta-\alpha}$$

Notice that in the case that α,β are integers, we get the same result as that of the classical derivative. We are now ready to introduce the definition of the Riemann-Liouville integral, after which defining the derivative is simple. Let $\alpha > 0$. Assume $f \in L_1[a,b]$ where L_1 denotes the Lebesgue space equipped with the 1-norm. Then for $t \in [a,b]$, we define the Riemann-Liouville integral of order α by

$$I_a^{\alpha}(f(t)) = \frac{1}{\Gamma(\alpha)}\int_a^t (t-\tau)^{\alpha-1}f(\tau)d\tau$$

We note that in the case $\alpha = 1$, we obtain

$$I_a^1(f(t)) = \frac{1}{\Gamma(1)}\int_a^t (t-\tau)^{1-1}f(\tau)d\tau = \int_a^t f(\tau)d\tau$$

which is simply the classical integral, as expected. We now use this definition to define the fractional derivative. Let $\alpha > 0$, and let n be the integer such that $n-1 \le \alpha \le n$. Then the α-th order Riemann-Liouville derivative of a function f defined on $[a,b]$ is given by

$$D_a^{\alpha}f(t) = \frac{d^n}{dt^n}(I_a^{n-\alpha}f(t))$$

where $\frac{d^n}{dt^n}$ is the classical n-th order derivative.

Caputo Derivative

The definition of a fractional derivative is a somewhat controversial topic within the field. Certain properties of different definitions may or may not be desirable in certain realms. For example, when solving an initial-value problem using the Riemann-Liouville definition, initial values for arbitrary-order derivatives may need to be known. This is undesirable when creating models for physical systems as these quantities are much more abstract and harder to interpret in a physical sense. In these realms, the Caputo definition is often more desirable. This is because initial conditions with this definition use derivatives of integer-order. Let us now introduce the Caputo definition. Let $\alpha > 0$, and n be the integer such that $n-1 < \alpha \le n$. Assuming the expression below exists, we define the Caputo derivative of α-th order of f to be

$$D_{*a}^{\alpha}f(t) = I_a^{n-\alpha}\frac{d^n}{dt^n}f(t)$$

where $I_a^{n-\alpha}$ is the usual Riemann-Liouville integral of $(n-\alpha)$-th order, and the * is placed to differentiate between the Caputo and Riemann-Liouville definitions. Note that the only difference between the two definitions is the order of differentiation and integration.

Lasota-Opial Theorem

We now state and prove the Lasota-Opial Theorem for a second-order, nonlinear, ordinary differential equation with its associated two-point boundary value problem. We first introduce some notation. For $a,b \in \mathbb{R}$, and function $f:(a,b) \times \mathbb{R}^2 \mapsto \mathbb{R}$, we define the ordinary differential equation (4.1) by

$$x''(t) = f(t,x(t),x'(t)) \qquad (4.1)$$

and the boundary conditions (4.2) by

$$x(t_1) = r_1, x(t_2) = r_2 \qquad (4.2)$$

where $a < t_1 < t_2 < b$. Conditions (A), (B) and (C) on the function f are given by

(A) f is continuous on $f:(a,b) \times \mathbb{R}$;

(B) $\forall (t_1,r_1), (t_2,r_2) \in (a,b) \times \mathbb{R}$ ($t_1 < t_2$), a solution to (4.1)-(4.2) is unique

(C) $\forall (t_0,x_0,m_0) \in (a,b) \times \mathbb{R}^2$, there exists a unique solution $x(t;(t_0,x_0,m_0)$ to (4.1) satisfying $x(t_0)=x_0$ and $x'(t_0) = m_0$

Theorem 4.1

Let $a,b \in \mathbb{R}$. If $f:(a,b)\times\mathbb{R}^2 \mapsto \mathbb{R}$ satisfies conditions (A), (B) and (C), then $\forall (t_1,r_1),(t_2,r_2) \in (a,b) \times \mathbb{R}$ (t_1,r_1) a solution to (4.1)-(4.2) exists.

Proof

Let $x(t,m)$ be a solution to (4.1) with $x(t_1) = r_1$ and $x'(t_2) = m$. Denote $T:\mathbb{R} \mapsto \mathbb{R}$ by $T(m)=x(t_2,m)$. From condition (C), it follows that T is a continuous mapping, so T maps into a connected subset of $\mathbb{R}$. From condition (B), T is an injection, so T maps into an open subset of $\mathbb{R}$. If the range of T, denoted by $T(\mathbb{R})$, is the entire real line, then $r_2 \in \mathbb{R}$. Thus there is a solution to the problem (4.1)-(4.2). So all that is left to show is that $T(\mathbb{R}) = \mathbb{R}$. Assume for the sake of contradiction that $T(\mathbb{R}) \neq \mathbb{R}$. Then we split into the case when $T(\mathbb{R})$ is bounded above. So let $M = \sup\{T(\mathbb{R})\}$. Choose an increasing sequence $\{m_k\}_0^\infty$ converging to M. From here, it follows (refer to [3] for complete details) that $M \in T(\mathbb{R})$. But then $T(\mathbb{R})$ contains its own supremum, so $T(\mathbb{R})$ is not open, a contradiction. The case when $T(\mathbb{R})$ is bounded below is similar, leading to the same contradiction with $\inf\{T(\mathbb{R})\} \in T(\mathbb{R})$. Therefore, it must be that $T(\mathbb{R})$ is neither bounded above nor below, so $T(\mathbb{R}) = \mathbb{R}$.

Remarks for the Fractional Case

While the form of the differential equation in the fractional case is similar to that in ordinary differential equations, there are some complications that arise within the fractional case. Most importantly, determining whether or not solutions are well-behaved is inherently harder due to continuity issues. For example, suppose f is continuous on [a,b]. Then by the Fundamental Theorem of Calculus, we know that the function $g:[a,b] \mapsto \mathbb{R}$ given by

$$g(t) = \int_a^t f(\tau)d\tau$$

is well-defined and continuously differentiable. However, if we were to consider an integral of order 1/2, the picture is different for g:[a,b] $\mapsto \mathbb{R}$ where

$$g(t) = I_a^{\frac{1}{2}}(1) = \frac{1}{\Gamma(1/2)} \int_a^t (t-\tau)^{-1/2}\, d\tau = 2\sqrt{t-a}$$

Now since the kernel $(t-r)^{-1/2}$ is singular at the upper bound, we can no longer guarantee continuous differentiability of g. This is easily shown by the function $f(t) = 1$, so we simply obtain

$$g(t) = I_a^{\frac{1}{2}}(1) = \frac{1}{\Gamma(1/2)}\int_a^t (t-\tau)^{-1/2}\,d\tau = 2\sqrt{t-a}$$

Computing its derivative:

$$g'(t) = \frac{1}{\sqrt{t-a}}$$

which is only continuous on $(a,b]$ but not $[a,b]$ as we had in the previous case. With respect to the Lasota-Opial Theorem, discontinuity at a is not an issue as it deals with an open interval. However, this example is a good illustration as to why extra care must be taken with fractional differential operators as a discontinuity occurs even with a function as simple as $f = 1$. For arbitrary functions, a strong Lipschitz condition must be made on the function and integral order to guarantee continuous differentiability of the fractional integral (see [1], Theorem 2.5). This contrasts with integer-order integration where the much weaker assumption of continuity implies continuous differentiability. These continuity issues suggest that a more precise function space must be defined for the analogous theorem.

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The Impact of Acute Hypoxic Exposure on Vascular Function in Young, Healthy Humans: A Review

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Abstract

Reactive hyperemia (RH), or the return of blood flow to vessels after occlusion, can be indicative of microvasculature health. Weakened function in the microvasculature is observed in populations that are more susceptible to cardiovascular disease. While the function of microvasculature can be impaired by many factors, the effect of acute isocapnic hypoxic exposure on reactive hyperemia is of interest. With isocapnic hypoxia, oxygen saturation is lower than normal, while carbon dioxide levels remain the same. When exposed to hypoxia blood vessels dilate, allowing for an increase in blood flow. Thus, the need for increased blood flow during a reactive hyperemia response may be greater when combined with hypoxia. However, at the same time, hypoxia itself is a stress to the system and can impair the mechanisms by which reactive hyperemia occurs.

The purpose of this review is to discuss how hypoxia affects vascular function, specifically by looking at blood flow changes during the reactive hyperemia response. This will be accomplished by considering how blood flow is regulated in the human body and how blood flow measurements are used to determine the health of vasculature. Additionally, the physiological role that hypoxic exposure plays at the level of the blood vessels, as well systemically, will be considered. Understanding these concepts allows for the discussion of how blood flow and vascular function are influenced by the combined effects of reactive hyperemia and hypoxia.

Physiological Determinants of Blood Flow

Blood flow is determined by various factors in order to supply sufficient amounts of oxygen to the tissue level. Blood flow measurements are often studied in the resistance vessels known as arterioles, since they are highly responsive and largely regulate flow. Blood flow can be calculated by looking at the pressure gradient across arterial and venous beds: ($pressure_{arterial}$ – $pressure_{venous}$) / (resistance). Resistance in the vessel is determined by 1 / (vessel radius)^4. As the radius of a blood vessel increases, known as vasodilation, resistance in that vessel decreases by a power of four. As resistance decreases, more blood will flow through the resistance vessel, and supply more blood (and therefore oxygen) to the tissue. The goal of the circulatory system is to maintain the matching of oxygen supply to oxygen demand, known as metabolic autoregulation, via changes in blood flow.

Mean Arterial Pressure

Mean arterial pressure (MAP), which is the driving force for blood, is determined by multiplying cardiac output by total peripheral resistance (TPR). MAP is calculated as an average of pressure over one cardiac cycle (Silverthorn, 2012). Heart rate, stroke volume and degree of resistance in blood vessels all influence MAP. Any changes in these factors will cause the driving force of blood to change. A change in vessel radius would also change MAP since

vessel radius and resistance have an inverse relationship, and total peripheral resistance is one of the determinants of MAP.

Vessel Radius and Resistance

As discussed earlier, vessel radius and resistance to blood flow have an inverse relationship. In response to changes in blood oxygenation, vessel radius will increase in order to decrease resistance to flow. This allows for increased blood flow to the level of the tissues. As vasodilation occurs and resistance decreases, TPR falls, which causes MAP to also decrease, explaining the mechanism discussed above. However, in order for blood vessels to regulate flow, the vascular smooth muscle cells must be able to contract and relax. This contractile ability determines vessel caliber, and is based on calcium concentrations within the vascular smooth muscle cells. In order for smooth muscle cells to contract, calcium ions must be present to initiate the movement. A muscle contraction occurs when myosin heads slide along actin filaments to create muscle tension; actin and myosin are two of the most basic components of muscle cells, whose movement causes muscle contractions (*Figure 1*). Ca^{2+} ions are released from the sarcoplasmic reticulum of smooth muscle cells, and bind to a protein called calmodulin (CaM). The Ca^{2+}–CaM molecule activates myosin light chain kinase (MLCK), which increases myosin-ATPase activity. Active myosin cross bridges slide along actin and cause muscle contraction (Silverthorn, 2012). As smooth muscle cells contract, they shorten, which will change the radius of the vessel and allow the vessel to regulate flow (Webb, 2003).

The stimulus for the calcium signal that initiates contraction can be chemical, mechanical or electrical. Hormones are chemical stimuli that can bind to certain receptors on the smooth muscle cell to cause contraction of the muscle. Mechanical stimuli include any stretching of the vascular smooth muscle, which would elicit a myogenic response and cause contraction. Lastly, electrical stimuli would come from a depolarization of the cell membrane, causing voltage-dependent calcium channels to open and initiate a muscle contraction (Silverthorn, 2012).

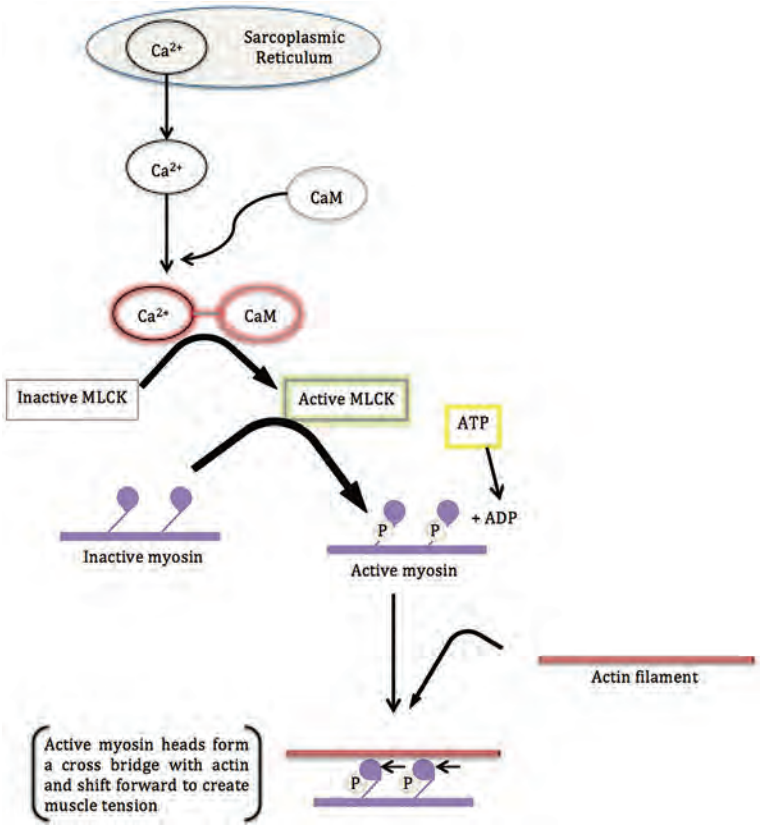

Figure 1. Stepwise representation of what occurs at the level of the muscle cell during a smooth muscle contraction. Courtesy of E. Kelsch, 2015.

Hemoglobin Saturation

A large determinant in oxygen supply to tissues is the O_2 saturation of hemoglobin molecules. O_2 saturation is directly related to the pO_2, or partial pressure of oxygen, in the blood. This association is represented by the oxy-hemoglobin dissociation curve (*Figure 2*), which describes the relationship between partial pressure of oxygen in the blood and percent oxygen saturation. This association is affected by changes in pH, pCO_2 and temperature. Each hemoglobin molecule has four binding sites for individual oxygen molecules. If a molecule of oxygen is bound at each site on a single hemoglobin molecule, it is 100% saturated. As pO_2 increases, hemoglobin's affinity for oxygen increases and O_2 saturation will rise. At the level of the alveoli in the lungs, partial pressure of oxygen is 100 mmHg. pO_2 remains around 100 mmHg in the arterial blood, and can drop to roughly 40 mmHg in venous blood. Therefore, since arterial pO_2 is about 100 mmHg, arterial O_2 saturation is noted as approximately 98% in a healthy human.

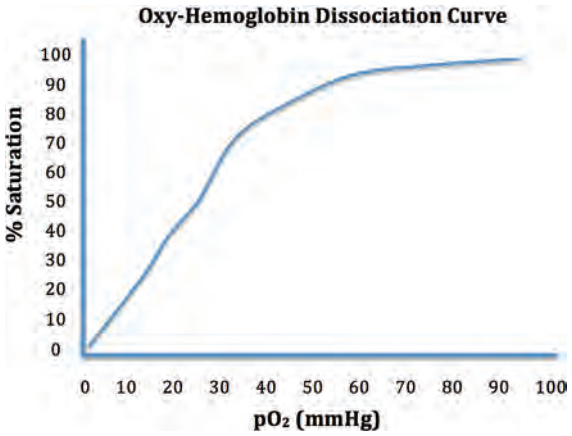

Figure 2. Oxy-hemoglobin dissociation curve. This shows the relationship between partial pressure of oxygen and percent saturation of oxygen in the blood. Courtesy of E. Kelsch, 2015.

Regulation of Blood Flow

Metabolic Autoregulation

Metabolic autoregulation is the body's way of regulating blood flow at the level of the tissue. This physiological mechanism works to match oxygen supply to the level of oxygen demand. Oxygen supply is determined as the product of blood flow and arterial oxygen concentration. The concentration of oxygen in blood is further determined by the product of hemoglobin content and percent O_2 saturation of the hemoglobin molecules. Oxygen demand is simply determined by the metabolism that occurs at the tissue level, quantified as VO_2, or oxygen consumption via aerobic respiration. Metabolic autoregulation works to keep the human body in homeostasis in terms of delivering enough oxygen to the tissues so that they can function to supply energy when needed. Oxygen supply can be changed by a number of factors: percent saturation on hemoglobin molecules, hemoglobin content in blood and blood flow. Depending on changes in any of these factors, blood supply will either increase or decrease to the tissue to match oxygen supply and demand of the tissue.

Measuring Blood Flow

With an understanding of how and why our body regulates blood flow to our tissues, it is important to identify what techniques are used to measure these changes in flow. Some techniques used to measure blood flow include Doppler ultrasound, dye-dilution technique and venous occlusion plethysmography. A Doppler ultrasound calculates the mean blood velocity and detects vessel edges to determine vessel diameter throughout continuous cardiac cycles (Naylor *et al.*, 2005). Any changes in blood flow would be noted by a change in blood velocity or vessel diameter. Dye-dilution technique is based on the idea that if an indicator (dye) is injected at a constant rate into the circulation system at a certain point and its dilution is estimated at some distal site, then rate of flow can be calculated (Payne, 1963). Rate of blood flow is determined by the amount of dilution that occurred in the dye. The technique of venous occlusion plethysmography will be discussed in depth below, as this particular method of measuring blood flow is widely used and ties into the vascular function test that will be explained later in this review.

Venous Occlusion Plethysmography

Venous occlusion plethysmography (VOP) is a technique used to take blood flow measurements in the forearm. This technique can be used to measure blood flow in response to various stimuli: exercise, reactive hyperemia, body heating and mental stress (Joyner *et al.*, 2001). A blood pressure cuff on the wrist is inflated to 200 mmHg to occlude, or block, blood flow to the hand (Greenfield *et al.*, 1963). This allows for monitoring of blood flow strictly in the forearm. A second blood pressure cuff is placed on the upper arm and cycles between inflation to 50 mmHg and deflation; an entire cycle lasts about 15 seconds. When the cuff is inflated, venous outflow is obstructed, while arterial inflow remains. During the time of cuff inflation, there will be a slight swell of blood flow in the forearm due to the venous occlusion. Once the cuff is deflated, venous outflow is reinstated, and arterial inflow remains free. Changes in blood flow are detected via a highly sensitive strain gauge placed around the forearm. The measurements of blood volume changes detected by the strain gauge are recorded in a graph-like form (*Figure 3*).

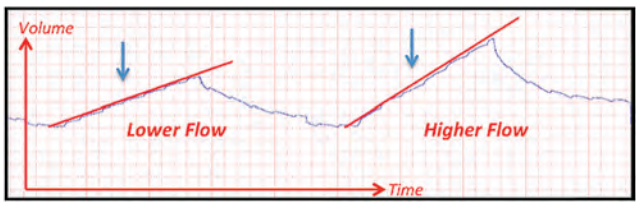

Figure 3. Changes in blood flow in the forearm, measured via venous occlusion plethysmography, are shown in this graph. As the BP cuff is inflated to 50mmHg, venous outflow is arrested, and blood volume increases (as shown by the arrows). If blood flow increases more rapidly in a shorter amount of time, the curve representing blood volume will have a steeper slope (higher flow curve). Courtesy of A. Crecelius, used with permission.

Measuring Vascular Function

There are various techniques used to measure vascular function in order to determine the health of blood vessels. Two popular non-invasive techniques used are reactive hyperemia and flow-mediated dilation. Reactive hyperemia looks at the peak flow response to an occlusion, while flow-mediated dilation measures the change in diameter of the brachial artery after occluding blood to the forearm (Flammer *et al*., 2012). Vascular function can also be determined by various exercise-induced tests, as well as drug infusions to stimulate dilation, which can be both endothelium dependent and endothelium independent (Joyner *et al*., 2015). However, one of the most common techniques used to measure vascular function is reactive hyperemia.

Reactive hyperemia is a widespread method of testing vascular function due to its ease, safety and repeatability. This technique uses the same cuff setup as VOP as described earlier. Reactive hyperemia works to repay oxygen to the tissues to make up for the oxygen deficit during occlusion (Joyner *et al*., 2001). This compensation of oxygen occurs via a rapid increase in blood flow to the forearm, due to vasodilation. The measurements of blood flow during VOP and reactive hyperemia can be viewed below (*Figure 4*).

Performing these techniques to measure vascular function is very important in order to determine vascular health. An impaired reactive hyperemic response has been linked to increases in cardiovascular disease risk (Flammer *et al*., 2012). Impaired reactive hyperemic response has also been measured in subjects with pre-established hypertension; this marker of endothelial dysfunction has been said to occur early in the course of cardiovascular disease (Wilkinson *et al*., 2001). If the body has an impaired response to an occlusion, there may not be sufficient blood supply getting to the deprived tissue area. This means that the area in question would not be receiving enough oxygen to match its required demand, and the tissue would not be at a normal level of functioning.

Hypoxia and its Effects

Hypoxia is a condition of low oxygen exposure. This phenomenon can be caused by a drop in atmospheric pO_2 while percent O_2 remains the same, as occurs in increasing altitudes. Hypoxia can also result from a change in the O_2 composition of the air, such as in a lab setting when gases are mixed to create low oxygen containing air. When a person is exposed to hypoxia, there is a deficiency in the amount of oxygen reaching the tissue, because there is a lower pO_2 in the blood. As pO_2 decreases, hemoglobin molecules have a lower affinity for oxygen and will not reach 100% O_2 saturation. With a lower percent O_2 saturation level, oxygen in the blood stream will decrease. The response will be an increase in blood flow in order to supply more oxygen to the tissue level. In order to increase blood flow, vasodilation will occur. Therefore, it is noted that the immediate response to acute hypoxia at rest is vasodilation (Casey *et al*., 2011).

Acute and Chronic Hypoxia

Although it has been found that the immediate physiological response to acute hypoxic exposure is vasodilation and increased blood flow, the same may not be true for chronic exposure. Several studies discovered that chronic exposure to hypoxia impairs vascular function (Chung *et al*., 2009). An experiment done by Chung *et al*. found that people with severe sleep apnea who are exposed to hypoxia while they sleep have decreased flow-mediated dilation when compared to normal subjects and, therefore, have impaired arterial elasticity. It has also been found that, when exposed to hypoxia, there is an increase in sympathetic nervous system activity (Marshall *et al*., 2004). As sympathetic nervous system activity increases, the reactive hyperemic response is reduced (Gilmartin *et al*., 2010). In a study performed by Gilmartin and colleagues, subjects were exposed to 14 nights

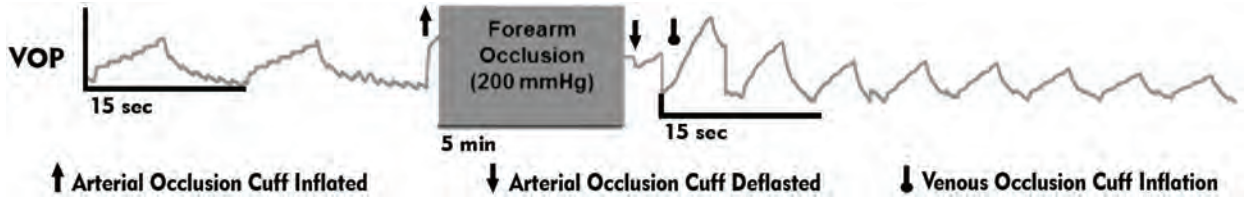

Figure 4. Blood flow measurements are taken during VOP and reactive hyperemia to detect any changes in blood volume. After five minutes of occlusion (200mmHg), blood flow measurements via VOP will resume to record the reactive hyperemic response. Courtesy of A.Crecelius, used with permission.

of poikilocapnic hypoxia (non-controlled CO_2 levels), and through tests of reactive hyperemia were found to have impaired responses to occlusion in terms of peak and total blood flow. However, this study does not take into account hypoxia alone, since CO_2 levels vary throughout, so the impact of hypoxia alone is still in question and requires further investigation.

Systemic Physiological Response to Hypoxia

The effects of hypoxia on the human body as a whole are also relevant. When a person is exposed to hypoxia, they will begin to hyperventilate. Hyperventilation is the body's attempt to increase the O_2 saturation in the blood to match the oxygen demand at the tissue level. As a person hyperventilates, they increase their rate of inhalation, allowing for more oxygen to enter the lungs and, therefore, into the blood. This can be further explained by the Bohr effect. The Bohr effect is associated with the oxy-hemoglobin dissociation curve; it is a phenomenon that explains the correlation between oxygen saturation of the blood and the presence of carbon dioxide. As the level of CO_2 falls, as it does during hyperventilation, hemoglobin molecules gain an increased affinity for O_2 molecules and become more saturated with oxygen. This explains why hyperventilation occurs in response to hypoxia as a way to increase O_2 saturation in the blood. Also, after chronic exposure to hypoxia, there is a systemic response of muscle sympathetic nervous system activity, which leads to increased mean arterial pressure and forearm vascular resistance (Gilmartin *et al*., 2008).

Significance

There are several diseased populations at risk for hypoxic exposure, as well as healthy people who are subject to exposure. People diagnosed with chronic obstructive pulmonary disease and sleep apnea are exposed to chronic hypoxia. Also, healthy humans who travel via commercial airline flights are at risk for hypoxic exposure due to decreased O_2 saturation (Cottrell *et al*., 1995). As discussed earlier, people exposed to hypoxia are at risk for future cardiovascular diseases and vascular dysfunction. It has also been found that vascular dysfunction is an early indicator of atherosclerosis (Anderson *et al*., 2011). Therefore, hypoxia can be noted as a significant physiological stress, and deserves to be studied more in depth.

Conclusion

Understanding vascular health is critical in order to determine future risk for cardiovascular diseases. There are several ways to test the function of vasculature in order to assess their health. One of the most widely used tests is reactive hyperemia. This test observes the reaction of blood vessels after an occlusion, and uses the peak response, also known as reactive hyperemia, or return of blood flow, as a measurement of vascular function.

Testing vascular health is important in populations of people who may be prone to vascular dysfunction. One example of a vulnerable population is people who have frequent exposure to hypoxia. As previously mentioned, people who experience systemic hypoxia are at risk for impairments of vascular health. Due to this fact, it is imperative to study the effects of acute hypoxic exposure in order to better understand how it affects vascular health. Based on the described responses to reactive hyperemia and acute hypoxia, it is unknown what occurs to vascular function and blood flow when these two stimuli are combined, and further investigation is required.

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Framing the Role of Provocation in the History of Modern Art

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Project Description

Art history is traditionally practiced as the study of finished objects. While such an approach grounds inquiry in the work of art itself, it does not take into consideration the broader context of art history as a discipline that charts the history of objects from conception to creation to reception. To address this situation, this research aims to illuminate the complex dynamics in thought, written expression, and even acts that exist before the actual production of art. Specifically, it advances the idea that in the history of modern art from the mid-nineteenth century to the present, the act of provocation has been of sufficient centrality as a catalyst to the artistic object that it merits consideration in terms of its own history, purposes and forms.

> *Provocation: the action of provoking or inciting anger, resentment, or irritation, especially deliberately; action, speech, etc., that provokes strong emotion; an instance of this.*[1]

Provocation is a strategy that has often been used by the modern artist. Indeed, provocation is at times so central to artistic purpose that it becomes the greater part of the work of art or the artistic process. An example is the work of Boris Lurie, as is evident from Lurie's *Railroad to America* of 1962. As a work that combines the sexual enticement of what Lurie called "pin-up girls" with photographs of exterminated bodies in a death camp during the Holocaust, *Railroad to America* provokes the viewer — at once to delight and to horror — through the presentation of two captivating realities: a woman slowly undressing herself, and an image that documents the harsh reality of mass murder. The question that Lurie's work raises is whether this provocation is merely a factor of reception or whether the will to provoke was at the root of the artist's intent and even constituted the greater part of the artist's actual production? In other words, is *Railroad to America* the whole of Lurie's specific work of art or merely the end product of a process of provocation that merits consideration as art history in its own right? The answers to these questions are to be found in the broader context of how provocation has been a catalytic factor in the history of modern art.

The provocative is an aspect that is regularly commented on in the discourse surrounding the study of modern art. Indeed, a notable presence and, at times, an increase in the very appearance of the term provocation can be found in art historical journals throughout the 20th and into the early 21st century. The graph feature on JSTOR outlines how often provocation occurs in published texts on a year-to-year basis.

1. *Oxford English Dictionary*, s.v. "provocation."

While there is a steady increase in the frequency of the term provocation over the period charted by the graph, the trend line especially increases in reference to texts from the 1950s onward (*Figure 1*). Particularly dramatic increases occur in the 1960s and 1970s, suggesting the importance of provocation in academic discourse about arts movements occurring during those decades. Although this graph does not take into consideration other publications or documentation such as books, art exhibition catalogs or artist statements, the tracking of provocation in journal literature provides a foundation for realizing the importance of the very concept of provocation in the artistic activity during these periods and during the broad history of modern art in general.

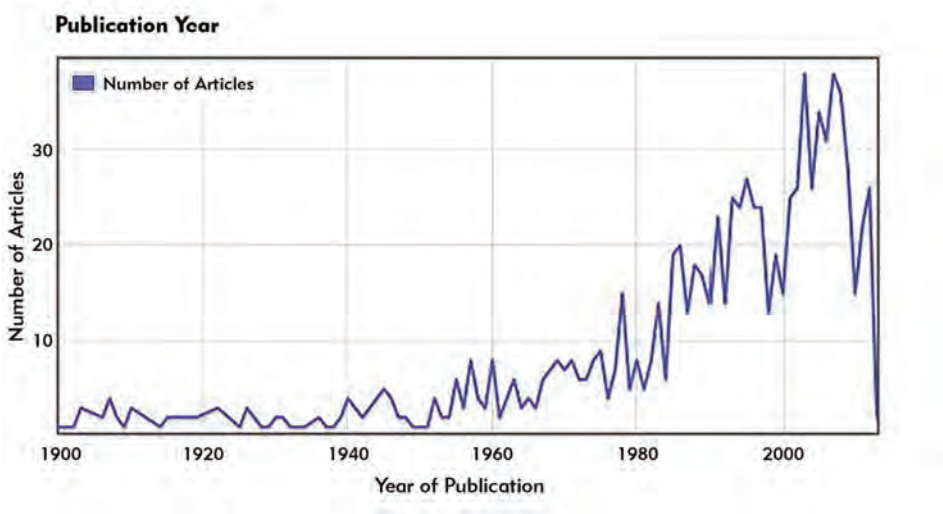

Figure 1. Photo courtesy of JSTOR.org.

Provocation and the provocative gesture have not been thoroughly analyzed by art historians and art critics when investigating and writing about modern art. Notably, the idea of the provocative gesture is conspicuously absent as a dedicated subject in scholarly works such as Arnason and Mansfield's *History of Modern Art* and Hal Foster's *Art Since 1900*.[2,3] While these works are survey textbooks, and do not represent a detailed accounting of how modern art has been discussed in the literature generally, they do constitute representative distillations of collective thought on the field and are therefore telling of what has or has not been addressed by art historians.

Somewhat deceptively the idea of the provocative, couched in terms of the element of shock value, has entered into the writings of certain art critics and art historians. Robert Hughes is an important example of the former. In *The Shock of the New*, Hughes writes about the history of modernism from 1880 to the present. Rather than provocation, Hughes uses the term shock because he tends to focus on the object itself and its aftermath in the history of reception. Because Hughes centers his discourse on this term that defines the aftermath of the art object, he would seem to underestimate the essential element of provocation before the object is produced.[4] What is missing in Hughes's discussion is that with the process of provocation, the modern artist on occasion has had a will to provoke even before the object has been made.

Art historians similarly tend to focus on the aftermath of reaction once the art object appears. An example would be art historian John Walker who, like Hughes, focuses on the aftermath of reaction to provocative art. In *Art & Outrage: Provocation, Controversy, and the Visual Arts*, Walker writes

2. H.H. Arnason and Elizabeth C. Mansfield, *History of Modern Art*, 7th edition (New Jersey: Pearson Prentice Hall, 2013).
3. Hal Foster, *Art Since 1900: Modernism, Antimodernism, Postmodernism*, 5th edition (U.S.: Thames & Hudson, 2005).
4. Robert Hughes, The Shock of the New (New York: Alfred A. Knopf, Inc., 1980).

about the scandalous reactions that occur after an object is made.[5] While he focuses on how shock is an identifiable strategy used by the modern artist to disturb audiences, the greater part of Walker's emphasis is on how works of art are received and not on how they are produced as provocations. For Walker, provocative art circulates in the controversial aftermath of post-artistic creation.

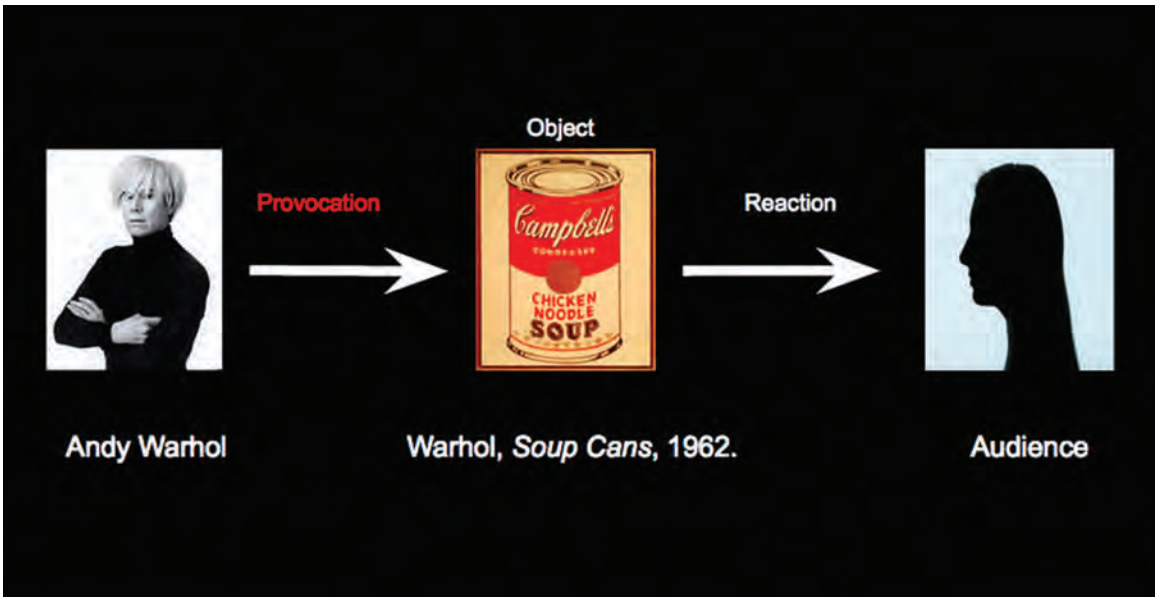

Figure 2. Photo courtesy of Kiersten Remster and Alamy, June 2015.

In general the writings of Arnason, Foster, Hughes and Walker reveal that art history, including the history of modern art, has been pursued as a study of finished objects. By examining the moment before the work of art exists, it is possible to explore the extent to which artists may not always have started with art and the object but have used the gesture of provocation as a critical preliminary step. An example is Andy Warhol and his *Soup Cans* of 1962. *Soup Cans* is an art object that entered the institutionalized settings of display in galleries and museums almost immediately upon its creation. The result has been that *Soup Cans* became strictly a finished and definable work of art seen by the public and written about by critics and art historians. Because the work of art is the only thing that is present in the art museum or gallery, any understanding of the creative process is lost. What is not seen in the institutionalized setting of display for *Soup Cans* is the process of the provocative gesture that motivated Warhol to make such a new and ultimately shocking thing.

The linear diagram in *Figure 2* proposes an alternative understanding of Warhol's work of art that gives interpretive weight to the provocative gesture before the actual creation of *Soup Cans*. The first arrow at the left reveals the unexamined space between Warhol and the moment of creating *Soup Cans*. The arrow on the right between *Soup Cans* and the audience shows the more traditional space between artistic creation and audience reaction. The purpose of this diagram is to suggest that the artistic significance for Warhol's modern provocative art is less in the object that is displayed in a gallery or art museum and more in what is not seen of the artistic process. Once the provocative gesture is identified, one then must contemplate how it is possible to historicize this less-than-tangible but crucial process of provocation.

The idea of examining the provocative in the process of modern art is informed by George Herbert Mead's concept in sociology of symbolic interactionism. Symbolic interactionism is the "social action lodged in acting individuals who fit their respective lines of action to one another through a process of interpretation."[6] Through symbolic interactionism meaning is created and found, analyzed and

5. John A. Walker, Art & Outrage: Provocation, Controversy, and the Visual Arts (UK: Pluto Press, 1999).

6. James Farganis, Readings in Social Theory: The Classic Tradition to Post-Modernism, 2nd edition. (United States of America: McGraw-Hills Companies, Inc., 1996), 262.

valued from the moment of an individual's motives and preliminary actions. Symbolic interactionism is applicable to the concept of the provocative in the creation of art because it suggests by extension that the artist's written, spoken or physical actions, and how those actions are received before the creation of an object, constitute an identifiable process that holds and projects meaning in and of itself.

Taking the concept of symbolic interactionism and its importance to art history one step further, we can look to the writings of sociologist Erving Goffman. Goffman writes about symbolic interactionism from the standpoint of revealing and examining the construction of motives. Goffman believes that we can look at human functions as dramaturgy, or viewing life as a metaphor for a theatrical performance, a concept akin to the "theatrical act" of provocation in the actions of artists.[7]

As art enters the institutionalized realm, understanding the process of provocation is lost. Visitors to a museum or gallery setting interact with a work of art and *per force* interpret its meaning in the present, but any understanding of the provocative gesture is concealed from their cogitation. The process is hidden behind the "stage curtain," as Goffman would refer to it, which is where the artist houses her or his beliefs, motives and intentions.[8] The art object that hangs on the museum wall is simply a prop placed on the front stage. Observers have the opportunity to experience the finished creation, but they are kept from seeing what is behind the traditional process, that is to say behind Goffman's curtain.

In effect, Warhol slipped into the institutionalized realm of finished art with much of the real matter and meaning of his art — the provocative aspects — left behind and eventually obscured by the "curtain" of museum and gallery display. Nonetheless, while provocation is central to Warhol's work, he is still a maker of art objects that have been persevered, valued and discussed as objects and as finite and determinable "monuments" in the history of modern art. This is markedly different from the experience of other modern artists whose works never entered the fixed art history as identifiable and valued objects. What has resulted is that many artists as modern provocateurs have been

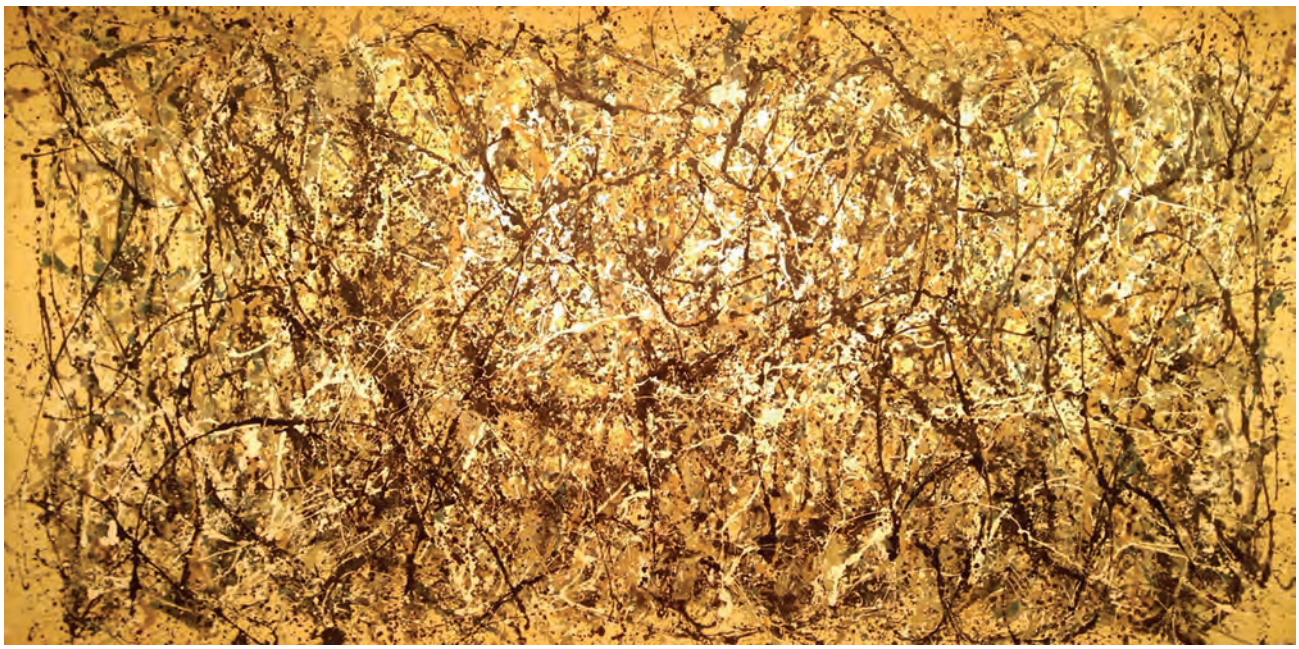

Figure 3. Photo courtesy of Kiersten Remster: Jackson Pollock *Number 31, 1950*. 1950. Museum of Modern Art.

7. Erving Goffman, The Presentations of Self in Everyday Life (New York: Random House, 1959).
8. Goffman, The Presentation of Self in Everyday Life.

left outside the art historical canon of remembered and recognizable artists identified with specific, finished works of art. In some cases, however, what remains in the historical record of these artists is ironically but tellingly the record of their provocations much more so than their actual works of art.

One such artist is Boris Lurie, whose works have never been entered into either the institutionalized realm of galleries and museums or the art historical literature. Put simply, Lurie has never had his rough edges of provocation sanded down or eliminated. A Russian-born Holocaust survivor, Lurie came to the United States in the late 1940s following the liberation of the Buchenwald concentration camp where both he and his father had been imprisoned. Arriving in New York City when Abstract Expressionism was the dominant art movement and Jackson Pollock (*Figure 3*) was the popular subject of conversation, Lurie was outraged. He was outraged less by the art itself than by the circumstances or promotion and patronage of that art that were variously tied to larger historical dynamics. In particular, he saw this art as representative of all that he perceived as wrong with America and how America was perpetuating imperialism and war that had brought about the very same problems that he had directly experienced during the Second World War.

As an act of resistance, Lurie started NO-art, a movement that commented on the social fabric and issues of society during the late 1950s. Lurie felt that the same atrocities that were being committed in the Korean War and, more broadly, the developing Cold War were repeats of the Holocaust. He intended his essentially outsider art to function as a voice of remembrance and resistance to what he saw as the faults of modern society. Lurie's art was not easy to look at, especially because it was so stridently confrontational. Indeed, his art was intentionally provocative, representing the "individual's nightmare of confronting the atrocities that we turn our heads away from and hide behind the collective façade of a prepackaged American dream."[9] His works were (and are) not aesthetically pleasing or pleasant to the eye, but rather they were made to provoke disturbances in his viewers as he cast a dark voice of social commentary outward to his anticipated audience. Lurie described his work as "pattern-breaking art" that is not new to the modern arts scene because confrontational art has been around "since the caveman and will continue to recur — and it will continue to be 'news' on each recurrence, for the reoccurrences are rare indeed and always violent but never capricious."[10] Lurie's art was disruptive and he wanted his creations to provoke a vivid response from the audience.

The critical aspect to understand about Lurie's work is that the ideas of art and creativity are not entirely embodied in his finished objects such as *Railroad to America*. Rather, the core of his art and creativity is in the act of provocation that antedates and is now hidden behind his conventionally "finished" works of art. As much as his finished work of art, Lurie's writings, experiences and thoughts on a dysfunctional society form the provocative gesture that commands attention and merits consideration as art historical artifact. For Lurie, his art was a strategy even before it became an object, with his objects being simply things, by-products of a creative process that begins and has much of its importance in the moment of provocation. As a result, the offensive and disruptive forms of Lurie's art was never welcomed through the doors of the established museums like the Whitney Museum of American Art or the Museum of Modern Art or writing about and contextualized in the standard discourses on the history of modern art.[11]

Boris Lurie serves as a case study for giving credence to and examining the larger phenomenon of the provocative in the history of modern art. His story also constitutes a warning and a remembrance that art history as traditionally conceived, presented and preserved hides and suppresses as much as it reveals and celebrates the significantly provocative moments in the history of modern art. It may just be that what is needed is an art history without objects, or an art history with objects temporarily held at bay, so that we might begin to see, appreciate and study as a subject in and of itself the full artistic process from provocation to object to audience reaction.

9. Museo Vostell Malpartida, Boris Lurie. (New York: Boris Lurie Art Foundation, 2014).
10. Boris Lurie, "Violence without Caprice in 'NO-Art,'" Leonardo 7, no. 4 (1974), doi: 10.2307/1573067.
11. Emanuel K. Schwartz and Reta Shacknove Schwartz, "NO-ART: An American Psycho-Social Phenomenon," Leonardo vol. 4 (1971), doi: 10.2307/1572298.

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An Investigation into Supercapacitor Design with Specific Focus on Energy Density

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Abstract

Supercapacitor technology has unique characteristics such as high power density and durability under a multitude of charge and discharge cycles. Applications of supercapacitors utilize these features to improve efficiency in electrical systems but such applications are limited by the low energy density of supercapacitors. Recent discoveries of advanced carbon materials have improved the energy density of supercapacitors, suggesting that further improvements in energy density can be made. This work reviews the current literature on supercapacitors and analyzes experimental data developed under this investigation to determine possible improvements. Specifically, the effects of salts on capacitance were investigated to determine the relationship between the charge carriers in the electrolyte and the overall capacitance. While the results indicate that such a relationship does exist, further investigation is necessary to delineate the root cause. Possible causes are discussed in the analysis section of this work.

Introduction

At this time fossil fuels remain the most energy dense natural energy storage material, an attribute that has led to their continued use over developing cleaner technologies. One clear example of this issue is apparent in the comparison of internal combustion engine vehicles versus electric vehicles. Electric vehicles are currently not capable of providing the same driving distance as internal combustion engines and require long charge times. These problems are a direct result of the intrinsic limitations of current battery technology, specifically Li-ion batteries. Supercapacitor technology could improve electric vehicles by providing a device for fast charging. Therefore, methods for improving the energy density of supercapacitors will be investigated to provide an energy storage solution.

Supercapacitors were first patented in 1957 by H. Becker but have seen many improvements since their introduction (Kötz and Carlen, 2000). For example, new materials in the form of graphene and activated carbons have demonstrated promising properties when applied to supercapacitors. These materials provide enormous surface area per unit volume, making them ideal electrodes for supercapacitors. In fact, using graphene electrodes and an ionic liquid electrolyte, researchers at Dalian University in China were able to create a supercapacitor with an energy density of 85.6 Wh/kg, a result comparable to the performance of nickel metal hydride batteries (Liu *et al.*, 2010). This achievement not only demonstrates the viability of supercapacitors but it also highlights a major challenge.

While being able to provide significant power density, supercapacitors struggle to provide the same energy density as batteries. In other words, supercapacitors can handle short bursts of power more effectively than batteries, but store less energy. These characteristics make supercapacitors ideal in applications like regenerative braking and managing load spikes in the electrical power grid. For example, a study at Clemson University determined that the use of supercapacitors in regenerative braking could improve the efficiency of a vehicle by 13.8 percent (Rotenburg *et al.*, 2011, p. 55). However, the limited energy density of supercapacitors is the primary justification for batteries being used as the preferred device for electrical energy storage. Despite current shortcomings like energy density, new research suggests that supercapacitors could provide an alternative to batteries for energy storage. An energy dense supercapacitor could theoretically offer the energy storage capabilities of a battery while providing the power density and durability of capacitors. This idea underlines the aim of this investigation, which is to explore new techniques for increasing the energy density of supercapacitors.

Supercapacitor Theory

Exploring supercapacitor theory not only helps to understand current supercapacitor technology, but it also offers insight into possible improvements to the design. As such, the first phase of this investigation involves examining the current design and theory of supercapacitors. Careful analysis of the current theory is conducted to reveal the underlying mechanisms at work.

From a macroscopic perspective, supercapacitors are very similar to batteries in that their components are arranged in a similar fashion. A supercapacitor consists of two electrodes with an electrolyte and separator placed between them. However, these components are most often made from different materials than what is found in batteries. For example, the electrodes are typically composed of carbon materials such as graphene or activated carbons. The electrolyte can take many different forms but usually falls into three categories based on the constitution of the solvent. These categories include aqueous electrolytes, organic electrolytes, and ionic liquids (Pandolfo *et al.*, 2013, p. 12). Each electrolyte has its advantages and disadvantages, but typically aqueous based electrolytes exhibit higher power while ionic liquids and organic solvents have higher operating voltages (Pandolfo *et al.*, 2013, ch. 2, p. 13-14). Electrolyte material choice is key in supercapacitor design because it is directly linked to the overall capacitance and operating voltage.

Factors like operating voltage and capacitance are important to consider when designing a supercapacitor because they are associated with the overall energy storage of the device. Equation (1) relates the energy stored (E) in a capacitor with the capacitance (C) and the operating voltage (V) and can be derived from fundamental relations (*Appendix 1*).

$$E = \frac{1}{2}CV^2 \tag{1}$$

It is evident in Equation (1) that in order to increase the energy stored on a capacitor, capacitance (C) and operating voltage (V) must be improved. These two variables, C and V, are the results of interactions of electrode and electrolyte on an extremely small scale. Thus, to improve the performance of a supercapacitor these nanoscale interactions attributed to energy storage must be explored.

Unlike batteries, supercapacitors do not primarily utilize oxidation-reduction reactions to store energy. Instead, supercapacitors store energy in what has been termed the "electric double layer" (Kötz and Carlen, 2000, p. 2484). Essentially this is the positioning of ions in the electrolyte along the surface of the electrode held in place by an electric field. As Dr. Gao reports, the electric double layer was first described by Hermann von Helmholtz in 1853 but has been revised in recent years (Gao, 2013, p. 12). Helmholtz described the separation of charges due to an electric potential resulting in charge buildup at the electrodes. The current model combines this idea with a diffuse layer of ions described by Gouy & Chapman, as communicated by Dr. Gao (Gao, 2013, p. 12). This combination is depicted in *Figure 1* where the Helmholtz layer (a) is combined with the diffuse layer in (b) to yield the Stern model of the electric double layer in (c) (Gao, 2013, p. 13).

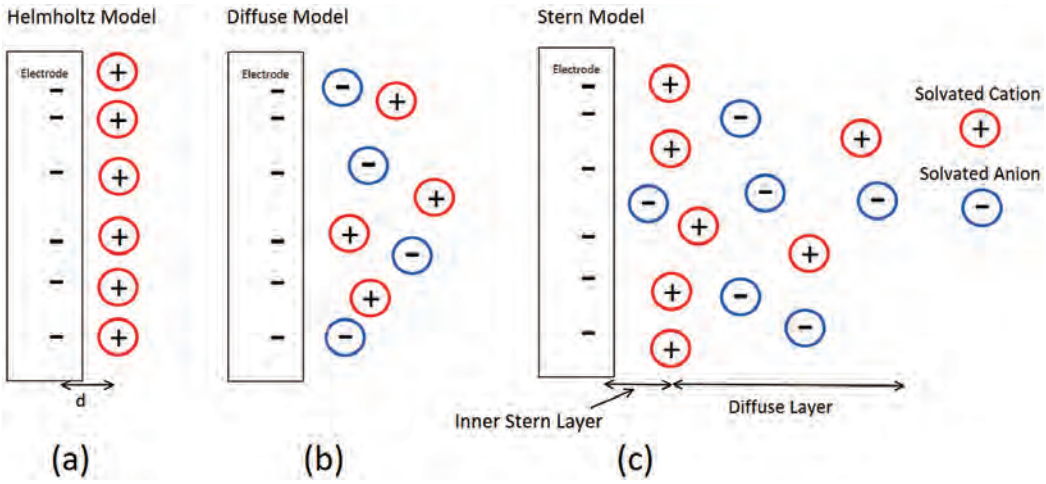

Figure 1. The components of the electric double layer described by Helmholtz (a), Gouy & Chapman (b), and Stern (c). (Gao, 2013, p. 13).

The electric double layer is the defining characteristic of supercapacitors as it is not found in conventional parallel plate capacitors. Despite this difference, the traditional equation for capacitance is still useful in understanding the superior performance of supercapacitors. In conventional capacitors, such as parallel plate capacitors, the total capacitance is given by Equation (2):

$$C = \frac{\varepsilon * A}{d} \qquad (2)$$

In Equation (2), A represents the total surface area of the electrodes, d is the distance between the plates, and ε represents the permittivity of the dielectric medium (Liao *et al.*, 2004, p. 7). Applying Equation (2) to the electric double layer reveals some key features that explain the higher capacitance achieved in supercapacitors. First, supercapacitors often use highly porous carbon materials as electrodes exhibiting a theoretical surface area greater than 1000 square meters per gram (Pandolfo *et al.*, 2013, p. 15). In addition to this feature, the distance between the charged ions and the electrode is an atomic distance, typically on the level of angstroms (Pandolfo et al, 2013, p. 7). When these values, the high surface area (A) and the small separation in the double layer (d), are inserted into Equation (2) the resulting capacitance is thousands of times greater than what can be achieved in parallel plate capacitors. For example, conventional capacitors typically exhibit capacitance in the range of 0.1 to 1000 µF while carbon based supercapacitors have a theoretical specific capacity of 62.9 F/g (Jayalaskshmi, 2008, p. 8).

It is clear that Equation (2) is useful in explaining important characteristics of supercapacitors that lead to higher capacitance. Yet it is important to note that Equation (2) is based on conventional parallel plate capacitors that do not possess liquid electrolytes. The absence of liquid electrolytes indicates that the electric double layer, the defining characteristic of supercapacitors, is not present in parallel plate capacitors. To apply Equation (2) more effectively, analysis must be performed on the derivation of the equation. The image presented in *Figure 2* portrays a model of a charged parallel plate capacitor and will be used in describing the derivation of Equation (2).

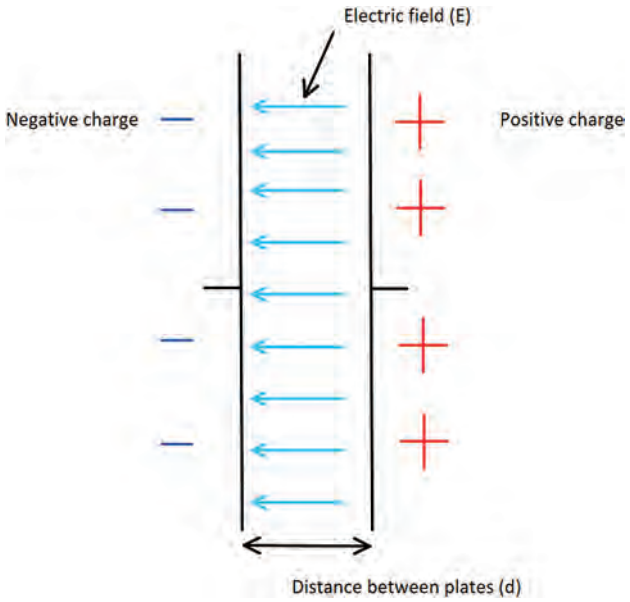

Figure 2. A parallel plate capacitor in the charged state.

Equation (2) can be derived using the following steps. This derivation is based on the works of Rod Nave of Georgia State University (Nave, 2015) and Sen-ben Liao of MIT (Liao *et al.*, 2004).

1. Begin with the definition of Capacitance, $C = \frac{Q}{V}$. This equation states that the capacitance is the amount of charge stored per volt.
2. Voltage can be broken down further. $V = \frac{J}{C} = \frac{work\ done}{charge} = \frac{F*d}{q} = Ed$. E represents the electric field between the plates and d is the distance between the plates.
3. Substituting this into the definition of capacitance yields: $C = \frac{Q}{V} = \frac{Q}{Ed}$. This indicates that the capacitance is related to the electric field generated between the plates.
4. The electric field inside the capacitor is simply the addition of the electric field generated by each plate. To determine the electric field generated by one of the plates Gauss's law must be applied.
5. Gauss's law states that the total electric flux out of a closed surface is equal to the charge enclosed divided by the permittivity. So $\varphi = E * A = \frac{Q}{\varepsilon_0}$ where φ is the electric flux, E is the electric field, A is the surface area of the enclosed shape, Q is the charge, and ε_0 is the permittivity of free space.
6. Assuming the plate to be an infinite sheet indicates that the electric field is perpendicular to the plate and the field generated at the edge of the plate can be ignored.
7. Enclose a section of the plate with surface area A in a cylinder. Each circle at the ends of the cylinder has the same surface area as the closed region (circle) of the plate. Since the electric field is perpendicular to the plate only the two circles at the ends of the cylinder must be considered for electric flux.
8. The total flux is therefore: $\varphi = E * 2A$ where A is the surface area of one of the ends of the cylinder (and also the surface area of the enclosed region of the plate).
9. Assume the plate to have a charge density: $\sigma = \frac{Q}{A}$ (Coulombs per square meter). The charge enclosed in the cylinder is therefore $Q = \sigma * A$ where A is the surface area of the enclosed region of the plate.
10. Substituting the equations from steps 8 and 9 into the equation from step 5 yields: $\varphi = E * 2A = \frac{\sigma * A}{\varepsilon_0}$. After simplifying the equation we are left with $E = \frac{\sigma}{2\varepsilon_0}$.
11. Assuming both plates are composed of the same material and have similar electrical properties, each plate can be considered to have the same charge density, σ. If both plates have the same charge density then the electric field generated by each plate is equivalent. This means that the electric field inside the capacitor is the electric field due to one plate multiplied by two. So the electric field inside the capacitor is $E = \frac{\sigma}{\varepsilon_0}$.
12. Substitute the equation from step 11 into the equation from step 3 yields: $C = \frac{Q}{V} = \frac{Q}{Ed} = \frac{Q\varepsilon}{\sigma d} = \frac{QA\varepsilon}{Qd} = \frac{\varepsilon A}{d}$. Thus equation 2 ($C = \frac{\varepsilon A}{d}$) is derived.

It is clear in the derivation of Equation (2) that many assumptions are made based on the symmetry of parallel plate capacitors. Since both electrodes are composed of the same material, they have similar electrical properties meaning the charge available on each plate is equivalent. With supercapacitors, more focus is placed on the relationship between the electrode and the electrolyte since these are the main components of the electric double layer. This comparison is presented in *Figure 3*.

In this comparison it is evident that the electric double layer doesn't exhibit the same symmetry as the parallel plate model. Instead of two charged plates the electric double layer is composed of dissociated ions in the electrolyte forming a layer at the electrode. This difference indicates that adjustments to Equation (2) may be necessary for an effective application of the equation.

One clear component of supercapacitors not accounted for in the equation is the existence of two double layers, associated with the two electrodes. It is apparent with the current

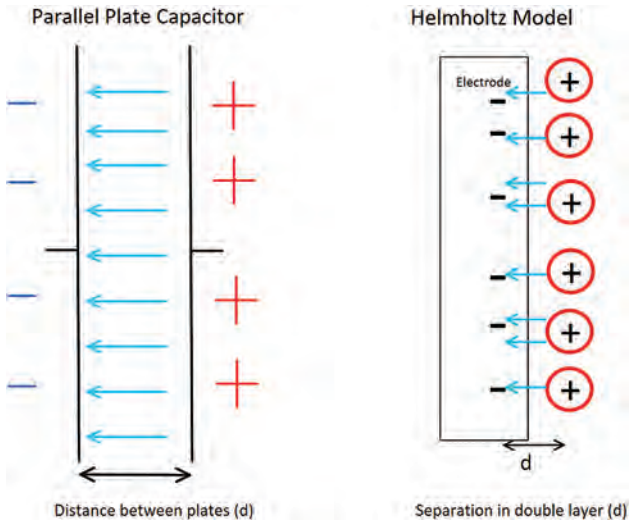

Figure 3. Comparison of a parallel plate capacitor and the electric double layer described by Helmholtz.

model that the double layer is forming on each electrode. Each double layer acts as a single capacitor meaning the overall capacitance is actually the result of two capacitors in series. Fortunately, this is easily accounted for by Equation (3) (Pandolfo *et al.*, 2013, p.13):

$$\frac{1}{C_{total}} = \frac{1}{C_{anode}} + \frac{1}{C_{cathode}} \qquad (3)$$

Besides highlighting the idea that two capacitors are present, Equation (3) also reveals that each electrode must be treated independently. Specific attention must be given to the components of the double layer at each electrode to understand the performance of the entire capacitor. As discussed earlier, the double layer consists of charged ions gathering at the surface of the electrode. This arrangement looks similar to a small parallel plate capacitor in that it features two charge carriers separated by a dielectric layer. However, in the electric double layer the charge carriers consist of the electrode and a collection of oppositely charged ions. This subtle difference reveals one of the main assumptions in the derivation of Equation (2). Since a parallel plate capacitor includes two electrodes of the same material, it can be assumed that each plate possesses the same charge density. In the electric double layer the two "plates", the electrode and the dissociated ions, possess different levels of available charge. To determine the charge density of the electrolyte Donne's model for charge density can be used (Donne, 2013, p. 22). The equation for this model is as follows:

$$\sigma = n^1z^1e + n^2z^2e + n^3z^3e + \cdots + n_iz_ie = \sum n_iz_ie \qquad (4)$$

In this equation n represents the number of ions, z represents the valence of each ion, and e represents the elementary charge value in coulombs ($1.602 * 10^{-19}$ Coulombs). Technically, it is difficult to know the exact value of n for each ion since the precise degree of dissociation is unknown. However, Equation (4) does suggest that the electrolyte charge density is limited by the number of ions present and the charge of each individual ion. Conversely, the charge available on the electrode is essentially very large when compared to the charge provided by electrolyte since charge can be placed on the electrode from an application of voltage by an external source. This is not to say that the charge on the electrode can reach astronomical values, rather it suggests that the limiting factor for the charge stored in the double layer is the charge density of the electrolyte.

The relationship between the electrode and electrolyte becomes clearer when analyzing the forces acting in the electric double layer. The electric double layer is held in place by the electrostatic force between opposite charges (Yu *et al.*, 2013, p. 3). This force can be modeled by Coulomb's law shown below:

$$F = \frac{kQ_1Q_2}{r^2} \qquad (5)$$

In Equation (5), Q_1 and Q_2 represent two charges acting on one another from a distance r while k is the electrostatic constant. For simplification purposes, analysis will be performed on a single ion in the electric double layer. Using this technique we assume that Q_1 is the charge of a single dissociated ion in the electrolyte. The value of this charge in coulombs would be the elementary charge multiplied by $1.602 * 10^{-19}$) (Yu *et al.*, 2013, p. 4). The value of Q_2 is the resulting charge on the electrode associated with the ion in the electrolyte. It is understood that "solvated ions in the electrolyte are attracted to the solid surface by an equal but opposite charge in the solid" (Miller *et al.*, 2008, par. 2). This indicates that the charge of Q_2 is equivalent to the charge of Q_1. Dr. Scott W. Donne, from the University of Newcastle, described this phenomenon as the "Image Force" (Donne, 2013, p. 47). According

to Dr. Donne, "The IF [Image Force] is the force between a charge and its induced charge. It is always attractive" (Donne, 2013, p. 47) (*Figure 4*). Dr. Donne describes this force using Equation (6):

$$IF = -\frac{(ze)^2}{4\pi\varepsilon\varepsilon_0(2x)^2} \quad (6)$$

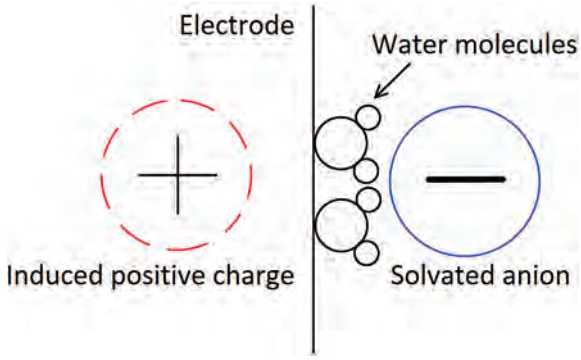

Figure 4. The "Image Force" as described by Dr. Donne. (Donne, 2013, p. 47).

With this equation z represents the valence charge of the ion, e is the elementary charge value in coulombs, and x is the distance between the ion and the electrode. Dr. Donne does not explicitly state how the equation was obtained. However, the many similarities between Equation (5) and Equation (6) suggest that this equation is a result of Coulomb's Law. The derivation of the "Image Force" using Coulomb's law is outlined below:

1. Begin with Coulomb's law: $F = \frac{kQ_1Q_2}{r^2}$
2. $k = \frac{1}{4\pi\epsilon\varepsilon_0}$ so $F = \frac{Q_1Q_2}{4\pi\epsilon\varepsilon_0 r^2}$ (k is Coulomb's constant)
3. Q_1 is the charge of the dissociated ion in Coulombs. To obtain this, multiply the valence z by the elementary charge value in Coulombs "e". So $Q_1 = ze$.
4. As discussed earlier, the charge of Q_1 and Q_2 are equivalent so $Q_1 = Q_2$.
5. This yields $F = \frac{(ze)^2}{4\pi\epsilon\varepsilon_0 r^2}$
6. The r value is the distance between the charges. If x is the distance from the ion to the electrode surface, then r = 2x.
7. Substituting yields $F = \frac{(ze)^2}{4\pi\epsilon\varepsilon_0(2x)^2}$
8. Since the charges are oppositely charged the force is attractive, so a negative sign must be added.
9. The resulting equation is:

$$F = -\frac{(ze)^2}{4\pi\epsilon\varepsilon_0(2x)^2} = IF$$

The "Image Force" equation is difficult to incorporate at the macroscopic level but it suggests that the strength of the electrostatic force is related to the charge of the ion and the distance between the charge and the electrode. As such, a smaller ion with higher charge should display higher capacitance because it can lead to a larger force. A stronger electrostatic force yields a more intense electric field, the main storage mechanism of a capacitor as described in the derivation of Equation (2) (Kötz and Carlen, 2000, p. 2492). To investigate the relationship between the charge of the dissociated ions and the overall capacitance an experiment was conducted using various salts in aqueous solutions. Based on the ideas discussed, the following hypothesis was made: Electrolytes with higher elementary charge shall exhibit higher capacitance.

Experimental Procedure

In most commercial supercapacitors the electrolyte of choice is usually a strong acid or base in an aqueous solution or an organic solution. However, in most cases the elementary charge of the dissociated ions is +1 and -1. In this experiment three salt solutions with increasing cationic charge were tested for capacitance and impedance using an impedance spectrometer. The resulting data were then analyzed to determine the relationship between elementary charge and capacitance.

The three salts selected for this experiment include sodium chloride, magnesium chloride, and ferric chloride (distilled water was also tested as a control). Each of these salts feature chlorine in an ionic bond with a cation of different theoretical charge. For example, a dissociated sodium cation possesses a +1 charge, a magnesium cation exhibits a +2 charge, and the iron cation has a +3 charge. This trend is also apparent in the number of chlorine atoms present in the chemical formula of each salt: $NaCl$, $MgCl_2$, $FeCl_3$.

Each salt solution was prepared in a .1 M solution of distilled water. A polyethylene separator was

soaked in each solution and placed between two stainless steel plates in a Scrosati cell. The cell was tested at three temperatures, -10° Celsius, 20° Celsius and 50° Celsius to monitor the effects of temperature on the capacitance. The test included the following steps:

1. Let cell stand at given temperature for 1 hour to ensure sample is at ambient temperature.
2. Charge the cell at a constant dV/dt (5 mV/second).
3. Hold the cell at 1 V for 5 minutes.
4. Discharge the cell at a constant dV/dt (5 mV/second) to monitor resulting current output.
5. Charge the cell at a constant dV/dt (5 mV/second).
6. Hold the cell at 1 V for 5 minutes.
7. Discharge the cell at a constant current (1 µA/second) to determine the charge stored on capacitor.
8. Steps 2-7 were then repeated 8 times to ensure the results were reproducible.
9. The cell was charged to 1 V and subjected to impedance measurements. The impedance measurement started at 1 MHz and ended at 25 Hz with an amplitude of 10 mV.
10. Steps 1-9 were performed at each temperature (-10° C, 20° C, 50° C).

The data from step seven was integrated to determine the overall charge stored. Using the definition of capacitance, the charge stored divided by one is the capacitance of the cell. Furthermore, data from the constant dV/dt discharge reveals the charge stored which can also be used to find the capacitance. The graph below summarizes the results using the constant current discharge method for computing capacitance.

The results from the experiment reveal two key findings. First, the capacitance of each electrolyte is temperature dependent. In *Figure 5* a trend is apparent where capacitance rises with increased temperature for each salt. The second finding becomes clear when analyzing the data recorded at each temperature in *Figures 6-8*. It is clear in each case that the solutions with higher elementary charge, magnesium chloride and ferric chloride, provided higher capacitance

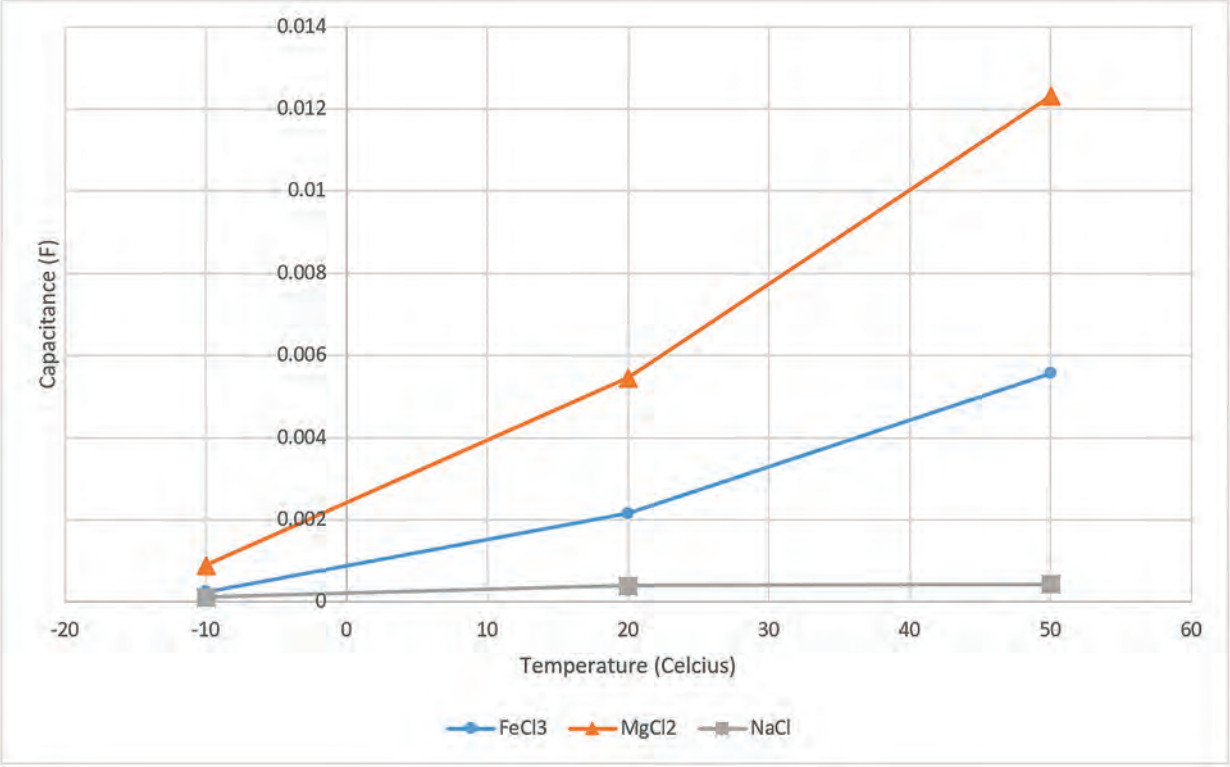

Figure 5. Average capacitance vs. temperature using constant current discharge method.

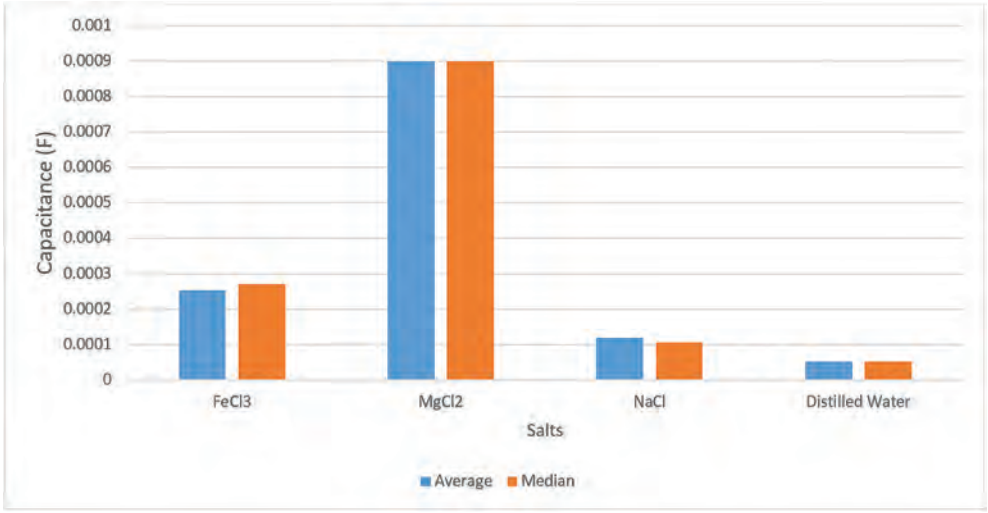

Figure 6. Capacitance results at -10 degrees Celsius.

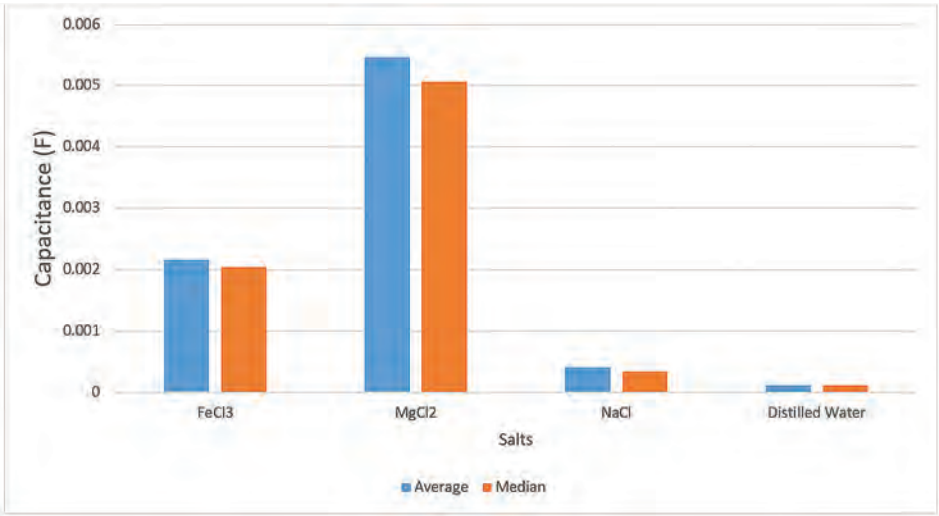

Figure 7. Capacitance results at 20 degrees Celsius.

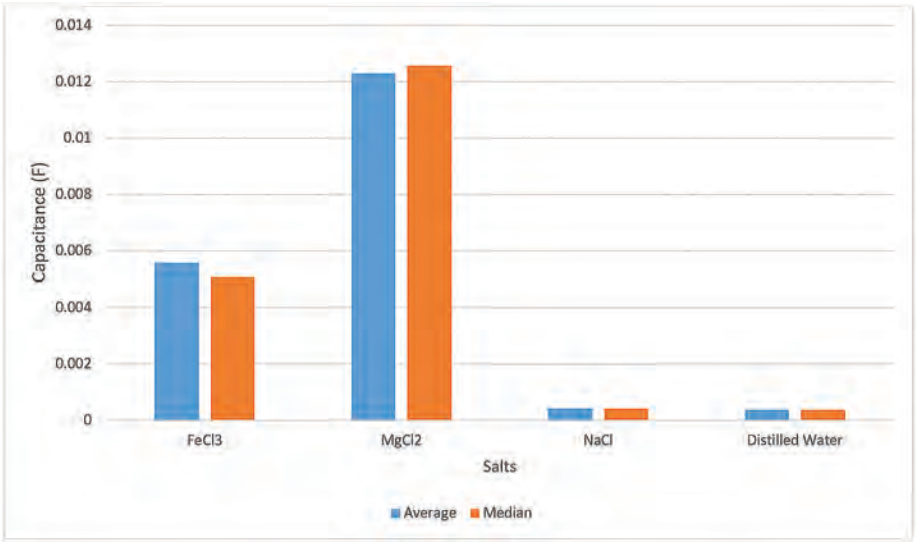

Figure 8. Capacitance results at 50 degrees Celsius.

than sodium chloride and distilled water. However, the samples did not completely behave as expected. Theoretically, if higher elementary charge leads to higher capacitance, ferric chloride should have provided the highest capacitance. Yet the results indicate that magnesium chloride exhibits the highest capacitance. This inconsistency with the original hypothesis may be due to several factors. To understand possible underlying causes the results must be analyzed and discussed.

Discussion

The experimental data reveal that there is a distinct difference in the behavior of the salts selected. Several factors may be considered to explain the behavior. For example, according to Equation (2), there are three characteristics that determine capacitance: surface area, dielectric constant, and the distance between plates.

Surface area can be ruled out as a factor since the same electrodes were used for each salt. The dielectric constant of each solution could be affected by the selected salt but experimental data typically shows a decrease in the dielectric constant of water when salt is added. In most cases "the static dielectric constant of a solution was observed to decrease with the salt concentration" (Gavish and Promislow, 2012, pg. 1). If the dielectric constant does in fact decrease with the addition of salts, then according to Equation (2) the overall capacitance should be decreasing when salts are added. Despite this argument suggesting that the dielectric constant is a nonfactor, it is difficult to completely rule out the dielectric effects of salts in aqueous solutions. Currently, there isn't enough data on the dielectric constants of magnesium chloride and ferric chloride in an aqueous solution to make a definite claim. Besides incomplete dielectric strength data, another issue is the manner in which current dielectric constants are obtained. Dielectric constants are measured using larger sample sizes that may not apply to supercapacitors. Since the interactions in a supercapacitor are on a nanoscale, the dielectric constant may be limited to a few molecules versus a large sample size. The relationship between dielectric strength and sample size is unknown for the tested solutions but it is understood that the dielectric constant of water decreases on a reduced scale (Gavish and Promislow, 2012, pg. 1). Since the electric double layer occurs on an atomic level the dielectric behavior of the electrolyte may be different than the currently available dielectric constants. Overall, the dielectric effects due to the addition of the selected salts are not the most likely reason for the behavior observed but additional testing is required before the role of the dielectric constant can be established.

The final variable in Equation (2) is the distance between the plates. While the distance between the electrodes is held constant by the separator, this distance is most likely not the distance in question. As discussed earlier, the distance described in the equation most likely applies to the width of the double layer. In other words, the distance between the dissolved ions and electrode surface. In this experiment the cations of the salts in solution have a different atomic radius which could lead to the observed behavior. Theoretically, the cations of each salt are progressively smaller when fully dissociated in solution (Imperial College London, 2015). This relationship could be described by *Figure 9*.

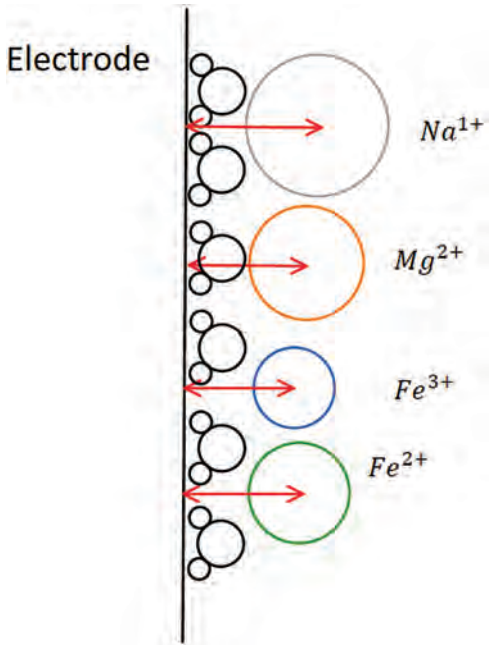

Figure 9. The smaller ionic radius of ferric chloride and magnesium chloride allow the ions to get closer to the electrode, depicted by the red arrows between the charge and the electrode. However, ferric chloride may not include fully dissociated Fe^{3+} cations leading to the larger Fe^{2+} ion.

As illustrated in *Figure 9*, the difference in ion size could allow the smaller ions of magnesium chloride and ferric chloride to position themselves closer to the electrode. Yet the data

shows that magnesium chloride performs the best even though it doesn't have the smallest ionic radius. One possible cause for the decreased performance of ferric chloride could be that the salt was not fully dissociated. If this was the case the dissolved ions would include larger ions instead of the smaller Fe^{3+} ion. The larger Fe^{2+} molecule depicted in *Figure 9* demonstrates how larger iron cations may be unable to position themselves as closely to the electrode. While this difference in ionic radius could be a factor in the observed results, further testing is required to reveal the true reason for magnesium chloride performing better than ferric chloride.

In the discussion leading to the experimental proposal it was suggested that Equation (2) may be incomplete in that elementary charge of the ion is not factored in. Using this logic, the induced force or image force would be greater with higher charge density in the electrolyte. This image force could help to describe the results seen in the experimentation. The data shows that magnesium chloride and ferric chloride, salts with higher elementary charge, perform better than sodium chloride. Theoretically, the fully dissociated cation of magnesium chloride is +2 and for ferric chloride it is +3. While this would explain why both salts performed better than sodium chloride, it does not account for magnesium chloride performing the best. If higher elementary charge led to higher capacitance, then ferric chloride should have performed the best. This inconsistency with the theory could be explained by the dissociation of ferric chloride, a factor that may be affecting the ionic radius as well. If the ferric chloride is not completely dissociating in the solution, then the resulting molecules would not feature a plus three charge. Instead the molecules might exhibit +1 or +2 charges, leading to a smaller induced force. If incomplete dissociation is indeed affecting the charge of the iron molecules then the data would still support the idea that higher charge leads to higher capacitance. The effect of the elementary charge on the overall capacitance should be further investigated with more samples to truly understand the relationship.

Conclusion

In this investigation aqueous salt solutions were tested for capacitance to determine a relationship between elementary charge and overall capacitance. The results indicated that such a relationship exists but a complete explanation for these results has not been determined. In summary, each salt sample demonstrated a temperature dependence with magnesium chloride performing the best at each temperature. The interesting results surrounding magnesium chloride suggest that further research should be conducted to better understand the capacitance of salt solutions. These properties could possibly increase the energy density of supercapacitors especially when paired with higher surface area electrodes.

Further Investigation

The experimental results presented in this report marks the first of a variety of tests that will be conducted to investigate improvements to supercapacitors. Elementary charge of the dissociated ions will continue to be investigated in further tests but other factors will need to be explored as well. For example, the solutions used in the experiment were all 0.1 M solutions. Other research, such as the paper written by Dr. Merrill, used higher concentrations with their salt solutions (Merrill *et al.*, 2014, pg. 1). In future tests, concentration will be investigated for its relationship to capacitance. Another factor that could lead to better performance is higher operating voltage. With this initial test, only aqueous solutions were used, thus limiting the operating voltage to around one volt. Subsequent tests will include other solvents with higher operating voltages such as organic solvents. Finally, it is well known that electrodes with higher surface area, such as activated carbons and graphene, exhibit higher capacitance. These materials shall also be investigated for capacitance and prefabrication methods. It is likely that one of these factors will lead to major improvements in supercapacitors. Still, the combination of improvements in each area of the design will lead to better overall performance.

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Appendix 1

Derivation of Energy Equation for a Capacitor

The following equations were used to compute the energy stored in a capacitor:

$$V_c = V_0 e^{\frac{-t}{RC}}$$

$$Q = CV_0 e^{\frac{-t}{RC}}$$

$$I = \frac{V_0}{R} e^{-\frac{t}{RC}}$$

Where Vc is the voltage of the capacitor, V_0 is the initial Voltage of the capacitor, I is the current due to the capacitor, Q is the charge on the capacitor, C is the capacitance of the capacitor, *R* is the resistance in the circuit, and t is the time in seconds.

To find the total energy release by the capacitor we can take the integral of the power function. To get the power due to the capacitor we simply multiply the voltage and current equation together. This yields:

$$Power\ (W) = \left(V_c = V_0 e^{\frac{-t}{RC}}\right) * \left(I = \frac{V_0}{R} e^{-\frac{t}{RC}}\right)$$

To get the total energy we simply integrate this function with respect to time:

$$Energy\ (E) = \int_0^\infty \left(V_0 e^{\frac{-t}{RC}}\right) * \left(\frac{V_0}{R} e^{-\frac{t}{RC}}\right) dt$$

$$E = \frac{{V_0}^2}{R} \int_0^\infty \left(e^{-\frac{2t}{RC}}\right) dt$$

$$Say\ u = -\frac{2t}{RC}, then\ du = -\frac{2}{RC}\ dt$$

$$E = \frac{-C{V_0}^2}{2} \int_0^\infty (e^u)\, du$$

$$E = \frac{-C{V_0}^2}{2} \left(e^{\frac{2(\infty)}{RC}} - e^{\frac{2(0)}{RC}}\right)$$

$$E = \frac{-C{V_0}^2}{2} (0 - 1)$$

$$E = \frac{C{V_0}^2}{2}$$

$$E = \frac{1}{2} CV^2$$

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The Promise of Justice: Are Public and Private Defense Equal?

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Abstract

This thesis will attempt to uncover whether or not differences in unclassified felony and first-degree felony case outcomes arise as a result of the type of counsel representing the defendant. The types of defense counsel addressed include public defenders, appointed counsel, and retained counsel. This research project specifically focuses on Butler, Clinton, Greene, Montgomery, Preble and Warren Counties, in order to cultivate large enough samples for statistical testing and to compare the systems of each county. The felony cases that will be examined include aggravated murder, murder, rape, voluntary manslaughter and kidnapping. Existing research (Cohen, 2014; Levine, 1975; Hartley, Miller, and Spohn, 2010; and Williams, 2002) largely shows that public defenders perform better than appointed counsel and roughly equal to retained counsel. In order to discover whether or not discrepancies between the three categories of defense attorneys exist in southwestern Ohio, county-level crime and court data encompassing type of counsel, charge(s) filed, and conviction and post-conviction outcomes including conviction, guilty plea, and incarceration rates and sentence lengths will be analyzed. In addition, select individuals that work in the criminal justice system, such as defense attorneys or clerks of court, will be interviewed or will complete a survey, in order to get a sense of how these dissimilarities actually manifest themselves and affect defendants beyond plea deals, trials and hearings.

Introduction

The question of whether defense counsel influences case outcomes has been studied repeatedly over the past sixty years, beginning in the aftermath of the *Gideon* decision (372 U.S. 335 (1963)). Because the decision intended to assure fair trials to all, any advantage or disadvantage that a defendant faces based on the type of counsel, whether said counsel is a public defender, appointed counsel, contract lawyer or retained counsel, is fundamentally a problem of legal process. Cases with the same characteristics should receive roughly the same outcomes, but, as detailed by Stephen Bright (1994), this is not always the case. In his study of defense counsel in capital cases, he discovered that criminal justice professionals in two states stated that cases where the defendant received the death penalty and cases where the defendant received a sentence of life in prison cannot be sorted out by sentence based on the facts of the case (Bright, 1994). Since the facts of the crimes and information about the offenders do not distinguish the cases, Bright (1994) posits that "abject poverty, debilitating mental impairments, minimal intelligence and poor legal representation" do. He explained stories of public defense lawyers who possessed inadequate experience, knowledge and resources to provide quality representation to the indigent defendants that they represented (Bright, 1994). Though these examples are from more than twenty years ago, whether public and private defense are equal in the present-day is still up for debate, as evidenced by the continued

inquiry on the subject (Anderson and Heaton, 2012; Beck and Shumsky, 1997; Cohen, 2014; Hanson and Ostrom, 1998; Hartley, Miller, and Spohn, 2010; Hoffman, Rubin, and Shepherd, 2006; Iyengar, 2007; Levine, 1975; Roach, 2014; Williams, 2002 and 2013; Willison, 1984). These studies have aided in shaping the research question at hand: are there differences in serious felony case outcomes based on type of defense counsel in southwestern Ohio?

Literature Review

Over the past six decades, many scholarly articles have been published, as the first titles published on this subject were written following the *Gideon* decision (372 U.S. 335 (1963)). The literature related to the present topic varies in terms of which types of counsel are compared, what geographical areas are studied and what kinds of data are utilized. Though the findings of each are varied, they still merit comparison. None of these articles capture the topic of this thesis exactly, as this thesis focuses specifically on serious felonies in southwestern Ohio. Some of the studies examined in this paper solely discuss felony cases; however, most of them discuss all types of felony cases. A few of the studies focus on smaller geographic areas comprised of less than five counties, but many cover larger areas than the six-county area that this thesis will analyze. These studies provide a stepping stone with which to begin inquiry on the subject, through qualitative and quantitative data analysis. The articles reviewed for this project have been divided into four subcategories, grouped by which kinds of defense counsel were studied, in order to more easily compare studies. Specifically, the studies have been broken down into these groups: those that compare all three types of counsel, those that compare public defenders to retained counsel, those that compare appointed counsel to retained counsel, and those that compare public defenders to appointed counsel. Though conclusions will be made within these four subcategories, those conclusions will then be compared to each other to show how all of the studies are connected. Note that the green equal sign in the following tables denotes a finding of no statistically significant difference, or that the outcome was equal regardless of attorney type, and that the red not equal sign denotes a finding of a statistically significant difference, or that the outcome was unequal based on attorney type.

First, consider the two studies that compared all types of lawyers, public defenders, appointed counsel, and retained counsel, to each other. Hanson and Ostrom (1998) and Cohen (2014) each compared and contrasted all three types of defense attorneys, but in different ways. While both examined felony cases, Hanson and Ostrom (1998) focused on "nine diverse courts" throughout the country that were handpicked to include courts with different types of indigent defense systems in both large and small communities for 1987, but Cohen (2014) focused on the largest counties in the United States by using the State Court Processing Statistics series for 2004 and 2006. These studies examined two of the same variables, conviction rates and incarceration rates, and they both found that indigent defenders are similar to privately retained defenders. However, these findings were not the same. Hanson and Ostrom (1998) discovered no significant difference in conviction rates for all types of attorneys, but Cohen (2014) found that assigned counsel's conviction rate was significantly higher than the rates for public defenders and retained counsel. Both found that clients of retained counsel are less likely to be sentenced to jail, but the statistical relationships differed between the two studies. In addition to this, Cohen (2014) studied incarceration lengths and found that, although sentence lengths between public defenders and retained counsel were not statistically different, clients with assigned counsel were statistically more likely to receive longer sentences than those with public defenders (*Figure 1*).

	Conviction Rate	Guilty Plea Rate	Incarceration Rate	Incarceration Length
Hanson and Ostrom (1998)	=	n/a	≠	n/a
Cohen (2014)	≠	n/a	=	≠

Figure 1. Findings of studies comparing all three types of counsel.

Next, five studies published between 1975 and 2013 discussed the similarities and differences between public defenders and retained counsel. Every study in this category focused on felony cases in different years from the 1970s until 2006 and they were all conducted in a specifically defined geographical area. Sources of data were varied. Levine (1975) used interviews with lawyers to gather his data, while the other researchers used county level data from various sources including, but not limited to, the Integrated Computerized Online Network (Hoffman *et al.*, 2006) and the State Court Processing Statistics series 1990-2006 (Williams, 2013). Hoffman *et al.* (2006) and Williams (2013) determined that there were differences in outcomes between the two types of counsel. Defendants represented by public defenders face more time in prison (Hoffman *et al.*, 2006), are more likely to be convicted and less likely to get their charges dismissed (Williams, 2013). Levine (1975), Hartley *et al.* (2010) and Williams (2002) all found that there were not statistically significant differences between public defenders and retained counsel (*Figure 2*).

	Conviction Rate	Guilty Plea Rate	Incarceration Rate	Incarceration Length
Levine (1975)	=	≠	n/a	n/a
Williams (2002)	n/a	n/a	=	=
Hoffman, Rubin, and Shepherd (2005)	=	n/a	n/a	≠
Hartley, Miller, and Spohn (2010)	n/a	n/a	=	=
Williams (2013)	≠	n/a	=	=

Figure 2. Findings of studies comparing public defenders to retained counsel.

Beck and Shumsky (1997) and Willison (1984) compared and contrasted retained counsel and appointed counsel. They went about their studies in dissimilar ways. Willison (1984) studied felonies and misdemeanors in one county during 1982, while Beck and Shumsky (1997) studied death-eligible cases in the entire state of Georgia from 1973 to 1978. Their conclusions were opposite, as Willison (1984) found that defendants represented by appointed counsel tend to face lighter sentences than defendants represented by private counsel, but Beck and Shumsky (1997) found that death sentences are more likely for defendants with appointed counsel (*Figure 3*).

	Conviction Rate	Guilty Plea Rate	Incarceration Rate	Incarceration Length
Willison (1984)	n/a	n/a	n/a	≠
Beck and Shumsky (1997)	n/a	n/a	≠	≠

Figure 3. Findings of studies comparing appointed counsel to retained counsel.

Lastly, three studies looked at public defenders and appointed counsel. Roach (2014) and Iyengar (2007) studied them on a national scale with data from the State Court Processing Statistics series 1990-2004 and from the Administrative Office of the U.S. Courts Criminal Docket from 1997-2002, respectively. Anderson and Heaton (2012) studied murder cases in Philadelphia from 1994-2005. They found that public defenders had lower conviction rates and shorter sentence lengths (Anderson and

Heaton, 2012). Roach (2014) exclusively studied felony cases at the state level, while Iyengar (2007) studied all criminal cases on the federal level. They both concluded that assigned counsel generates worse outcomes than public defenders in conviction rates and sentence lengths. In addition to this, both of these researchers explored the economic implications of attorney wages. Iyengar (2007) found that the closer appointed counsel's wages are to the market wage, the better they perform. Roach (2014) discovered that the outside option for both high and low quality appointed counsel can affect outcomes. Specifically, that if the outside option for high quality attorneys dips, or for low quality attorneys rises, appointed counsel will perform better than if it had stayed the same (*Figure 4*).

	Conviction Rate	Guilty Plea Rate	Incarceration Rate	Incarceration Length
Iyengar (2007)	≠	≠	n/a	≠
Anderson and Heaton (2012)	=	≠	n/a	≠
Roach (2014)	≠	n/a	n/a	≠

Figure 4. Findings of studies comparing public defenders to appointed counsel.

Patterns in the Literature

The general conclusion from these twelve studies is that the results are varied and inconsistent. Though only three concluded that indigent defense is not different from private defense, the remaining studies that demonstrate inconsistencies between the two do not show that all case outcomes are significantly different. While Beck and Shumsky (1997) showed that appointed counsel yielded worse outcomes than retained counsel, Hoffman *et al.* (2006) identified that appointed counsel was indistinguishable from retained counsel. Cohen (2014) demonstrated that appointed counsel yields the least favorable outcomes, which is consistent with the research of Iyengar (2007) and Roach (2014). Levine (1975), Williams (2002) and Hartley *et al.* (2010) found that public defense appeared to be adequate, while Williams (2013) found the opposite. Clearly, the results of research on this subject have evolved as the years have passed, but they still have not become consistent. Perhaps, over time and with the undertaking of more research on attorney types and their influences on case outcomes, the literature will begin to more clearly take a position as to whether or not attorney type matters. This thesis attempts to add to the existing research and influence that direction that the literature may take.

Methodology

This project utilizes a mixed methods approach. Data for this thesis will be gathered primarily from the available public records of Butler, Clinton, Greene, Montgomery, Preble and Warren Counties in southwestern Ohio. Data will be gathered on all cases for which an unclassified or first-degree felony was the original charge filed from 2000 through 2015 in these six counties. The information included will be attorney type, charge(s) filed, and conviction and post-conviction outcomes, including conviction rates, guilty plea rates, incarceration rates and incarceration lengths. The data will be analyzed to determine whether statistically significant differences between types of counsel exist in the sample counties from southwestern Ohio. Data will be collected from online public records, to the extent that it is available, and through contacting the clerks of court in each of the six counties. The analysis will reveal whether there are significant differences in each outcome of interest based on the type of defense counsel present for each case.

The secondary method of data collection is interviews with criminal justice professionals about the nature and style of criminal defense employed by each type of attorney. Specifically, comments from a public defender, a private lawyer who takes court appointments and a private lawyer who does not take court appointments will provide context as to how each attorney type generally approaches providing criminal defense services. Additionally, these interviews may highlight differences that may exist at the county-level as demonstrated by the data analysis, particularly because the system of indigent defense varies by county.

In order to conduct these interviews, an Institutional Review Board proposal will be submitted, detailing the sequence of interview questions and how the answers to those questions will be used in the final thesis manuscript. The interviews are intended to add color to the case data that will be presented, and they will look for professional thoughts and opinions about the different types of representation from defense attorneys. In conducting these interviews, comments from six to twelve defense attorneys would be ideal. These lawyers will be recruited in a variety of ways: through public defender staff lists located online; through utilizing the "attorney of record" section in the public records for the cases from recent years; and through snowball sampling, recommendations from attorneys at the end of interview regarding who should be recruited next. The interviews could be conducted in assorted ways, whether in person, over the phone, or through the internet. Every effort to accommodate the schedules of the attorneys that may participate will be taken in order to recruit enough participants. Before the participants begin to answer questions they will be shown or read out loud (over the phone interviews) a document regarding informed consent to ensure that they are aware of the following: how their responses will be used, how their information will be stored, and the fact that they can end the interview at any time.

Preliminary Conclusions

Although data has not yet been analyzed and the interviews have not yet been conducted, through considering the studies discussed in the literature review, a noticeable pattern that public defenders and retained counsel are more or less equal and that appointed counsel yields the least favorable outcomes appears. While this is based on studies that were conducted across the nation, a few of the studies contained data from Ohio counties.

Also, the data from the Ohio Office of the Public Defender on appointed counsel fees for felony cases from March 2013 through February 2014 shows that the average appointed lawyer made less than $50 an hour and less than $1,000 per case. Though this represents all felony cases, not just unclassified and first-degree felonies, the implications of financial incentives could arise. Furthermore, two counties use appointed counsel as the primary system for providing indigent defense, three counties use the public defender as the primary system and one county uses a mix of the two systems. This variance could impact the conclusions at the end of this study.

Overall, this first stage of research has provided insights on how studies in this field are conducted, the limitations of gathering data from public record and how indigent defense systems function. By reading the twelve empirical studies discussed in this literature review, the process of research in the criminal justice field as a whole and the field of indigent defense has become clearer. Reading about how other researchers designed and carried out their studies has given me a sense of the different kinds of research methods available and how they are used in reality. I've seen how multiple methods have been used in conjunction and how one method on its own can be used. As a result of this, I will be able to more intelligently design my study so it will be aligned with the accepted methods of the criminal justice field.

While learning how other researchers designed their studies, I also learned that some of them also experienced problems with gathering data from public record. Cohen (2014) and Hoffman *et al.* (2006) specifically mentioned having to exclude cases because of missing data, which showed me that even professional researchers cannot always collect data to the extent that they desire. Because of this, I will not be as discouraged when difficulties with gathering data occur in the future stages of my research.

Lastly, reviewing the existing literature has shown how indigent defense systems across the nation function. Many, if not all, of the articles detailed how the indigent defense system in question functioned, and I began to see similarities and differences between them. Specifically, in their study of nine diverse courts, Hanson and Ostrom (1998) explained each system that they studied, including the types of indigent defense system (public defender, assigned counsel, or contract attorneys) present in each court, level of funding (state or county), how indigency is determined, and the percentage of felony cases that are handled by indigent defenders. This study opened my eyes to the complexities of the criminal justice system and showed me that there are multiple ways to create a public defense delivery system.

Future Research

With this new knowledge in mind, this research will continue to be developed. In the fall semester, the Institutional Review Board proposal will be completed and submitted for review, and potential interviewees will be identified. In putting together the Institutional Review Board proposal, interview questions will be developed to create a complete, yet concise, interview to conduct with defense attorneys in later stages. Also, data will be collected, either from online public records or from clerks of court. Though online data from some counties is more readily available than from other counties, all clerks of court will be contacted either by phone call or letter in order to ensure that no relevant cases are left out.

In the spring semester, the gathering of case data will continue. Additionally, it will be coded for analysis after the best statistical tests are identified. This will require reading about what tests are typically used for this type of study and determining what level of significance should be used to determine whether or not there are statistically significant differences between attorney types. Interviews will be conducted during this time as well. The interview process may need to be stretched into the summer or fall, depending on availability and willingness of attorneys to participate. At the end of next summer, progress will be evaluated and the next steps for the final stage of research will be considered and defined, based on the amount of progress that has been made.

An Introduction to the Ohio Indigent Defense System

State Statutes

Indigent defense systems in Ohio function on two levels: state and county. At the statewide level is the Office of the Ohio Public Defender, which includes a nine member commission (Ohio Rev. Code § 120.01). This commission is comprised of appointments from the governor and the state supreme court (Ohio Rev. Code § 120.01). The governor appoints five of the nine members — the chairman, who must be an attorney; and four general members, two from each major political party; and at least two attorneys (Ohio Rev. Code § 120.01). The supreme court appoints the remaining four members, following the same criteria as the general members appointed by the governor. The duties of the commission include appointing the state public defender, establishing rules for the conduct of county public defenders and appointed counsel and establishing minimum qualifications of appointed counsel (Ohio Rev. Code § 120.03).

As outlined in Ohio Rev. Code § 120.04, the state public defender must be an attorney with at least four years of experience and at least one year of practice in Ohio. His or her duties include appointing assistant state public defenders, making sure that the guidelines set by the public defender commission are adhered to, establishing provisions related to the reimbursement of counties for public defense expenses and determining indigency (Ohio Rev. Code § 120.04, Ohio Rev. Code § 120.05).

At the county level, there may be a county public defender commission, but it is not a requirement (Ohio Rev. Code § 120.13). If the county commissioners decide to establish a county public defender commission, it is comprised of five members (Ohio Rev. Code § 120.13). Three of the members are

appointed by the board of commissioners, one of whom must be an attorney; the remaining two are appointed by the judge of the court of common pleas, one of whom must be an attorney (Ohio Rev. Code § 120.13). The duties of the county public defender commission are as follows: appoint the county public defender (unless the county contracts with the state public defender or a non-profit organization for all services), submit an annual report to the county commissioners and the Ohio public defender commission and maintain standards established by the Ohio public defender commission (Ohio Rev. Code § 120.14).

The person that the county public defender commission chooses to appoint as the county public defender must be an attorney with at least two years of experience and at least one year of practice in Ohio (Ohio Rev. Code § 120.15). His or her responsibilities include appointing assistant county public defenders and determining indigency of potential clients (Ohio Rev. Code § 120.15). In lieu of using a county public defender to provide defense for indigent defendants, appointed counsel can handle all the cases (Ohio Rev. Code § 120.16).

The state public defender, county public defender and appointed counsel can all provide representation to indigent clients. The state public defender can represent any indigent person who faces charges that carry consequences of the potential loss of liberty when requested by a county public defender or the court (Ohio Rev. Code § 120.06). The representation provided by a county public defender is more comprehensive than that provided by the state public defender. County public defenders can represent anyone that a state public defender can represent, at any stage of proceedings following arrest, detention, indictment, or service of summons (Ohio Rev. Code § 120.16). He or she may request that the state public defender represent an indigent defendant if the interests of justice so require (Ohio Rev. Code § 120.16). Finally, the county public defender, through the county commission, must submit his or her expenses to the state public defender within sixty days after the last day of the month in which the expense is incurred (Ohio Rev. Code § 120.18). Both types of public defenders can appoint counsel for the defendant or allow the defendant to choose his or her own personal counsel as co-counsel (Ohio Rev. Code § 120.06, 120.16).

When appointed counsel provides representation for the indigent defendant, the attorney can either be chosen by the defendant or appointed by the court (Ohio Rev. Code § 120.33). Regardless of whether counsel is appointed or chosen, he or she is paid according to the fee structure created by the county commissioners (Ohio Rev. Code § 120.33). In order to set the fee structure, the county commission shall request it from the bar association, after which it can be amended as the commissioners see fit (Ohio Rev. Code § 120.33). The exception to these two provisions occurs during capital cases, when appointed counsel is paid according to the fee schedule established by the supreme court that is approved by the county commissioners (Ohio Rev. Code § 120.33).

In order for the public defender to determine whether a defendant qualifies as indigent, the defendant must file an affidavit of indigency (Ohio Rev. Code § 120.36). There is a fee of $25 associated with the affidavit, but that fee can be waived by the court if the person cannot pay the fee or if the person would suffer undue hardship as a result of paying it (Ohio Rev. Code § 120.36). Though the court requests that this fee be paid, Ohio Rev. Code § 120.36 states that the inability, refusal, or failure to pay it cannot disqualify someone from receiving legal assistance.

Standards for Representation

There are various standards that defense counsel must meet when representing their clients which are spelled out in the Performance Standards for Criminal Defense Representation in Indigent Criminal Cases, a document published by the Office of the Ohio Public Defender. Though all standards are relevant, the standards that are especially pertinent to this thesis include general standards and standards related to investigation, plea negotiation, trial preparation and sentencing hearings.

The first chapter pertains to general standards. According to standard 1.1, defense attorneys should "provide zealous and effective representation for their clients at all stages of the criminal process," as this is their "most fundamental obligation" (Office of the Ohio Public Defender [OPD]). Defense lawyers that defend indigent clients need to have "sufficient experience or training," though the

standards do not explain what this means (OPD). Additionally, defense attorneys must know the substantive criminal laws and laws of procedure and how they are applied in Ohio (OPD). Finally, before an attorney agrees to represent an indigent client, he or she must make sure that he or she has enough "time, resources, knowledge, and experience" to effectively defend the potential client (OPD).

Chapter four explains standards related to investigation. First and foremost, a defense attorney has an obligation to promptly investigate each case that he or she takes on, including whether the charges are correct and whether the client knows about the ways his or her lawyer could present a defense (OPD). In addition to this, counsel should "secure the assistance of experts where it is necessary or appropriate" (OPD). Discovery is also included in this chapter; standard 4.2 states that defense counsel should seek discovery of potential exculpatory and mitigating information, names of witnesses for the prosecution and relevant information about them, and statements made by the accused, just to name a few things (OPD).

Chapter six focuses on the plea negotiation process. As provided by standard 6.1, defense counsel should talk with his or her client about the "possibility and desirability" of a plea bargain in lieu of going to trial. When considering a plea, counsel should know what kinds of pleas that can be entered, as well as their advantages and disadvantages and whether they are "binding on the court and prison and parole authorities" (OPD). According to standard 6.4, before a guilty plea is entered, the defense attorney must ensure that his or her client realizes the implications of said plea, and that he or she receives a description of the agreement, the rights he or she will waive, and the punishments that he or she could face as a result of the plea (OPD).

Chapter seven lays out the standards for trials, including preparation and jury selection. In standard 7.1, the guidelines state that the defense lawyer should have all relevant and necessary documents in his or her possession throughout the trial, and that he or she must consider how his or her actions will potentially affect the defendant at sentencing if he or she is deemed guilty (OPD). When it comes to voir dire, the defense attorney should "establish a relationship with the jury" and present his or her client in a light that enables the jurors to see him or her positively (OPD). Jury selection should be undertaken in a way that does not reveal information about the defense to the prosecution (OPD). Before the trial, the attorney should determine whether it is beneficial to cross-examine each witness and, if it is deemed beneficial, a plan should be created in regard to each witness that will be cross-examined (OPD). When developing a defense strategy, the defendant should be involved (OPD). Lastly, as standard 7.6 states, in the closing argument, the defense attorney should "consider highlighting weaknesses in the prosecution's case and describing favorable inferences to be drawn from the evidence" (OPD).

The last chapter that is relevant to this thesis is chapter eight, which discusses standards related to sentencing hearings. First, standard 8.1 states that whether the sentencing hearing is a result of a guilty plea or a guilty verdict at trial, all "reasonably available mitigating and favorable information, which is likely to benefit the client, is presented to the court" during the hearing (OPD). Counsel should know what the sentencing options are, as well as the consequences of those sentences (OPD). In preparation for the hearing, attorneys should let their clients know what the sentencing requirements are, if applicable, as well as the options and alternatives and their consequences (OPD). The client should also be aware of his or her right to speak at the hearing and the sentences that his or her attorney will ask the court to consider, as stated in standard 8.3 (OPD). Lastly, the attorney should fully advocate for the requested sentence and his or her client's interest and take the necessary steps to do so (OPD).

When reading these standards it is important to note that none of the points made are clear across the board requirements of counsel. As stated in the introduction, they are intended to be flexible, because every case is different (OPD). The words "should" and "shall" are used for the actions that are deemed "absolutely essential to providing quality representation" (OPD). However, these actions will not always be necessary, so acting on the standards that are phrased this way may still depend on the individual attorney's discretion (OPD). Additionally, the standards are intended to be comprehensive, but not exhaustive (OPD). The importance of the standards is to provide a measuring

tool for the performance of indigent defenders, whether they are public defenders, assistant public defenders, or appointed counsel (OPD).

Comparing Crime Rates

When comparing the level of criminal activity in Ohio to that of the region and that of the United States as a whole, the FBI's Uniform Crime Report (UCR) data is used. Though this data is collected from agencies so it can be compared across states, there are still problems with accuracy. First, not all crimes are reported to the police, and not all agencies report to the FBI; obviously, unreported crimes will not be factored in (Siegel & Worrall, 2015). Definitions of crimes differ between states, meaning that not every state will classify them in the same way as the FBI (Siegel & Worrall, 2015). Also, because of the hierarchy rule, only the most serious crime in an incident gets reported as part of the UCR, even if it is counted separately by the local police department (Siegel & Worrall, 2015). Therefore, when comparing the number of crimes committed in Ohio to the number of crimes committed in other states in the East North Central Region (as identified by the Crime in the United States 2013 report) or to the number of crimes committed in the United States as a whole, it must be known that since the UCR does not measure all criminal activity, the comparisons will not be completely accurate.

The crime statistics that will be compared are those of murder and rape, as these are the only crimes studied in this thesis that are reported as Part 1 crimes in the UCR. According to the *Crime in the United States* 2013 report, the murder rate in Ohio declined by five percent to 3.9 per 100,000 population (Office of Criminal Justice Services, 2014 [OCJS]). This rate is lower than the rate for both the East North Central Region (which includes Illinois, Indiana, Michigan, Ohio, and Wisconsin) and for the United States, which had rates of 4.9 and 4.5, respectively (OCJS, 2014). The rape rate in Ohio in 2013 was 34.9, lower than the East North Central Region (40.1) and similar to the United States (34.4) (OCJS, 2014). A change in rate was not reported because the FBI changed its definition of rape between 2012 and 2013, so comparisons in the statistics could not be made (OCJS, 2014).

The six counties that will be studied in this thesis can also be compared using UCR statistics collected in 2012. Montgomery County had the highest murder rate, at 6.1 per 100,000 population (OCJS). The counties with the lowest murder rate were Clinton and Warren, with a rate of 0 per 100,000 population (OCJS). Greene County (1.8), Butler County (2.7), and Preble County (5) fell in the middle of the spectrum (OCJS). The county with the highest rape rate was Butler County, with a rate of 51.4 per 100,000 population, and the county with the lowest rate was Clinton County, with a rate of 16.7 per 100,000 (citation). Warren County (17.3), Preble County (17.6), Greene County (31.3), and Montgomery County (47.3) fell in the middle of the spectrum (OCJS). Clearly, the crime rates of each individual county vary. However, the demographic characteristics of each county vary as well, from primarily rural (Clinton and Preble) to primarily urban (Montgomery), with Butler, Greene, and Warren being a mix of the two, which has an impact on crime rates.

All of these characteristics – both of the indigent defense systems working in Ohio and of the six counties that have been identified – will impact this thesis. The structure of the indigent defense systems will likely have the largest influence, as the system directly impacts how defense representation is rendered and how attorneys are able to defend their clients. The standards of representation set guidelines for what attorneys need to do in order for their representation to be considered "adequate," even though the guidelines do not always set clear requirements, and can sometimes depend on the situation and the attorney's professional discretion. The measures of criminal activity connect to this thesis, as do the geographic comparisons of the counties, because the types of crimes committed and the number of crimes committed will definitely impact the statistical analysis. The effects that each of these characteristics have on the thesis will be further examined once data is collected.

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An Analysis of the Educational Systems in Finland and the United States: A Case Study

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Abstract

International assessments have drawn attention to discrepancies in student achievement scores between countries. Due to the relatively new introduction of these tests, scholarly research on the subject has developed as the tests have produced more results. A shared goal of a majority of the research regarding international student achievement is to establish quality educational systems. As the United States continuously ranks at or slightly above or below average on the Programme for International Student Assessment (PISA) over the past twelve years, Finland's consistent success has drawn worldwide attention. For this reason, Finland is the educational system benchmark for this undergraduate thesis. The goal of this honors thesis is to identify specific approaches for improving the American educational system in order to provide every student with a quality education. This thesis reviews literature relating to comparative education, numerical data relating to the context (politically, demographically, and economically) and education structure in each country, and the respective educational programs under review in each country. The literature review addresses key components of success, which are further explored through data collection, including interviews, government documents, universities' curricula, and more. An analysis of the components of the two educational systems will lead the researcher to produce a guide identifying where and what to modify in the American programs based on key components of the Finnish programs.

Introduction of the Problem

There are a multitude of articles addressing the "Finnish Phenomenon;" this phenomenon is based on the scores from the Programme for International Student Assessment (PISA). PISA is a triennial international student achievement test first administered in 2000. The results were curious in that Finland preformed first in reading, fourth in science, and fifth in math, as shown in *Tables 1* and *2*. By 2003 Finland was either first or second in all categories. Finland's success was dubbed "the Finnish Phenomenon" due to mediocre performance on other international assessment prior to PISA. Across the Atlantic the United States had less impressive scores: in 2000 the U.S. ranked sixteenth in reading, twentieth in math, and fifteenth in science.

Table 1. Finland's 2000 and 2003 PISA Scores. Data from OECD and OECD/UNESCO Institute for Statistics, 2003.

		2000	2003
Finland	Reading	1	1
	Mathematics	5	2
	Science	4	1

Table 2. The United States' 2000 and 2003 PISA Scores. Data from OECD and OECD/UNESCO Institute for Statistics, 2003.

		2000	2003
United States of America	Reading	16	18
	Mathematics	20	28
	Science	15	22

The multiple readings that led the researcher to this phenomenon noted there were specific factors scholars have identified as contributing to Finland's success on PISA. Although there were many, some inside and some outside the school systems sphere of influence, the researcher noticed many scholars pointing to two programs in particular: part-time special education and teacher education. The researcher, from personal experience and knowledge of the United States' educational system, knew of American equivalents of these Finnish programs: Response to Intervention in the United States resembles part-time special education in Finland and teacher education is comparable in both countries. The researcher, at this time, formed the following research questions to create a comparative analysis of the educational systems in Finland and the United States:

1. How does teacher education in Finland and the United States compare?
2. How do the structure and process of implementation compare for Part-Time Special Education and Response to Intervention in Finland and the United States respectively?
3. How can the United States learn from the Finnish programs in terms of structure, program specifics, and implementation?

Review of the Literature

The review of the literature relates to components addressed in the comparative analysis (e.g. the Programme for International Student Assessment (PISA), country contexts, program overviews). The literature review begins with an overview of each country, Finland and the United States of America, to offer a historical and current perspective of national factors affecting education. Change in educational philosophy and practice often happens within economic, political, and social changes within the country and the world. The overview is followed by a summary of the literature published on each program which addresses the first and second research questions: Response to Intervention in the United States, Part-Time Special Education in Finland, and teacher education in the United States and Finland.

Overview of Finland and the United States

The educational systems of the United States and Finland lie in an economic, political, social, and structural context. The progression of educational change follows changes in other areas of American and Finnish life. Gaining their independence in 1776 and 1917, respectively, the United States of America and Finland shaped early national education to serve cultural purposes: one to form democratic citizens and the other to foster nationalism through a unified national language. Race, language, curriculum, and structural changes correlate with cultural, political, social, and economic changes in a county. Largely, education in Finland and the United States, and every other

country, has been and will continue to be used as a mode of transferring current cultural values and needs to the next generation. Even though this is a comparative analysis of similar programs in Finland and the United States, one cannot help but notice the contextual differences between the two countries, as shown in *Table 3*.

Table 3. Contextual and educational overview of the United States and Finland. Data from The World Bank, 2010-2014.

Category	United States of America	Finland
	Basic Information	
Independence	1776	1917
Total Area (sq. km)	9,147,420.0	303,900.0
Population	309,347,057.0	5,363,352.0
Government	Republic	Republic
Gross Domestic Product (USD)	14,964,372,000,000.0	247,814,569,536.4
Average Income Per Capita (USD)	48,374.1	46,205.2
Income Inequality (%)*	41.1	27.8
	Education	
Official Entrance to Primary Education (yrs.)	6	7
Teachers in Primary Education (Number)	1,794,812	24,736
Percentage of teachers in primary education who are female (%)	87	79
Annual statutory teacher starting salaries in primary public institutions (USD)	36,858	29,029
Government expenditure on education as % of GDP (%)	5	7

*World Bank defines Income Inequality: "GINI index measures the extent to which the distribution of income or consumption expenditure among individuals or households within an economy deviates from a perfectly equal distribution.

Response to Intervention and Part-Time Special Education

Wayne Sailor (2009) defines Response to Intervention: "Response to Intervention (RTI) is best understood as a model used to guide efforts to teach (intervention) based on measures of pupil progress (response) and grounded in the idea of prevention" (p. 3). Barbara Ehren *et al.* further note Response to Intervention is not concrete in that definitions, practices, and ideals are not uniform in every practicing school, district, or state. Even the definition of RTI is ambiguous in that the International Reading Association, the National Research Center on Learning Disabilities, the National Association of State Directors of Special Education, and the National Center for Learning Disabilities all have characteristically similar but differently worded definitions (Ehren *et al.*, 2009).

The Individuals with Disabilities Education Act, first enacted in 1997 and later reauthorized in 2004, used language that would allow educators to use scientifically-based methods of identification for special needs. Up until this point, the discrepancy model was the main mode of identification; it did not allow for intervention time before diagnosis, leading to a high number of students identified or misidentified with special needs or learning disabilities. IDEA gave funding and allowance for early intervention measures to prevent the need for special needs identification.

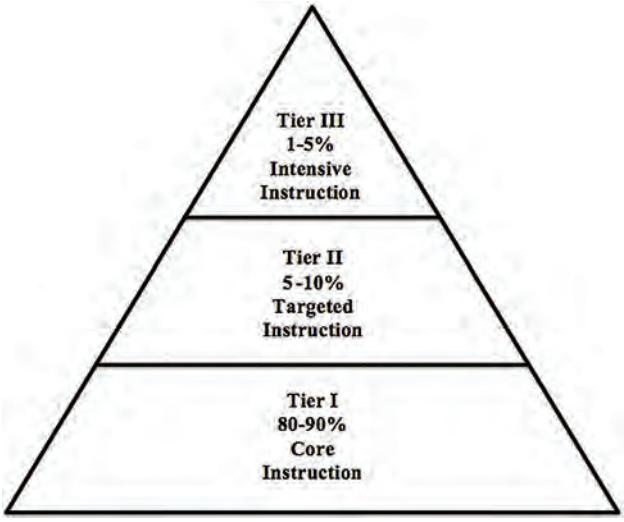

Figure 1. Response to Intervention. Adapted from Fuchs, Fuchs and Vaughn , 2008.

Fuchs, Fuchs and Vaughn (2008) state, "There are three-tiered RTI models and four-tiered models, and even with those models not everyone agrees as to what the different tiers may refer to" (p. 106). Divided usually into three tiers, each represents a level of intervention administered to a student, as shown in *Figure 1*. The base, Tier I, comprised of approximately 80% of the student population, receives minor intervention throughout the day. Students can receive this intervention as the teacher circulates the classroom throughout the lesson. Tier II, targeted support, is given to approximately 5% -10% of students. Students in this tier separate into groups of usually two to five for group instruction with a teacher (Mellard, McKnight, & Jordan, 2010). Tier III, controversial in definition, is considered either the last intensified intervention before identification or identification itself.

Students in the United States do not need an Individual Education Plan (IEP) to receive instruction under the RTI model. That is just it; students are receiving various intervention methods in RTI to delay identification with special needs or a learning disability. Douglas Fuchs, Lynn Fuchs and Sharon Vaughn argue an educator's philosophy regarding RTI can be understood through how many tiers they believe to comprise RTI: "Those who see RTI as mainly about disability identification want fewer tiers. Those who see RTI primarily in terms of early intervention and prevention want more tiers" (2008, p. 3).

The origins of part-time special education in Finland lie within two larger phenomenon: the Comprehensive School Act of 1968 when Finnish schools transitioned from a two-track system of separate elementary and middle schools to a nine-year comprehensive system (Kivirauma & Ruoho, 2007) and the Special Education Strategy adopted in 2010 (Thuneberg *et al.*, 2014). The new Comprehensive School Act created new heterogeneous classrooms of students who previously were separated into academic and vocational tracks. The heterogeneous classroom created a demand for extra support to keep the lower achieving students at the regular curriculum pace (Kivirauma & Ruoho, 2007). Over the years, the number of referrals to special education skyrocketed. Thus, a necessary reform to the special education system took place.

The new Special Education Strategy (SES) was officially adopted in 2010 but first talked about in 2007. Between those years, the structure of SES was established in the triangular form shown in *Figure 2*. General support takes place within the classroom where a teacher takes time to attend to individual student needs on the spot. In-depth intervention is not administered at this time, so if a student does not quickly respond to the teacher's intervention they are moved to the second category, intensified support. Sahlberg (2015) defines the practices of the second category by stating, "[it] consists of remedial support by the teacher, co-teaching with the special education teacher, and individual or small-group learning with a part-time special education teacher" (p. 66). When students still do not respond to intensified support, they are

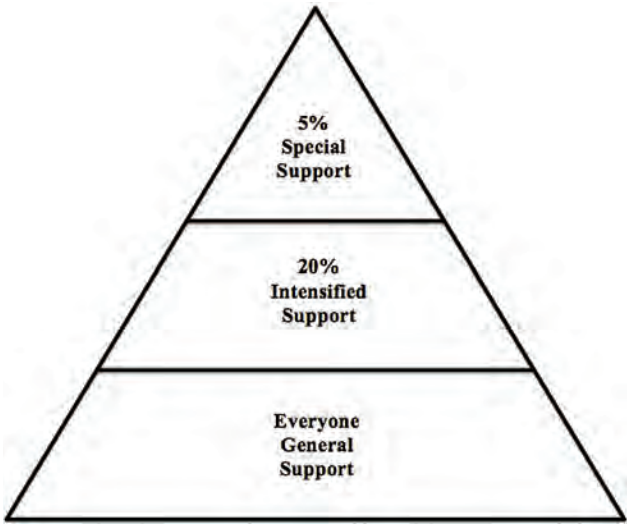

Figure 2. Special Education Strategy. Adapted from Thuneberg *et al.* 2013.

moved to special support, which varies between students. This category can look like inclusive intervention within the classroom or segregated intervention in a special needs room.

Recipients of part-time special education differ in that they do not need an IEP or an identified disability (Hausstätter & Takala, 2010); Takala *et al.* (2009) state, "[part-time special education students] do not have special education status; they are not considered disabled, but they are in need of short-term special education" (p. 162). For example, a student with low second-grade reading comprehension scores may not be autistic or dyslexic but only need one-on-one assistance to perform at grade level. This student would benefit in short-term special education for whatever amount of time it takes to get to second-grade reading level.

Teacher Education

Teacher education in the United States has been under scrutiny and reform for many years. It did not evolve until the beginning of the seventeenth century. Until then, religiously involved men or the few men educated at the collegiate level were drawn into the teaching force. Education, in an agrarian society that did not place much value on education, was available to the select few whose family valued and could afford it (Lucas, 1997). The establishment of teacher training colleges was heavily opposed in the beginning. Teaching was not a well-paid or respected profession, thus the dedication of time and money towards teacher training seemed illogical (Lucas, 1997). Training institutes occurring during the summers, although a popular method of further instruction, lasted only a short time until normal schools rose in the mid-eighteen hundreds (Lucas, 1997). Normal schools provided three to four yearlong curriculum-based teacher education (Lucas, 1997). Christopher Lucas believes that establishment of university-based teacher training that has its earliest foundations in the 1870s was due to the need to produce highly educated administrators and leaders for educational institutions.

The United States offers two paths for teacher education: university-based and practice-based. The Council for the Accreditation of Educator Preparation accredits a majority of the university-based teacher education programs in the United States. Teach for America and the Boston Teacher Residency are examples of practice-based options aside from a university. These programs offer teacher certification in short amounts of time whether one has or has not received prior teacher preparation. This thesis does not delve into the debate between the two teaching pathways, but looks solely at the prominently taken pathway in the United States: university-based teacher education.

One of the many criticisms of teacher education in the United States is the acceptance criteria into teacher training programs, specifically university-based teacher training programs. Christopher Lucas referred to the lax acceptance criteria, saying, "If the nation is to have first-rate teachers, it is said, colleges and universities offering preparatory programs must "tighten up" standards and make admission criteria more stringent. Only the brightest and best applicants should be accepted" (1997, p. 12). Although university-based teacher training is a generic method of training across the United States, the variety of program specifics is wide. Application criteria, levels for certification, endorsements offered, amount of practicum time, and more vary from one program to another.

Unlike the United States, many scholars have credited success on international student achievement tests to Finnish teacher education. The differences between each country's programs vary, as shown in *Table 4*. The Finnish educational system prides itself on the autonomy and respect it gives its teachers. As stated above, many scholars contribute this and Finland's success on international student achievement tests, to the structure of teacher education programs. Conjoining theory and practice in a research-based education program solidifies a never-ending professional attitude to discovery and learning.

A university-based teacher preparation program became the sole way of becoming certified for teaching in Finland in the 1970s (Sahlberg, 2015). A university-based teacher preparation program in Finland is at minimum five years in length: three years to acquire a bachelor's degree and two years for a master's degree, with a master's thesis (Sahlberg, 2015). The master's thesis contributes to many aspects of teacher development. Sahlberg (2007) believes that a master's thesis empowers

Table 4. Key Variations Between Finland and the United States' Teacher Education Programs.

United States of America	Finland
Application process varies	Two-fold application process
Selectivity varies by institution	Highly selective
Optional undergraduate thesis	Research-based teacher education
Practical experience in the classroom varies between programs in time and extent	Practical experience in the classroom is uniform between programs in time and extent

the educator to continue in various levels of the field of education other than teaching. This removes the constraints of immobility that can exhaust teachers within the first few years of teaching. A master's thesis also contributes to the notion of research-based education many Finns value as a strength in the teacher education program.

The application process to one of the eight government-funded Finnish teacher education programs at the university level is broken down into three stages: First, at the nation-wide level, students participate in an exam. The purpose of the exam, according to Silander and Valigarvi, is to measure candidate's ability to understand, analyze, and apply knowledge to educational practices (2013). Scores are distributed to universities where the second stage takes place. Unique to each university, interviews are conducted to demonstrate cooperation, creativity, and adaptability to the teaching profession. Stage three is a culmination of the candidate's academic and personal resúme, combining Matriculation Examination scores, stage one scores, upper-secondary school extra-curricular activities relating to education, and more (Sahlberg, 2015). It is at that point one is either accepted to or denied from a teacher preparation program.

Methodology

The following three research questions guide the research and methodology for this undergraduate thesis:

1. How does teacher education in Finland and the United States compare?
2. How do the structure and process of implementation compare for Part-Time Special Education and Response to Intervention in Finland and the United States respectively?
3. How can the United States learn from the Finnish programs in terms of structure, program specifics, and implementation?

Research Design

A qualitative case study was chosen as an appropriate design for multiple reasons. Part-time special education, Response to Intervention and both teacher education programs are bounded programs currently resting in and affected by the context of Finland's and the United States' educational systems as a whole. A case study is designed to allow in-depth descriptive discovery of influential factors. Merriam reiterates this design by stating, "a case study concentrates on many, if not all, the variables present in a single unit…it is impossible to identify all the important variables ahead of time" (1988, p. 7). From there, the researcher can use inductive reasoning to create a descriptive analysis based on a literature review and an interpretive analysis based on collected data (Merriam, 1988, p. 19).

The intended results of the thesis, a theory of translation for international educational practices and implementation methods, foster the use of qualitative data. Merriam defines the use for qualitative data to a case study by stating, "[it is] primarily concerned with process rather than outcomes or products" (1988, p. 19). This thesis is not looking at the outcomes produced by each program, but

rather, it is looking at the progression of implementation and modification. Since qualitative research rests heavily on interviews, the researcher becomes the vessel through which data is collected, sorted, and analyzed.

Participants

The researcher, through the Berry Summer Thesis Institute during the summer of 2015, was able to conduct six interviews of persons relating to Response to Intervention and comparative education. Participants include personnel at the school building, district, and national levels, creating a personal view of educator interaction with the program. The researcher will continue collecting data from interviews into the 2015-2016 academic year, and conduct interviews with United States and Finnish personnel in various positions of involvement relating to specified programs in the areas of: Response to Intervention, Part-Time Special Education, American teacher education, and Finnish teacher education.

Data Collection and Analysis

Data were pulled from two categories: interviews and primary source documents. Interviews included participants from varying categories, as listed above in the participant section. Each interview was recorded and transcribed by the researcher. Primary sources included documents such as district policy guides, universities' teacher education curriculum, government documents relating to programs, and more. The data will be coded for themes that will contribute to an understanding of key components of Finland's and the United States' programs under study in this thesis.

Conclusion

This undergraduate honors thesis began with a literature review directed by previous and current knowledge relating to comparative education, specifically relating to Finland's and the United States' results on international student achievement tests. The researcher completed a review of the literature comprised of scholarship and research relating to each country and educational programs within it. A contextual foundation of each country and its history creates a framework to understand historical educational changes. The researcher presented the three questions guiding this undergraduate thesis, creating a comparative educational analysis of specific programs within the Finland and United States' educational systems. The methodology for comparison is based on a qualitative case study that allows the researcher to identify factors that affect current educational programs. Primary sources comprise the data collection, ranging from interviews at various positions of involvement, government documents, curricula, and more. The researcher is looking for Finnish program specifics, such as indicators of duration, development, and key factors that can inform changes to existing similar programs in the American educational system. Finally, the researcher will continue into the 2015-2016 academic year to complete research; the end result aims at identifying, modifying, and producing a guide for key Finnish components necessary for inclusion to insure program success in the United States. The guide will identify where and what to modify in the American programs based on key components of the Finnish programs. For example, Finnish and American teacher education programs require a certain average amount of practical experience hours in the classroom. Because each country already has this similar practice, the guide will distinguish the average amount of hours in Finland programs, which could translate into existing American teacher education programs to replicate Finland's average amount of hours. A similar format will apply to other aspects of teacher education and the Response to Intervention and Part-Time Special Education comparison.

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Educating the Whole Child

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Abstract

Under-resourced children often experience multiple stressors in their everyday lives that can negatively impact their performance in schools. However, numerous research studies have shown that the most significant protective factor for under-resourced children is a caring, adult relationship. This study focuses on what needs to happen in the teacher-student relationship in order for the teacher to be that protective relationship. Building the teacher-student relationship aids all children, not just under-resourced children, in developing the academic, social and emotional skills necessary to be successful in the classroom. Development and presence of academic perseverance and an academic mind-set of students are the two central skills analyzed and integrated into this study to support teachers in better educating the whole child.

Introduction

While the United States has one of the strongest economies in the world, there is still a sizeable population of its youth that grows up in poverty. Growing up in poverty creates obstacles for development and thriving, which more affluent students often do not have to experience. Education is crucial in order to have a chance to escape the cycle of poverty that is so prominent in the United States. However, the pathway to a quality education is overshadowed by the end result alone: GPAs, test scores and national standards. A teacher can do so much more than merely give tests and grades. A student needs to have a reason to work for those test scores; a student needs to have someone to help them acquire the strategies necessary to get there. A student needs perseverance, grit, determination and tenacity. They need a mind-set that reinforces the belief that they are strong, capable and necessary. Teachers have the ability to aid students in cultivating those beliefs and attributes to help them find a reason to work toward the end goal of education and thrive as human beings. The purpose of this literature review is to present ways in which adults, particularly educators, can help under-resourced children succeed through the development of noncognitive skills fostered through relationships. Understanding how educators can lead under-resourced and impoverished youth to the path that leads to success is crucial for the future of our nation's youth.

Discussion

Poverty as it affects the outcome of children is nothing new. In 1964, President Lyndon Johnson proclaimed a War on Poverty with the intention to provide funding to programs that would work to alleviate and prevent poverty. Additionally, there was a push to provide people with quick access to jobs that required minimal skills (Babcock, 2014, p. 4). In 1968, the minimum wage was $1.60/hour, which is the equivalent of $10.56/hour today. "Because the minimum wage is not indexed, the

difference between the price-indexed level of $10.56 and actual level of $7.25 represents an erosion of more than 31 percent in the minimum wage" (Babcock, 2014, p. 4). The earnings from a full-time, low-wage job can no longer cover the average rent of a two bedroom apartment in the nation's lowest-cost urban neighborhoods (Children's Defense Fund, 2014).

Today, conditions are relatively unchanged; many families are living in poverty struggling to make ends meet. Among all age groups, children are the most likely to be poor. "Every fifth child (16.1 million) is poor, and every tenth child (7.1 million) is extremely poor. Children are the youngest age group and the younger they are the poorer they are" (Children's Defense Fund, 2014, p. 4). When a child is poor, that child is in the perfect place to continue the devastating cycle of generational poverty and to experience all the negative outcomes that come with poverty. For example, children of poverty are more likely to be homeless; "homeless children are more likely to go hungry, with one-third reporting that they skip meals; they are more than twice as likely as middle-class children to have moderate or severe and chronic health problems; and are more than twice as likely to repeat a grade in school, to be expelled or suspended, or to drop out of high school" (Children's Defense Fund, 2014, p. 28). Additionally, children of poverty experience a "lack of early school readiness; poor school performance and social and emotional development in middle childhood; lower grades, higher crime convictions and pregnancy in teen years; and lower college completion, earnings and independent household formation in young adulthood, which cumulatively severely impair the ability to attain economic independence in adulthood" (Babcock, 2014, p. 6). Poverty is affecting the way children develop, which in turn affects how they learn and prevents them from truly flourishing as individuals.

In addition to the many obstacles listed, one of the most significant obstacles to learning of children of poverty is what researchers call "toxic stress." The Crittenton Women's Union and Harvard's Center on the Developing Child have both included the brain science of poverty in their research, which is largely connected to education. The human body naturally reacts to stimuli that are considered "stressful" to the body, and this stress response is completely normal. The prefrontal cortex of the brain is the area of the brain associated with many of the analytic processes necessary to solve problems, set goals and effectively execute plans. This part of the brain receives signals on how to act from the limbic system, which triggers emotional reactions to environmental stimuli (Babcock, 2014, p.6). Additionally, the brain produces certain chemicals, such as adrenaline and cortisol that cause a physiological response to stress. However, the trouble occurs when these chemicals, specifically cortisol, are activated for extended periods of time. Babcock highlights the implication of this overreaction and says, "when the limbic brain is overactive and sending out too many powerful brain signals of desire, stress, or fear, the prefrontal brain can get swamped and the wave of emotion can drown out clear focus and judgment" (2014, p. 6). Taking in new learning material in class is an insurmountable task because the brain is unable to clearly focus on the task at hand. Harvard's Center on the Developing Child says, "if this so called 'toxic stress' continues and is not mitigated by adequate adult support, it can literally rewire children's brains, disrupting their social competence and ability to succeed in school and in life and increasing the likelihood of low educational achievement, unstable employment, adult poverty, and involvement in the criminal justice system" (2015, p. 2). For students in school, this hyper activation of the stress response can cause impulsive decision making and can cause a child to become overwhelmed and to shut down. As a result, these students struggle to find the energy to become interested in coursework (Duncan-Andrade, 2015). Stress for children of poverty is constant and has lasting effects in the classroom and later in life.

Entering school far less prepared than their more affluent peers is an additional obstacle to learning. It is important for children to have "serve and return" conversations, meaning the caregiver asks questions or begins conversations that call for a child to have a well thought out response (Babcock, 2014). This seems like a fairly simple task for a parent. However, low-income parents often spend more time away from their children because they are juggling multiple jobs, spending significant periods of time in transit, searching for secure housing or navigating complex public-assistance bureaucracies (Shanks & Robinson, 2011). Children whose parents are constantly working to make ends meet miss out on critical time with their parents which leaves them struggling as they enter school. "By the time a child in a very low income family reaches age 4, she will have heard only

two words for every seven that a child in a higher-income family has heard. By the time children in families with very low incomes enter kindergarten, they are 12 to 14 months behind in language and pre-reading skills, compared with children in higher-income families, where reading books and engaging in regular conversations with adults help build much larger vocabularies" (Hart & Risley, 2004). A child who is a low-income kindergartner is already starting behind, making him or her more likely to struggle in school, which leads to lower paying jobs and a higher likelihood of that child continuing the cycle of poverty.

Though the tremendous amount of adversity under-resourced youth experience is often overwhelming, there is research that heavily supports the definite necessity of relationships in the lives of youth. The Crittenton Women's Union, Harvard's Center on the Developing Child and multiple other individual researchers have shown that the most protective factor for a child to be resilient when facing adversity is a stable, caring, committed, adult relationship (Center on the Developing Child, 2015). All it takes is a single, strong and significant relationship in order to help a child of poverty overcome the multitude of hurdles standing in the way, including challenges presented by the home environment. Educators are the adults children see most often besides caregivers. Educators have the privilege and the power of potentially being that crucial relationship.

Though the research is clear that a strong, adult relationship is necessary, it is not always clear as to what is involved in that relationship. However, the Search Institute, an organization dedicated to learning what children need to succeed, is in the process of creating a framework for what is called a developmental relationship. A developmental relationship is a connection between a young person and an adult or peer, which "positively shapes the young person's identity and helps the young person develop a thriving mind-set" (Search Institute , 2015b, p.1) and aids young people in attaining the "psychological and social skills that are essential for success in education and in life" (Search Institute, 2015b, p.1). The Search Institute and a multitude of other researchers, say that these relationships are important because "the number and intensity of developmental relationships in young people's lives is linked to a range of positive educational outcomes… and can increase student engagement and improve academic motivation" (Search Institute, 2015a, p.1). This relationship framework is especially important for educators because the relationship described can greatly affect student learning and achievement. However, an important point to consider is that this relationship helps students attain "*psychological* and *social skills* that are *essential* for success in education and in life" (Search Institute, 2015, p.1). Teachers can provide exceedingly vital relationships not only because of the school content they teach, but also because they have the power to be the developmental relationship that encourages these crucial social and emotional skills for success. In fact, children who enter kindergarten with below-average language and cognitive skills, like many under-resourced youth do, are most likely to catch up only if they are physically healthy and have strong social and emotional skills (Vandivere, Pitzer, Halle, Hair, 2004). This is where teachers can help improve the lives of under-resourced youth. Though this is very powerful research, there are some flaws to this theory as well. Students are only with a teacher for one year before they move on to the next grade. In the context of education, there has not been specific or deliberate research in order to address how teachers can extend that relationship. With that in mind, it is important to note that research strongly indicates that students will work harder if they care about what they are doing and will work harder if they care about the person they are working for. This is why educators matter so much to under-resourced youth even in the single year they are with them in the classroom. Teachers have the power to help children see the importance of hard work, effort and school content so they can truly pursue a future where they can flourish; they have the privilege to nurture and form young minds to see failure as a tool for their success instead of an indication of their incompetence. In other words, teachers are in a unique position to aid students, especially under-resourced students, in developing what are called "noncognitive skills."

Research on noncognitive skills shows high-test scores and GPA are not what defines a student as successful or intelligent. In fact, a study done by Geiser and Sanelices (2007) at the University of California found that "high school grades were a stronger predictor of both college GPA and likelihood of college graduation than students' SAT scores, class rank and family background" (Farrington, *et al.*, 2012, p.4). This is true because grades "reveal qualities of motivation and perseverance as

well as the presence of good study habits and time management skills. It is these qualities, more so than content knowledge, that signal which students are likely to excel in their studies and persevere in their schooling" (Farrington, *et al.*, 2012, p.4). Whether a child has been labeled as "smart" by a standardized test or whether a child comes from an under-resourced, low socioeconomic situation has nothing to do with how successful this child will be. Benjamin Bloom, an educational researcher who studied high achievers, said that even by early adolescence future accomplishment cannot be predicted from current ability, and only continued motivation and commitment, along with their network of support took these achievers to the top (Dweck, 2012). Far more than results of previous tests or the situation a child comes from, what matters most for students are relationships nurturing skills such as motivation, perseverance, time management skills, study skills, mind-set, etc. These are the noncognitive skills necessary for a child that teachers can help cultivate in their students in order for children to succeed in school and in other important aspects of life.

Though there are countless skills labeled as "noncognitive skills" there are five that have been identified as being the most important for educational attainment. The University of Chicago Consortium on Chicago Research (CCSR) and the Raikes Foundation produced a critical literature review on the importance of certain noncognitive factors for academic success. The development of these skills is not just important for the success of children of poverty but can be useful for all students. CCSR's "five general categories of noncognitive factors related to academic performance: (1) academic behaviors, (2) academic perseverance, (3) academic mind-sets, (4) learning strategies and (5) social skills" (Farrington, *et al.*,2012, p.6).

FIGURE 2.1

A Hypothesized Model of How Five Noncognitive Factors Affect Academic Performance within a Classroom/ School and Larger Socio-Cultural Context

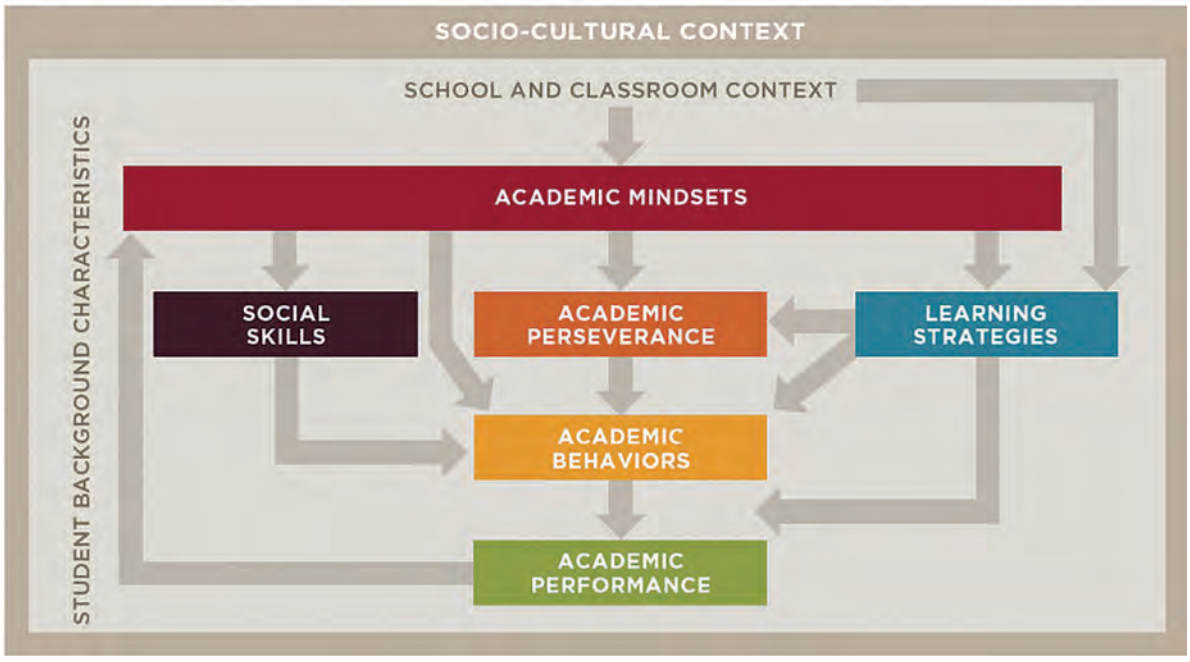

Figure 1. Farrington, C.A., Roderick, M. Allensworth, E. Nagoka, J., Keyes, T.S., Johnson, D.W., & Beechum, N.O. (2012). Teaching adolescents to become learners. The role of noncognitive factors in shaping school performance: A critical literature review. Chicago University of Chicago Consortium on Chicago School Research.

These are the skills that educators can help develop. CCSR created a flow chart (*Figure 1*) that illustrates how the five noncognitive skills work together and affect academic performance. The goal is the green box, strong "academic performance." Academic performance is measured by the yellow box, "academic behaviors" and quantities such as GPA and grades. Academic behaviors are

"those behaviors associated with being a 'good student'" (Farrington, *et al.*, 2012, p.8). For example, coming to class with proper materials, paying attention in class, studying, regular class attendance, etc., are strong academic behaviors. These are "visible, outward signs that a student is engaged and putting forth the effort to learn" and it is "how students develop and demonstrate their content knowledge and academic skills" (Farrington, *et al.*, 2012, p.15). The other four noncognitive factors all work together and lead to stronger academic behaviors, which in turn lead to stronger academic performance.

Again, all five noncognitive factors work together and are necessary in order to help students achieve higher academic goals. However, for the purpose of this study, the two noncognitive factors that will be focused on the most are "academic perseverance" and "academic mind-set" because of the strong mutually reinforcing relationship between the two. The factors of perseverance and mind-set have received strong evidence and support in research exploring their importance in the classroom (Farrington, *et al.*, 2012, p.15).

Academic perseverance is a "student's tendency to complete school assignments in a timely and thorough manner, to the best of one's ability, despite distractions, obstacles or level of challenge" (Farrington, *et al.*,2012, p.9). There are many different terms related to "academic perseverance" such as resiliency, delayed gratification, self-discipline, self-control, academic tenacity and grit. Angela Duckworth, a researcher at the University of Pennsylvania in the department of Psychology, has published information on "grit" widely accepted by the education community. Her research on grit and perseverance has been crucial for the understanding of the importance of perseverance when trying to accomplish goals, specifically in the classroom. She claims that grit is having the stamina to work hard, day in and day out, no matter what (Duckworth, 2013). It is working hard to achieve a long-term goal (Farrington, *et al.*, 2012, p.21). Grit is a skill necessary to complete work when it seems overwhelming or useless, it is a skill that athletes, academics, students and all people need in order to overcome obstacles. Grit keeps a person moving forward when instinct tells a person to stop.

In order for a student to be "gritty" in the classroom, they have to have the self-control to do so. Self-control is the student's ability to "avoid impulsive behavior and fulfill *short-term* obligations" (Farrington, *et al.*, 2012, p.21). If a child does homework instead of watching T.V. or studies instead of going out with friends, this child is exercising proper self-control. Grit is having the ability to exercise self-control every day, no matter the obstacles.

Duckworth claims grit is more important for success than a student's innate ability to succeed in school. In a recent TED talk, Duckworth talked extensively about how the educational community needs to understand learning from a motivational perspective. IQ, test scores and GPA are some of the skills schools know how to measure most accurately because academic indicators are easily measured. However, similar to the previous studies mentioned, Duckworth and her team spent time studying successful people and found that intelligence and IQ do not necessarily mean success. She said:

> My research team and I went to West Point Military Academy. We tried to predict which cadets would stay in military training and which would drop out. We went to the National Spelling Bee and tried to predict which children would advance farthest in competition. We studied rookie teachers working in really tough neighborhoods, asking which teachers are still going to be here in teaching by the end of the school year, and of those, who will be the most effective at improving learning outcomes for their students? We partnered with private companies, asking, which of these salespeople is going to keep their jobs? And who's going to earn the most money? In all those very different contexts, one characteristic emerged as a significant predictor of success. And it wasn't social intelligence. It wasn't good looks, physical health, and it wasn't IQ. It was grit (Duckworth 2013).

Duckworth's main argument is grit predicts success more than talent. Duckworth even went into schools and measured grit against characteristics such as family income, standardized achievement test scores and how safe kids felt when they were at school, and found grittier kids were significantly more likely to graduate. Being "gritty" is necessary to be successful at anything, including

school. So when it comes to academics, it seems that educators should not be focusing only on a child's intelligence and test scores. Nurturing students to become "gritty" is what is going to make them successful. However, the question is, "how do we create gritty students?" Duckworth says that the best idea she has heard so far in helping to developing gritty kids has come from the University of Stanford's Carol Dweck (2013) on the development of a "growth mind-set."

Academic mind-set is the second noncognitive skill related to academics, which is foundational for learning. Dweck has made incredible strides on changing the way educators, parents and athletes view the power of mind-set. In Dweck's book, *Mind-set: The New Psychology of Success*, she says a growth mind-set is "based on the belief that your basic qualities are things you can cultivate through your efforts…. A person's true potential is unknown (and unknowable); that it's impossible to foresee what can be accomplished with years of passion, toil and training" (2012, p. 7). Additionally, "the passion for stretching yourself and sticking to it, even (or especially) when it's not going well, is the hallmark of the growth mind-set" (2012, p.7). With this definition of mind-set, it is easy to understand how academic perseverance and academic mind-set have such a strong reciprocal relationship. For example, if a student keeps failing history tests, this student is not going to get better without gritting his/her teeth and continuing to work hard through the tough times. Moreover, the student is not going to power through the tough times if he/she does not have the proper mind-set to stay motivated in order to finish the class successfully. Mind-set and perseverance/grit are two crucial noncognitive skills that need one another. The mind-set a person adopts for themselves will greatly affect the way they lead their life and how they persevere (Dweck, 2012, p.6). The important question for educators now is, "how can I help cultivate a growth mind-set in my students?" Developing a growth mind-set is one of the most significant ways educators have been cultivating a positive academic mind-set in students.

In order to help create a growth mind-set in students, it seems that teachers almost need to adopt a growth mind-set themselves. Teachers need to believe that every student can do well. In her book Dweck talks about a teacher named Jaime Escalante who went into one of the worst high schools in Los Angeles and taught inner-city Hispanic students college-level calculus and eventually took them to the top of the national charts in math. Dweck said, "With his growth mind-set he asked, '*How* can I teach them?' not '*Can* I teach them?' and '*How* will they learn best?' not '*Can* they learn?'" (2012, p. 64). This teacher knew that these students were capable of more than for what they were given credit and he used that to help them succeed.

In addition to educators themselves having a growth mind-set, it is important to be aware of the mind-set teachers are promoting in students. The opposite of growth mind-set is a fixed mind-set which can be dangerous in the classroom. A person with a fixed mind-set is someone who believes his/her intelligence, traits and talents are "fixed" and nothing can change those innate talents. These people are afraid of challenges because they are afraid they will look bad if they fail (Dweck, 2012). Fear of being incorrect in a classroom seems as if it is a miniscule problem, but this fear of failure is strongly connected with a fixed mind-set that is incredibly powerful in preventing students from being "gritty" and pushing forward in their educational aspirations.

In order to properly display the power of mind-set and how it can be preventative or supportive to learning, Dweck did a study with 4-year-olds. She gave the student an easy jigsaw puzzle. After the students finished the given jigsaw puzzle, they had the option of trying a newer, harder jigsaw puzzle or the same jigsaw puzzle they had already completed. The children with the fixed mind-set chose the same jigsaw puzzle because they knew they could not fail while students with the growth mind-set chose the new jigsaw puzzle because it provided a challenge and an opportunity to learn (Dweck, 2012). People with a fixed mind-set see obstacles as indications of failure instead of opportunities to thrive. This mind-set can negatively influence the classroom. Fixed mind-set students may be afraid to answer questions in class because they do not want to be wrong. They also do not want to put in any effort because if a concept does not come naturally that must mean they are not smart enough to understand. The key for teachers in the classroom is to help students understand failure differently and to help them develop the kind of mind-set that enables them to thrive. Dweck has proven how this noncognitive skill is essential for not only academic success, but also the overall success of a child in fulfilling their potential.

One of the most significant ways teachers can influence mind-set is through the use of language, specifically how students are praised. Dweck states that educators need to praise effort and process, not outcomes or innate abilities. If a child grows up being told he/she is smart, there is a chance that child will not feel it is necessary to work hard because finding a task challenging is an insult to his/her intelligence. Dweck says adults should praise children for qualities they can control such as effort. Those praised for their innate brainpower might develop the sense that hard work is not necessary (2012). Expressing pride in a child for working hard will help that child much more than expressing pride in them for being smart. If students are praised for their hard work they are more likely to repeat that behavior but praising a child for how "smart" they are means they will work to maintain that image of smart. So, in the classroom it is important to be aware of not only the way failure is addressed, but also the way success is addressed. Educators can have a very significant influence on children and their mind-sets simply through the way a teacher praises a student.

Conclusion

Poverty has a significant negative impact on the development and academic success of a student; however, how teachers build relationships with those students can develop noncognitive skills that can be of monumental importance for their future ability to thrive. Teachers have a tremendous amount of influence on the lives of their students and are one of the most significant adult figures children have, especially when coming from a background of poverty. It is important for teachers of under-resourced youth to be aware of the influence they have so they can learn to provide necessary support, encouragement and motivation. Teachers have the power to be the relationship that helps equip students with the noncognitive tools they need in order to approach academic work with the mind-set associated with the determination to succeed and the drive to persist in times of frustration. Educators cannot merely teach content and believe that is a job well done. Teachers need to be a source of strength and understanding for students in addition to a source of knowledge. They need to focus not on a student's struggles and impossibilities, but rather focus on a student's potential, focus on developing the skills a child already has so they can eventually develop the skills they do not have. Simply creating a classroom environment that welcomes failure instead of condemns it, and celebrates improvements and hard work instead of the end results can make a substantial difference in how students approach academic work. Finally, under-resourced students need the type of teachers who focus not on developing content-based knowledge alone, but focus on the development of the human person as a whole; moreover, they need teachers who are under the permanent impression that every single child is capable of fulfilling their potential.

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