Mommy Fishues

A Thesis
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Bachelor of Arts

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Acknowledgements

> Any situation in which some individuals prevent others from engaging in the process of inquiry is one of violence.
>
> *Paulo Freire*
> *Pedagogy of the Oppressed*

I have been so very privileged to have the experience of attending college and writing a thesis. Any success I have had has been based in my access to extracurriculars, present parents, and supportive friends. I would like to thank:

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Preface

Science has a history as an oppressive institution. That being said, I think that science has the ability to liberate individuals through challenging the notion of self determination. I hope that this thesis is found to be accessible and at least makes one think of how plastic we are to our day-to-day experiences.

* The introductory information in this thesis is generalized. There are almost always exceptions in biology

List of Abbreviations

ACTH	Adrenocorticotropin hormone
CRH	Corticotropin-releasing hormone
GR	Glucocorticoid receptor
HLG	High licking & grooming
HPA	Hypothalamic-pituitary-adrenal
LLG	Low licking & grooming
PCR	Polymerase Chain Reaction
PVN	Pareventricular nucleus
qPCR	Quantitative Polymerase Chain Reaction

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Abstract

The preface pretty much says it all.

Dedication

And in our hearts
How beautiful the flames that will
flare up in a ring

Chika Sigawa
"Mountain Range"

For Langston.

Introduction

A defining feature of living organisms is that they are able to respond to stimuli in their environment. In other words, they behave. Each behavior requires an external stimulus, or multiple stimuli, that triggers a chain reaction of internal responses, changing how an organism exists in its environment. In understanding why an animal responds to a stimulus in the way it does, there are two places to start. One can look to the organism's genotype *i.e.*, was this behavior inherited genetically from its parents? Or one can look to the organism's upbringing *i.e.*, was this behavior learned in response to the enviornment, over the course. Traditionally, these two possibilities have been thought of as seperate and exclusive, as in the phrase *nature* vs *nurture*.

The dichotomy of nature and nurture as we know it today has its unfortunate beginnings in the field of eugenics. The phrase was popularized by the father of eugenics, Francis Galton, in the late 19th century in an effort to understand if human "ability" was heritable. He defined nature as "all that a man brings with himself into the world" and nurture as "every influence from without that affects him after his birth." (Galton, 1874) While there was not yet a concept of DNA, both Darwin's theory of evolution and Mendel's inheritence experiments were in circulation. The interest in nature *vs* nurture remained within developmental psychology until late in the 20th century when behavioral and developmental neurosciences were popularized.

In the early and mid 20th century, the fields of animal behavior and genetics were being revolutionized in ways that would ultimately contribute to the modern debate of nature and nurture (Krubitzer & Kahn, 2003). In the 1930's a pioneering behavioral scientist by the name of Nikolaas Tinbergen began studying behaviors holistically, as a product of individual experience and evolution. He was interested in creating a scientifically rigorous way by which to observe and comment on behavior. What emerged was the modern field of ethology and a set of four categories to study a behavior through: causation (mechanism), survival value (adaptation), ontogeny, and evolution (Tinbergen, 1963). Tinbergen's four questions were important in examining a single behavior as a product of an individual's experiences and that individual's lineage. That being said, there was still a gap in understanding how molecular biology contributed to all of this.

Abstract concepts of DNA and RNA as a heritable molecule had been proposed by the early 20th century in response to heritability studies (Koltzoff, 1934; Hershey & Chase, 1952), but it wasn't until Francis Crick and James Watson published a study in 1953 on the structure of DNA (notabley, the study relied heavily on prior work by Rosalind Franklin) that the field of modern genetics really began (Watson

& Crick, 1953). Using information about base pairs and amino acids published by other labs at the time, in 1955 Crick proposed the central dogma of genetics. This crucial concept states that DNA is translated into RNA, which is then transcribed into amino acids that are linked together to form proteins.

The last big step in getting to our current concept of nature and nurture was the popularization of epigenetics. Epigenetics, in short (this concept will be more deeply explored in Chapter 1), refers to the factors that change the ability of DNA to be transcribed, contributing to changes in gene expression. Much of modern behavioral sciences is aimed at understanding how the environment influences an organisms epigenome.

Because we now understand gene expression is often altered by the environment, our notion of nature *vs* nurture becomes rather arbitrary. Behaviors can instead be thought of as an intertwining of nature *and* nurture. Rather than understanding the ratio of environmental to genetic influence on a behavior, we can instead examine how certain genotypes make an organism more vulnerable to enviornmental influences or how the environment influences the quantity of a protein produced.

Put simply, this thesis will examine how maternal care (a stimulus) affects stress response (a behavior) in a mouth-brooding fish. I will begin by explaining epigenetics and physiological components of the stress response. Then I will give a brief summary of what is already known about maternal care's influence on behavior, before diving into the novel research I conducted. I will conclude with the implications of my study and future directions for this line of research.

Chapter 1

Stress, Epigentics, and Early-Life Experience

1.1 The Mechanisms of Stress

If you have made it this far in life, you have at some times felt *stressed*. Stress can be defined as the body's response to and recovery from a threat that disrupts homeostatis (Bodegom). We often times think about stress negatively; however our ability to respond to stressors is essential for our day-to-day lives. It is the exposure to chronic levels of stress which disrupts our ability to live well.

1.1.1 The Hypothalamic-Pituitary-Adrenal Axis

The stress response is made possible by the existence of the hypothalamic-pituitary-adrenal (HPA) axis. Activation of the axis by a stressor leads to a cascade of central and peripheral responses that help in directing the body towards allostasis.

The hypothamus is a region of the midbrain known for its role in maintaining allostasis through its involvement in stress, appetite, circadian rhythms, and sexual behavior. In response to stressors, the peraventricular nucleus (PVN) of the hypothalamus secretes corticotropin-releasing hormone [footnote on hormones] (CRH) and vasopressin.

The pituitary gland is directly ventral to the hypothalamus and is a main regulator of hormone release. It has notable roles in the stress response as well as in reproductive functions. The binding of vasopressin and CRH in the anterior pituitary leads to the secretion of adrenocorticotropin hormone (ACTH).

ACTH enters the blood stream and travels to the adrenal cortices, which are a region of the adrenal glands. Upon stimulation by ACTH, the adrenal glands secrete corticosteroids.

Corticosteroids are a class of steroid hormones that can bind to glucocorticoid receptors (GRs) and mineralocorticoid receptors. GRs are found throughout the brain, notabley within the hypothalamus and pituitary where they play a negative feedback role for the HPA axis. Additionally, GRs are found in prominant forebrain structures, such as the hippocampus and prefrontal cortex. The G-coupled protein

receptors, when activated, act as a transcription factor in the nuclear and mitochondrial genome, making them capabe of largely influencing the brain's epigenome (mito paper).

1.2 Chronic Stress

Chronic stress is associated with an extensive amount of psychological and immune diseases.

1.2.1 Epigenetic Changes from Stress

1.2.2 Pro-inflammatory Stress Response

1.2.3 Early Life Stress

1.3 Long Term Effects of Early Life Experience

1.3.1 Maternal Care and Stress

The lab of Michael Meaney has done years of groundbreaking work on how maternal care alters the stress response in rats. Rat mothers exhibit consistent differences in the time spent licking and grooming their young during their first week of life (Meaney, big paper citation 28). This difference takes place during a critical period of the rats neural development. As a result, pups reared by high licking and grooming (HLG) mothers and low licking and grooming (LLG) mothers have distinct phenotypes and epigenomes (Meaney epigenome).

In 1997, Meaney's lab examined how ciculating stress hormones differed in pups reared by HLG and LLG mothers. (Meaney, gluc). HLG pups had reduced circulating levels of ACTH and corticosterone in response to restraint stress. Additionally, HLG pups appeared to have enhanced regulatory feedback in stressful situations, as they surpressed ACTH to a greater extent after being pre-treated with corticosterone. HLG pups also developed lower GR expression in the hippocampus as adults. In a complementary study, the lab found a distinct behavioral phenotype between the two groups (behavioral). Rats reared by HLG dams exhibited more exploratory behavior, as measured by an open field paradigm, compared to those reared by LLG dams. Additionally, LLG pups exhibited a longer latency to start of eating when placed in a novel environment compared to HLG pups. One year later, Meaney cross-fostered pups from HLG and LLG mothers. As a result, pups born to LLG dams, but reared by HLG dams had a similar phenotype to those born to and reared by HLG dams (transgenerational). These findings indicated that maternal care can influence offsprings' responses to stress as adults.

In 2004, Meaney published a paper on the epigenetics of the above discoveries (Meaney epigenome). He found that the epigenetic state of the GR promoter gene was altered by maternal licking and grooming. This difference in methylation state

was contingent on the rearing, not the biological, mother.

1.3.2 The Gap in Our Knowledge

Chapter 2

The Experiment

2.1 Methods

2.1.1 The Fish

The parental generation of the focal juveniles originated from a wild-caught stock of *A. burtoni* collected from Lake Tanganyika. Social groups containing males and females of the same generation were kept in DIMENSIONS tanks at a temperature of TEMP and a pH of PH. Each tank's bottom was covered in gravel and terra cotta pot pieces were placed in the tank to act as shelters and territory markers. Females were monitored for mouth brooding behavior and were randomly assigned to an experimental condition. All females were collected within the first three days of brooding.

In the unseparated condition, mothers were removed from their home tank, weighed, and measured. They were then placed in small tanks containing gravel and a piece of terra cotta pot. Mothers continued to brood their young until the fry were old enough to regularly leave their mother's mouth, at which point the mother was removed from the tank to prevent her from eating the fry.

In the separated condition, mothers were weighed and measured and then the eggs were manually removed from their mouths by gently pulling down their bottom lips. The eggs were then placed in a flask within a tank containing gravel. Once the eggs developed into freely moving fry, the flask was removed from the tank and a piece of terra cotta was added.

Behavioral testing began approximately 130 days after the brooding mothers were placed into experimental conditions.

2.1.2 Behavioral Tests

Prior to behavioral testing, each brood was moved in their home cage to the testing area. They were allowed to adjust to the lighting for 10 minutes.

Boldness Assay

Boldness, or willingness to explore novel and open environments, is often used as a measure of stress. Animals that are stressed tend to freeze in place, seek cover, and

avoid open spaces. Focal fish were placed in a novel aquarium containing gravel and a terra cotta shelter. They were allowed to acclimate for 10 minutes before their behavior was scored for another 10 minutes. Boldness was measured on three axes: time spent in top half of tank, time spent frozen, and time spent under the shelter.

Aggression Assay
Because *A. burtoni* are highly social fish, we were interested in how maternal separation would affect their social behavior. Individual broods were transferred into a novel aquarium containing only water. The fish were allowed to acclimate to the new environment for 10 minutes before scoring began. The number of charges, bites, and chases between fish that occurred in 10 minutes were recorded and divided by the number of fish in the brood to create a score. Broods that only had one fish in them were excluded from this paradigm.

2.1.3 Gene Expression Assay

Directly following behavioral testing, fish were measured and quickly euthanized via decapitation. The brains were then extracted and placed into RNAlater, and stored at 4 °C. **Troubleshooting** Prior to working with experimental fish, an age-matched brood was used to troubleshoot behavioral testing. Following that, the brood was euthanized using MS-22 to practice brain extractions. Two of the extracted brains were used for gene expression troubleshooting. RNA was extracted from the brains and reverse transcribed into cDNA. All of the primer sets were tested using PCR on a gradient of melting temperature using this cDNA. The most effective melting temperature was then selected for qPCR.

Table 2.1: Genes and Corresponding Primers Used for qPCR

Target	Forward	Reverse	T_m
gr2	TGC CTC TGT CAC TGC CAC CGT AG	AGT CGT CTG CGT CTG AAG TAA CTG	60.9 °C
gr1a	TCA TAA GAT CTG TTT GGT GTG CTC	GTA GTT GTG CTG GCC TTC AAC	
gr1b	TGT TGG CTT CTC CGG TTC ATC AC	GTT GTG CTG GCC ATC TGT GTT T	60.9 °C
rfl23	TGC TGA TGC CCA ACA TCG GTT	TCT TGG AGG AGA CAT TGT GGG	55.7 °C

2.2 Results

Chapter 3

What Does this Mean?

Chapter 4

The Rest

4.1 References, Labels, Custom Commands and Footnotes

It is easy to refer to anything within your document using the `label` and `ref` tags. Labels must be unique and shouldn't use any odd characters; generally sticking to letters and numbers (no spaces) should be fine. Put the label on whatever you want to refer to, and put the reference where you want the reference. LaTeX will keep track of the chapter, section, and figure or table numbers for you.

4.1.1 References and Labels

Sometimes you'd like to refer to a table or figure, e.g. you can see in Figure 6.2 that you can rotate figures . Start by labeling your figure or table with the label command (`\label{labelvariable}`) below the caption (see the chapter on graphics and tables for examples). Then when you would like to refer to the table or figure, use the ref command (`\ref{labelvariable}`). Make sure your label variables are unique; you can't have two elements named "default." Also, since the reference command only puts the figure or table number, you will have to put "Table" or "Figure" as appropriate, as seen in the following examples:

As I showed in Table 6.1 many factors can be assumed to follow from inheritance. Also see the Figure 6.1 for an illustration.

4.1.2 Custom Commands

Are you sick of writing the same complex equation or phrase over and over?

The custom commands should be placed in the preamble, or at least prior to the first usage of the command. The structure of the `\newcommand` consists of the name of the new command in curly braces, the number of arguments to be made in square brackets and then, inside a new set of curly braces, the command(s) that make up the new command. The whole thing is sandwiched inside a larger set of curly braces.

In other words, if you want to make a shorthand for H_2SO_4, which doesn't include

an argument, you would write: `\newcommand{\hydro}{H$_2$SO$_4$}` and then when you needed to use the command you would type `\hydro`. (sans verb and the equals sign brackets, if you're looking at the .tex version). For example: H_2SO_4

4.1.3 Footnotes and Endnotes

You might want to footnote something.[1] Be sure to leave no spaces between the word immediately preceding the footnote command and the command itself. The footnote will be in a smaller font and placed appropriately. Endnotes work in much the same way. More information can be found about both on the CUS site.

4.2 Bibliographies

Of course you will need to cite things, and you will probably accumulate an armful of sources. This is why BibTeX was created. For more information about BibTeX and bibliographies, see our CUS site (`web.reed.edu/cis/help/latex/index.html`)[2]. There are three pages on this topic: *bibtex* (which talks about using BibTeX, at `/latex/bibtex.html`), *bibtexstyles* (about how to find and use the bibliography style that best suits your needs, at `/latex/bibtexstyles.html`) and *bibman* (which covers how to make and maintain a bibliography by hand, without BibTeX, at at `/latex/bibman.html`). The last page will not be useful unless you have only a few sources. There used to be APA stuff here, but we don't need it since I've fixed this with my apa-good natbib style file.

4.2.1 Tips for Bibliographies

1. Like with thesis formatting, the sooner you start compiling your bibliography for something as large as thesis, the better. Typing in source after source is mind-numbing enough; do you really want to do it for hours on end in late April? Think of it as procrastination.

2. The cite key (a citation's label) needs to be unique from the other entries.

3. When you have more than one author or editor, you need to separate each author's name by the word "and" e.g.
   `Author = {Noble, Sam and Youngberg, Jessica},`.

4. Bibliographies made using BibTeX (whether manually or using a manager) accept LaTeX markup, so you can italicize and add symbols as necessary.

5. To force capitalization in an article title or where all lowercase is generally used, bracket the capital letter in curly braces.

[1] footnote text
[2] Reed College (2007)

6. You can add a Reed Thesis citation[3] option. The best way to do this is to use the phdthesis type of citation, and use the optional "type" field to enter "Reed thesis" or "Undergraduate thesis". Here's a test of Chicago, showing the second cite in a row[4] being different. Also the second time not in a row[5] should be different. Of course in other styles they'll all look the same.

4.3 Anything else?

If you'd like to see examples of other things in this template, please contact CUS (email cus@reed.edu) with your suggestions. We love to see people using LaTeX for their theses, and are happy to help.

[3]Noble (2002)
[4]Noble (2002)
[5]Reed College (2007)

Chapter 5

Mathematics and Science

5.1 Math

TEX is the best way to typeset mathematics. Donald Knuth designed TEX when he got frustrated at how long it was taking the typesetters to finish his book, which contained a lot of mathematics.

If you are doing a thesis that will involve lots of math, you will want to read the following section which has been commented out. If you're not going to use math, skip over this next big red section. (It's red in the .tex file but does not show up in the .pdf.)

5.2 Chemistry 101: Symbols

Chemical formulas will look best if they are not italicized. Get around math mode's automatic italicizing by using the argument `$\mathrm{formula here}$`, with your formula inside the curly brackets.

So, $Fe_2^{2+}Cr_2O_4$ is written `$\mathrm{Fe_2^{2+}Cr_2O_4}$`
Exponent or Superscript: O^-
Subscript: CH_4

To stack numbers or letters as in Fe_2^{2+}, the subscript is defined first, and then the superscript is defined.
Angstrom: Å
Bullet: $CuCl \bullet 7H_2O$
Double Dagger: ‡
Delta: Δ
Reaction Arrows: $\longrightarrow$ or $\xrightarrow{solution}$
Resonance Arrows: $\leftrightarrow$
Reversible Reaction Arrows: $\rightleftharpoons$ or $\xrightleftharpoons{solution}$ (the latter requires the chemarr package)

5.2.1 Typesetting reactions

You may wish to put your reaction in a figure environment, which means that LaTeX will place the reaction where it fits and you can have a figure legend if desired:

$$C_6H_{12}O_6 + 6O_2 \longrightarrow 6CO_2 + 6H_2O$$

Figure 5.1: Combustion of glucose

5.2.2 Other examples of reactions

$NH_4Cl_{(s)} \rightleftharpoons NH_{3(g)} + HCl_{(g)}$
$MeCH_2Br + Mg \xrightarrow[below]{above} MeCH_2 \bullet Mg \bullet Br$

5.3 Physics

Many of the symbols you will need can be found on the math page (`http://web.reed.edu/cis/help/latex/math.html`) and the Comprehensive LaTeX Symbol Guide (enclosed in this template download). You may wish to create custom commands for commonly used symbols, phrases or equations, as described in Chapter 4.1.2.

5.4 Biology

You will probably find the resources at `http://www.lecb.ncifcrf.gov/~toms/latex.html` helpful, particularly the links to bsts for various journals. You may also be interested in TeXShade for nucleotide typesetting (`http://homepages.uni-tuebingen.de/beitz/txe.html`). Be sure to read the proceeding chapter on graphics and tables, and remember that the thesis template has versions of Ecology and Science bsts which support webpage citation formats.

Chapter 6

Tables and Graphics

6.1 Tables

The following section contains examples of tables, most of which have been commented out for brevity. (They will show up in the .tex document in red, but not at all in the .pdf). For more help in constructing a table (or anything else in this document), please see the LaTeX pages on the CUS site.

Table 6.1: Correlation of Inheritance Factors between Parents and Child

Factors	Correlation between Parents & Child	Inherited
Education	-0.49	Yes
Socio-Economic Status	0.28	Slight
Income	0.08	No
Family Size	0.19	Slight
Occupational Prestige	0.21	Slight

If you want to make a table that is longer than a page, you will want to use the longtable environment. Uncomment the table below to see an example, or see our online documentation.

Table 6.2: Chromium Hexacarbonyl Data Collected in 1998–1999

Chromium Hexacarbonyl			
State	Laser wavelength	Buffer gas	Ratio of $\frac{\text{Intensity at vapor pressure}}{\text{Intensity at 240 Torr}}$
$z^7P_4^\circ$	266 nm	Argon	1.5
$z^7P_2^\circ$	355 nm	Argon	0.57
$y^7P_3^\circ$	266 nm	Argon	1
$y^7P_3^\circ$	355 nm	Argon	0.14
$y^7P_2^\circ$	355 nm	Argon	0.14
$z^5P_3^\circ$	266 nm	Argon	1.2
$z^5P_3^\circ$	355 nm	Argon	0.04
$z^5P_3^\circ$	355 nm	Helium	0.02
$z^5P_2^\circ$	355 nm	Argon	0.07
$z^5P_1^\circ$	355 nm	Argon	0.05
$y^5P_3^\circ$	355 nm	Argon	0.05, 0.4
$y^5P_3^\circ$	355 nm	Helium	0.25
$z^5F_4^\circ$	266 nm	Argon	1.4
$z^5F_4^\circ$	355 nm	Argon	0.29
$z^5F_4^\circ$	355 nm	Helium	1.02
$z^5D_4^\circ$	355 nm	Argon	0.3
$z^5D_4^\circ$	355 nm	Helium	0.65
$y^5H_7^\circ$	266 nm	Argon	0.17
$y^5H_7^\circ$	355 nm	Argon	0.13
$y^5H_7^\circ$	355 nm	Helium	0.11
a^5D_3	266 nm	Argon	0.71
a^5D_2	266 nm	Argon	0.77
a^5D_2	355 nm	Argon	0.63
a^3D_3	355 nm	Argon	0.05
a^5S_2	266 nm	Argon	2
a^5S_2	355 nm	Argon	1.5
a^5G_6	355 nm	Argon	0.91
a^3G_4	355 nm	Argon	0.08
e^7D_5	355 nm	Helium	3.5
e^7D_3	355 nm	Helium	3
f^7D_5	355 nm	Helium	0.25
f^7D_5	355 nm	Argon	0.25
f^7D_4	355 nm	Argon	0.2
f^7D_4	355 nm	Helium	0.3
Propyl-ACT			

State	Laser wavelength	Buffer gas	Ratio of $\frac{\text{Intensity at vapor pressure}}{\text{Intensity at 240 Torr}}$
$z^7P_4^\circ$	355 nm	Argon	1.5
$z^7P_3^\circ$	355 nm	Argon	1.5
$z^7P_2^\circ$	355 nm	Argon	1.25
$z^7F_5^\circ$	355 nm	Argon	2.85
$y^7P_4^\circ$	355 nm	Argon	0.07
$y^7P_3^\circ$	355 nm	Argon	0.06
$z^5P_3^\circ$	355 nm	Argon	0.12
$z^5P_2^\circ$	355 nm	Argon	0.13
$z^5P_1^\circ$	355 nm	Argon	0.14
Methyl-ACT			
$z^7P_4^\circ$	355 nm	Argon	1.6, 2.5
$z^7P_4^\circ$	355 nm	Helium	3
$z^7P_4^\circ$	266 nm	Argon	1.33
$z^7P_3^\circ$	355 nm	Argon	1.5
$z^7P_2^\circ$	355 nm	Argon	1.25, 1.3
$z^7F_5^\circ$	355 nm	Argon	3
$y^7P_4^\circ$	355 nm	Argon	0.07, 0.08
$y^7P_4^\circ$	355 nm	Helium	0.2
$y^7P_3^\circ$	266 nm	Argon	1.22
$y^7P_3^\circ$	355 nm	Argon	0.08
$y^7P_2^\circ$	355 nm	Argon	0.1
$z^5P_3^\circ$	266 nm	Argon	0.67
$z^5P_3^\circ$	355 nm	Argon	0.08, 0.17
$z^5P_3^\circ$	355 nm	Helium	0.12
$z^5P_2^\circ$	355 nm	Argon	0.13
$z^5P_1^\circ$	355 nm	Argon	0.09
$y^5H_7^\circ$	355 nm	Argon	0.06, 0.05
a^5D_3	266 nm	Argon	2.5
a^5D_2	266 nm	Argon	1.9
a^5D_2	355 nm	Argon	1.17
a^5S_2	266 nm	Argon	2.3
a^5S_2	355 nm	Argon	1.11
a^5G_6	355 nm	Argon	1.6
e^7D_5	355 nm	Argon	1

6.2 Figures

If your thesis has a lot of figures, LaTeX might behave better for you than that other word processor. One thing that may be annoying is the way it handles "floats" like tables and figures. LaTeX will try to find the best place to put your object based on the text around it and until you're really, truly done writing you should just leave it where it lies. There are some optional arguments to the figure and table environments

to specify where you want it to appear; see the comments in the first figure.

If you need a graphic or tabular material to be part of the text, you can just put it inline. If you need it to appear in the list of figures or tables, it should be placed in the floating environment.

To get a figure from StatView, JMP, SPSS or other statistics program into a figure, you can print to pdf or save the image as a jpg or png. Precisely how you will do this depends on the program: you may need to copy-paste figures into Photoshop or other graphic program, then save in the appropriate format.

Below we have put a few examples of figures. For more help using graphics and the float environment, see our online documentation.

And this is how you add a figure with a graphic:

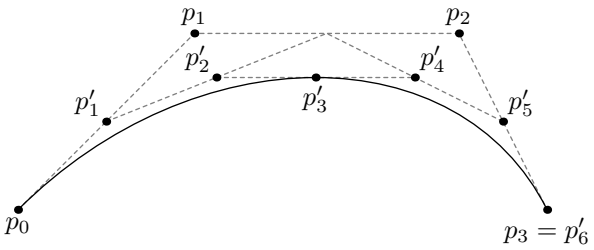

Figure 6.1: A Figure

6.3 More Figure Stuff

You can also scale and rotate figures.

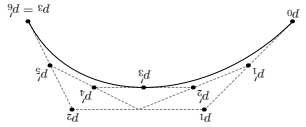

Figure 6.2: A Smaller Figure, Flipped Upside Down

6.4 Even More Figure Stuff

With some clever work you can crop a figure, which is handy if (for instance) your EPS or PDF is a little graphic on a whole sheet of paper. The viewport arguments are the lower-left and upper-right coordinates for the area you want to crop.

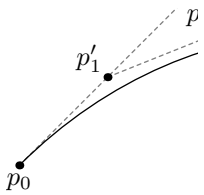

Figure 6.3: A Cropped Figure

6.4.1 Common Modifications

The following figure features the more popular changes thesis students want to their figures. This information is also on the web at `web.reed.edu/cis/help/latex/graphics.html`.

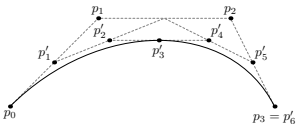

Figure 6.4: Subdivision of arc segments. You can see that $p_3 = p'_6$.

Conclusion

Here's a conclusion, demonstrating the use of all that manual incrementing and table of contents adding that has to happen if you use the starred form of the chapter command. The deal is, the chapter command in LaTeX does a lot of things: it increments the chapter counter, it resets the section counter to zero, it puts the name of the chapter into the table of contents and the running headers, and probably some other stuff.

So, if you remove all that stuff because you don't like it to say "Chapter 4: Conclusion", then you have to manually add all the things LaTeX would normally do for you. Maybe someday we'll write a new chapter macro that doesn't add "Chapter X" to the beginning of every chapter title.

4.1 More info

And here's some other random info: the first paragraph after a chapter title or section head *shouldn't be* indented, because indents are to tell the reader that you're starting a new paragraph. Since that's obvious after a chapter or section title, proper typesetting doesn't add an indent there.

Appendix A

Genetics Crash Course

Appendix B

The Basics of the Brain

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