ABSTRACT

Resurrecting *Tyrannosaurus rex*

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After the first successful extraction of ancient DNA from a fossilized Quagga in 1984, the subsequent development of PCR (Polymerase Chain Reaction) technology opened up a plethora of possibilities in the field of molecular paleontology. Supplied with fragmented ancient genomes, some scientists acted as if the days of resurrecting dinosaurs were a few technical difficulties away. Theories surfaced on the possible applications of ancient DNA technology, and some, such as creating tactical dinosaurs for the U.S. military, were outrageous. A less ridiculous idea surfaced in the form of Michael Crichton's *Jurassic Park*, published in 1990. Coupled with Steven Spielberg's 1993 feature film adaption, the *Jurassic Park* series created a world in which genetically-engineered dinosaurs roamed once again as theme park attractions on a billionaire's private island, and explored the possible outcomes of a "Jurassic Park" experiment. *Jurassic Park* ignited scientific debate over the technological feasibility, environmental impact, and ethical questions of a "Jurassic Park" experiment. This thesis continues that conversation by asking, could resurrecting a dinosaur be a productive environmental enterprise, other than a mere display of power over Nature? Focusing on *Tyrannosaurus rex*, this thesis combines a brief survey the current state of dinosaur genetic research, with analyses of rewilding with large predators, to discuss whether or not scientists should ever attempt to re-create a *T. rex* in the future.

APPROVED BY DIRECTOR OF HONORS THESIS

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Dr. Jennifer Good, Director of University Scholars

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RESURRECTING *TYRANNOSAURUS REX*

A Thesis Submitted to the Faculty of

Baylor University

In Partial Fulfillment of the Requirements for the

Honors Program

By

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TABLE OF CONTENTS

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When Pres. Starr spoke about the formative years of college that would shape us unsuspecting freshman into functional adults, I believe that I considered "formative" and "easy" to be equivalent terms. Looking back on my four years here at Baylor as a University Scholar, I realize that these "formative years", while filled with some great moments, sound much loftier on paper than they are in reality. During these difficult years, I faced challenges that seemed insurmountable. As I continue to the next phase of life, I would like to thank the people who helped me overcome the mountains in my life, and who encouraged me to take the next step in graduate school.

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INTRODUCTION

The Jurassic Park Experiment

The study of dinosaurs and ancient DNA has the allure of time travel. It offers a potential avenue for climbing into the past and bringing magnificent animals back into the modern world. This thesis explores the feasibility and ethical concerns of resurrecting a dinosaur as popularized by Michael Crichton's *Jurassic Park*. By examining the concept an implementation of rewilding theory, this thesis analyzes how a predator such as *T. rex* might impact a modern ecosystem, and whether its reintroduction could be useful to the modern world.

Chapter one summarizes Michael Crichton's *Jurassic Park* novel and Steven Spielberg's *Jurassic Park* film, and their context within popular culture and scientific discovery (Crichton 1990, Spielberg 1993). In the world of *Jurassic Park*, scientists use modern paleontology and molecular genetics to re-create viable dinosaur embryos from ancient dinosaur DNA. An assessment of the ancient DNA field follows to determine if the field is capable of supporting a *Jurassic Park*-like dinosaur experiment.

In a meta-analysis of studies from 1972 to 2004, Pääbo et al. summarized the field of ancient DNA research to identify and explain the molecular processes that can damage DNA over millennia (Pääbo et al. 2004). Ancient DNA can escape these processes under certain fossilization circumstances, but this is highly unlikely. As a result, most ancient DNA sequences do not survive intact for more than one million years, and those that do, are irreparably damaged and fragmented.

In addition to the scarcity of undamaged samples, ancient DNA research is further complicated by questions of sample origin. Most fossils are covered with material from fungi, bacteria, and the environment. Researchers could mistakenly isolate DNA from one of these contaminants in the fossil. In one study, Woodward et al. claimed to have isolated DNA from a Cretaceous dinosaur fossil, but Hedges et al. proved that the gene sequences were actually from fungal contamination of the fossil. This study showed that caution is paramount in ancient DNA research, because sample contamination could occur during the DNA extraction process (Hedges et al. 1995).

Due to contamination concerns and the relative scarcity of useful samples, ancient DNA research stalled until 2005, when Schweitzer et al. found soft tissue preserved in a *T. rex* fossil. by This discovery proved that ancient protein (and possibly DNA) could survive molecular degradation processes in certain fossilization circumstances. Future studies of these soft tissue samples could provide new insight into dinosaur biology, and supplement existing ancient DNA research (Stokstad 2005). However, without future discoveries, the ancient DNA field is incapable of providing sufficient genetic information to characterize an ancient species, and of supporting a dinosaur experiment.

Chapter two summarizes the current consensus on *T. rex*'s ecological role. Paleontologists previously debated over whether *T. rex* was a scavenger or a predator, but an analysis by Brusatte et al. indicates that *T. rex* had filled both ecological roles. Their analysis of T. rex's biomechanics and musculoskeletal adaptations demonstrate that the dinosaur was an ecological opportunist: an active predator that would scavenge for food if given the opportunity (Brusatte et al. 2010).

In support of the classification by Brusatte et al. in 2010, DePalma et al. provide conclusive evidence of *T. rex*'s predatory behavior. They found a *T. rex* tooth embedded in fossilized hadrosaurid vertebrae (DePalma et al. 2013). The hadrosaurid lived for some time after an unsuccessful attack by a *T. rex*, which indicated that the *T. rex* had engaged in active predatory behavior. They note that this finding does not imply that *T. rex* never scavenged, and support Brusatte et al.'s classification of the dinosaur as an ecological opportunist (DePalma et al. 2013).

Horner et al. conducted a massive survey of the ontogeny and morphology of *T. rex* specimens from the Hell Creek Formation in Montana (Horner et al. 2011). Their study revealed that *T. rex's* skull morphology changed dramatically throughout its lifetime. As a result, the dinosaur's musculoskeletal suitability for active predation shifted during development (Horner et al. 2011). Chapter two closes by summarizing *T. rex*'s role as an ecological opportunist, and suggests that modern cases of rewilding with carnivores may indicate how *T. rex* would act in a modern environment.

Chapter three evaluates the planning, execution and research of recent attempts to rewild large predators. These studies provide a framework for understanding how large predators initiate ecological interactions. In their report on the origins of rewilding for conservation, Soulé et al. utilized the keystone species theory and the "top down" hypothesis to show how large predators influence ecosystems through top-down ecological interactions. (Soulé et al. 1998). The rewilded Yellowstone park wolves study verifies that rewilding a top predator restores necessary ecological interactions to an ecosystem, and positively influences its biodiversity (Soulé et al. 1998).

While rewilding typically uses recently-extirpated species, in 2006 J. Donlan's Pleistocene rewilding proposal advocates rewilding parts of North America with

African megafauna (Donlan 2006). These African animals are proxies for species that existed in North America during the Pleistocene era 13,000 years ago, and Donlan believes that rewilding them will restore North American ecosystems to Pleistocene levels (Donlan 2006). This plan attracted harsh criticism from the scientific community, particularly from Rubenstein et al., who countered Donlan's proposal with evidence that Pleistocene rewilding is impractical, ecologically infeasible, and possibly destructive (Rubenstein et al. 237). This proposal is the best example of one that would precede working with dinosaurs, and exemplifies the difficulties inherent when rewilding with species that were absent for long periods of time.

Finally, these assessments of contemporary and Pleistocene rewilding are utilized to predict how *T. rex* might influence an ecosystem if it were reintroduced to the modern world. Sampson et al.'s analysis of *T. rex*'s paleoecology revealed that it was successful in a variety of paleoenvironments (Sampson et al. 471). With chapter two's data on *T. rex*'s behavior and ecology, chapter three posits that *T. rex* could survive in a range of environments, and may regulate ecosystems in a top-down manner, similar to that of modern top predators (Soulé et al.1998). However, previous rewilding attempts demonstrate that a *T. rex* would be dangerous to rewild, and its size and strength would make it difficult to contain and control. *T. rex* would also probably destroy any modern ecosystem in which it was placed, and should not be released in any proximity to civilization.

CHAPTER ONE

Resurrecting a Dinosaur

"Don't you see the danger John, inherent in what you're doing here? Genetic power's the most awesome force the planet's ever seen but you wield it like a kid that's found his dad's gun!" – Jeff Goldblum as Dr. Malcom in *Jurassic Park*

The Jurassic Park Phenomenon

When Indominus rex[1] smashed through a fence and terrorized stiletto-wearing scientists[2] in the 2015 film *Jurassic World*, a new generation experienced the incredible dinosaurs that captured Spielberg's audiences of *Jurassic Park* in 1993 ("Box Office History" 2015). Based on Michael Crichton's book of the same title, *Jurassic Park* essentially transformed dusty museum fossils into vibrant, breathtaking, and terrifying dinosaurs. Previous films such as *Walking with Dinosaurs*[3] attempted to bring dinosaurs to life as well, but never imagined them within a modern context and environment.

Built on a lush private island by an eccentric billionaire, *Jurassic Park's* fictitious dinosaur laboratories rely on the evolving disciplines of modern paleontology and molecular genetics to re-create viable dinosaur embryos. Once the embryos hatch in the laboratory, the growing dinosaurs live peacefully in the idyllic park until the fateful visit

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[1] *Jurassic World* scientists created a genetically chimeric dinosaur called "Indominus Rex".

[2] Trevorrow received harsh criticism for requiring the film's main female character to sprint through the jungle, *I. rex* in pursuit, while wearing five-inch stiletto heels (Mendelson 2015).

[3] A nature documentary-type series about dinosaurs.

of two paleontologists, Drs. Grant and Sattler, and a cynical chaos theorist Dr. Malcom[4]. The subsequent escape of Sue, the resident *Tyrannosaurus rex,* begins a domino effect resulting in the park's eventual collapse (Crichton 1990). Although they were cautionary tales against rash use of scientific power, the *Jurassic Park* franchise revived popular interest in dinosaurs[5], and in the chance of resurrecting them via genetic engineering.

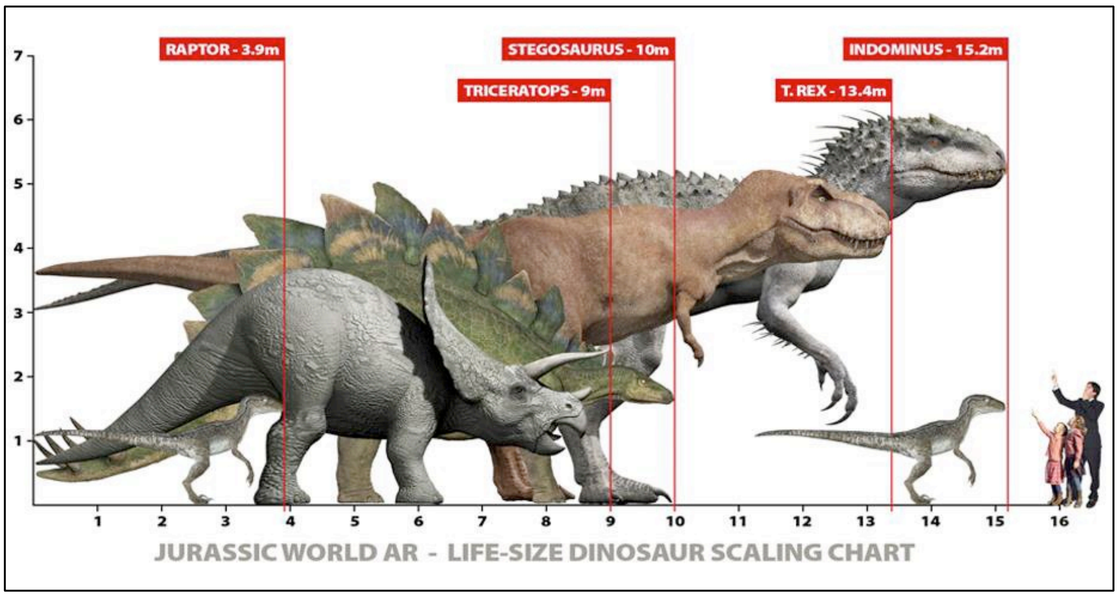

Figure 1: *Jurassic World's* dinosaurs, including the *T. rex* from *Jurassic Park*[6].

Resurrecting a Dinosaur

Although fossils can provide information about an animal's death, diet, and health, these inferences are hardly definitive. As evidenced by the current debate over

[4] Alan Grant played by Sam Neill; Ellie Sattler by Laura Dern; Ian Malcom by Jeff Goldblum.

[5] A Google Ngram search demonstrates that "T. rex" and "Tyrannosaurus rex" surged in popularity directly after the publication of Crichton's novel and the release of Spielberg's film.

[6] *Jurassic Park* 1993; *Jurassic World* 2015.

*T. rex'*s ecological role as a scavenger or predator[7], multiple teams studying the same fossil often reach opposite conclusions. However, recently researchers discovered that fossils hold a new dimension of information beyond their morphology: dinosaur DNA. Whereas in *Jurassic Park*'s laboratories, dinosaur DNA is purified from extracts of amber-entombed mosquitoes, technicians today obtain dinosaur DNA by isolating it from a solution of pulverized fossil bones.

DNA and the Genetic Code

An organism's deoxyribonucleic acid (DNA) codifies its genetic information into a sequential arrangement of nucleotides. A nucleotide consists of a phosphate group, a deoxyribose monosaccharide, and one of four nucleobases: adenine (A), thymine (T), guanine (G), and cytosine (C). Each polynucleotide[8] strand is held together by covalent bonds[9] between the phosphate group of one nucleotide and the sugar group of another, thereby creating DNA's alternating sugar-phosphate backbone. As shown in Figure 3, hydrogen bonds between pairs of nucleobases (A pairs with T; G pairs with C) coil the two sequentially complementary[10] strands together into the molecule's signature alpha helix structure. Therefore, by arranging its nucleotide sequence into functional units

[7] Two groups of archeologists studying the same class of *T. rex* skeletons reached opposite conclusions about its ecological role and diet: one claims *T. rex* was a scavenger, while the other advocates that it was an apex predator. See Ruxton et al. 2003, and Depalma et al. 2013.

[8] Polynucleotide: each strand of DNA is a polymer of nucleotide units.

[9] Covalent bonds are formed by association of orbitals and essentially sharing of electrons, they are recognized as the strongest type of bond.

[10] Each DNA molecule is composed of two strands with complementary sequences.

called "genes", a single DNA molecule carries most of the necessary genetic instructions for an organism's development, function, and reproduction.

When a cell needs a protein or expresses a particular gene, an enzyme called RNA polymerase synthesizes a complementary RNA[11] molecule from RNA nucleotides using DNA as a template. As a nucleic acid, RNA is similar to DNA but incorporates ribose as its monosaccharide (instead of deoxyribose), and replaces the nucleobase thymine (T) with uracil (U). Once the DNA's nucleotide sequence has been transcribed into pre-messenger RNA (mRNA), the pre-mRNA is then modified and edited by the spliceosome enzyme complex. Non-essential portions called "introns" are excised by the spliceosome, and the remaining essential "exons" are spliced together into mRNA. After a few modifications, the mRNA is translated by a ribosome into a polypeptide, which folds and twists in the cytoplasm to produce a functional protein. Figure 2 below summarizes how genetic information codified in DNA is transcribed into mRNA, which is then translated into an enormous array of proteins that compose a viable organism.

[11] Ribonucleic acid, has ribose as its sugar, and composes its sequences of A, G, C, and Uracil (U) instead of T (Thymine).

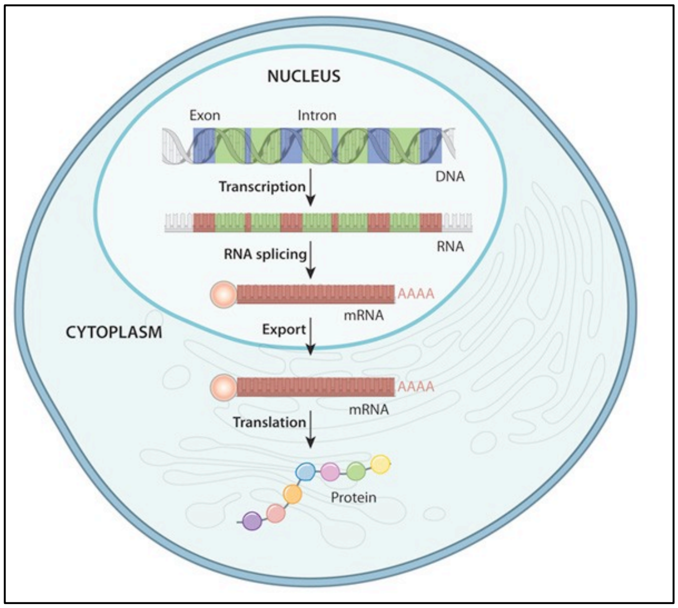

Figure 2: The process of transcribing by which DNA is transcribed into RNA, edited to produce mRNA, and translated into protein ("Gene Expression" 2014).

Obtaining Ancient DNA

In 1984, researchers celebrated the first successful extraction of ancient DNA sequences from the fossil remains of a Quagga (Pääbo et al. 645). This event fueled public curiosity about the array of possibilities that were now unlocked by the discovery of ancient genomes. However, the excitement may have overshadowed the relative scarcity of these prehistoric sequences.

Ancient DNA fragments are difficult to obtain even with modern technology. The most widely-used technique (from 1984 to today) for extracting genetic material from fossils is quite destructive and invasive. Technicians first remove the top layer of the fossilized bone to clear its surface of any human, microbial or fungal material[12] (Stoneking 1259). The remaining inner layers of bone are then pulverized, dissolved into

[12] Removing the top layer of bone in theory would clear the sample of any human, microbial, or fungal etc. DNA on its surface, and hopefully prevent sample contamination.

a solution, and treated with various chemical reagents. After a series of purifications, technicians can isolate dinosaur DNA fragments from suspension in the final solution (Pääbo et al. 653).

As DNA-extraction techniques improved in efficiency over decades, researchers were able to harvest genetic fragments from the fossils of extinct Pleistocene mammoths, ground sloths, and cave lions (Debruyne et al. 2003; Greenwood et al. 2001; Burger et al. 2004). But in the prototypical phase of the first extraction attempts, the DNA samples obtained from fossils were dismally small, and each bone-destroying attempt produced only one sample for laboratory analysis. Consequently, these experiments had no room for error, and running multiple tests to verify the DNA's prehistoric origins was impossible (Pääbo et al. 646).

The Monumental Invention of PCR

Limited to the scarce genetic samples obtained through fossil-extraction techniques, ancient DNA research remained stagnant until the invention of Polymerase Chain Reaction (PCR) technology in 1985 (Kondratas 1992). PCR is a relatively simple tool that allows scientists to copy a single DNA molecule billions of times over. When the DNA is heated in a test tube, its double helix structure unwinds and allows custom-built primers[13] to attach to complementary portions of its sequence ("PCR" 2016). An enzyme complex called DNA polymerase then associates with the DNA-bound primer and adds nucleotides onto its end (Adenine pairs with Thymine, and Cytosine with

[13] Primers are single-stranded DNA molecules. Their sequences are complementary to certain portions of the target DNA. When a primer attaches to the original DNA molecule, it serves as a starting point from which DNA polymerase can begin copying the strand.

Guanine) to form a new copy of the original complementary strand[14]. One PCR cycle produces two DNA molecules, each composed of one original strand, and one new strand.

By amplifying an invaluable sample into millions of disposable copies, PCR enables extensive laboratory analysis of "the miniscule amounts of highly degraded DNA … that can typically be recovered from ancient specimens" (Stoneking 1259). Accordingly, PCR became the "key molecular technique in ancient DNA research" (Pääbo et al. 670). Furthermore, the technology's customizable primers allowed geneticists to replicate any part of the fragment at will. Eventually, unlimited access to a copies of ancient DNA samples allowed molecular geneticists to test theorized evolutionary relationships by sequencing PCR-amplified DNA fragments, and comparing the ancient sequences to modern gene sequences.

Challenges of Decaying DNA

Unfortunately, DNA's chemical and molecular properties limit the length of time that it can survive intact. In a letter to *Science* in 1995, H. Zischler states that "DNA is not expected to survive over millions of years except, perhaps, under extraordinary conditions" (Zischler et. al 1193). In a living cell, enzymatic processes continually anabolize and repair DNA molecules until the cell's death (Pääbo et al. 646). When the cell dies, cytoplasmic compartments release catabolic enzymes that rapidly degrade the cell's macromolecules and nucleic acid[15] (Eglinton 1991). Over extended periods of time

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[14] Commonly *Taq polymerase,* a protein complex that copies DNA, whose natural function is to replicate a cell's genome before mitosis (Pääbo et al. 648; "PCR" 2016).

[15] Nucleic Acid is another term for the DNA molecule.

afterwards, spontaneous chemical reactions occur in the absence of cellular repair machinery and cause DNA damage to accumulate until the genome "loses its integrity and decomposes, [resulting in] an irreversible loss of nucleotide sequence information" (Pääbo et al. 647).

On exceedingly rare occasions, DNA can escape enzymatic and microbial degradation if it is either (1) adsorbed to a mineral matrix, or (2) in a tissue that becomes rapidly desiccated after death (Pääbo et al. 646). In the first instance, adsorption is a process by which a large organic molecule experiences a sort of "adhesive absorption" into the material around it. Through a variety of complex chemical reactions at multiple points on its structure, a DNA molecule adheres to mineral grains in soil and becomes incorporated into a mineral matrix, thereby shielding it from destructive chemical processes such as microbial respiration (Keil et al. 2014).

In the second instance, extremely cold temperatures can freeze-desiccate and thereby preserve ancient tissues, as observed in Pleistocene bones that were preserved by permafrost (Willerslev et al. 2004). The freezing conditions thermodynamically prevent water molecules from spontaneously catalyzing chemical reactions that would destroy the DNA's molecular structure. However, these extreme temperatures must remain constant for the DNA preservation to last. Unfortunately for molecular paleontologists, even in ideal frozen environments, the DNA in desiccated tissues "is thought to survive no longer than 1 [million years]" (Willerslev et al. 2004).

In the absence of these ideal conditions, isolating relatively undamaged DNA is an exceedingly rare occurrence (Pääbo et al. 2004). While some types of molecular damage can fundamentally change the original nucleotide sequence into missense or nonsense sequences, other types can break the molecule apart and render it incompatible with PCR primers. Consequently, the greater the amount of molecular degradation of a DNA molecule, the less likely it is that the surviving nucleotide sequence is an accurate representation of the original genetic code.

Types of Damage Found in Ancient DNA

Fundamental elements of DNA's molecular structure leave it particularly susceptible to chemical degradation over long periods of time. For example, free radicals created by background radiation[16] can disrupt double bonds in the fundamental ring structures of purine (A and G) and pyrimidine (T and C) bases[17]. Once ring fragmentation occurs, the ring structures' highly electronegative nitrogen and oxygen atoms are unavailable to form the hydrogen bonds that facilitate base pairing, and thereby hold the two strands of a DNA molecule together.

As shown in Figure 3, guanine requires three hydrogen bonds to pair with cytosine, and adenine requires two hydrogen bonds to pair with thymine. Blocking hydrogen bond formation renders the base incapable of pairing with its complement, and could result in fragmentation of the ancient DNA molecule. Accordingly, free radical-catalyzed ring disruption also interferes with PCR by preventing the primer from binding to the DNA template (Pääbo et al. 648). Without the primer's association with the prehistoric DNA strand, DNA polymerase cannot begin the copying process. Therefore,

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[16] Background radiation occurs normally from exposure to UV radiation in sunlight; the high-energy waves in UV radiation excite electrons in certain atomic species and create free radicals, or atoms with an "extra", highly-energetic electron. This electron can be quite destructive to molecules in its vicinity by catalyzing spontaneous chemical reactions.

[17] Purines: Adenine and Guanine; Pyrimidines: Thymine, Cytosine, Uracil (in RNA).

DNA that contains nucleobases with ruptured ring structures cannot be accurately amplified by PCR.

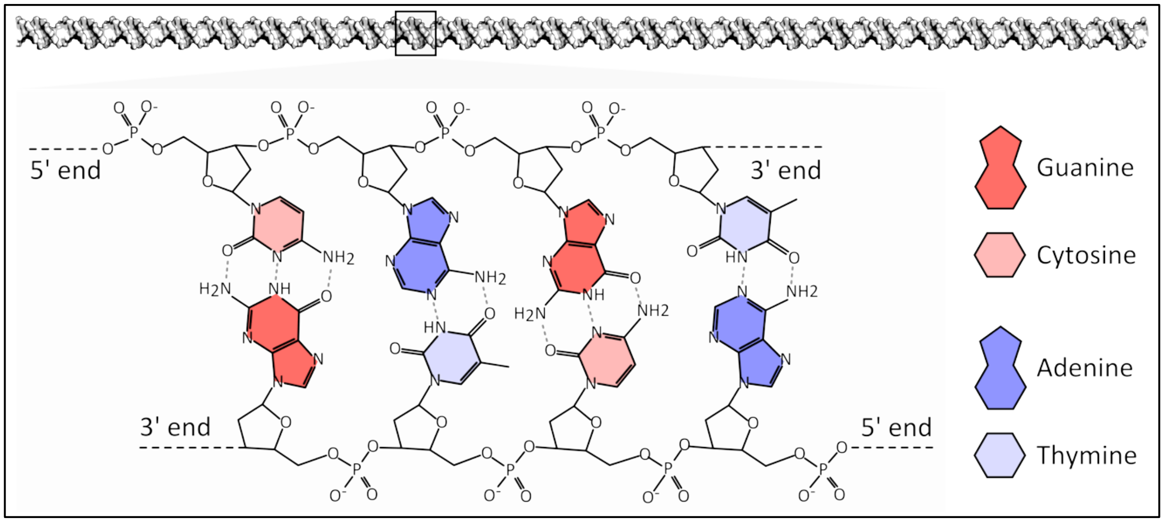

Figure 3: DNA's molecular structure. Purine bases (A and G) have two ring structures; pyrimidines (T and C) have one. Dashes denote hydrogen bonds; lines denote covalent bonds. Each side runs in relative opposition, and the nucleotide sequences are complementary. (Shafee 2016).

While the formerly mentioned processes can damage intrinsic portions of the DNA's structure, other types of molecular decomposition can create lesions that break the molecule into pieces. For example, in the presence of water, hydrolytic cleavage of phosphodiester bonds ruptures the DNA's sugar-phosphate backbone at random (Lindhal 1993). Other slow, destructive chemical processes can result in the loss of nucleotides and amino acid-catalyzed racemization (Karlstro et al. 1973; Poinar et al. 1996). The combined effects of these decomposition reactions break the ancient DNA into short fragments of around 100 to 500 base-pairs[18] (Hofreiter et al. 2001). Unfortunately,

[18] The size of a DNA molecule is often described by the number of base pairs, or the number of nucleotide pairs, that it contains.

the more fragmented the genome, the more difficult it is to properly order the segments into functional sequences.

Lastly, spontaneous reactions can occur between primary amino acids on the ends of decaying proteins and nucleic acid of prehistoric DNA. These processes create Maillard products that form cross-links and tangles between molecules, and subsequently block DNA polymerase during a PCR procedure (Vasan et al. 1996). Also, the stress induced by Maillard product cross-links puts additional strain on the already unstable DNA, and contributes to its fragmentation as well. Figure 4 summarizes common types of damage observed in ancient DNA.

Type of damage	Process	Effects on DNA	Possible solutions
Strand breaks	Degradation by microorganisms Nucleases in the postmortem cell Other chemical processes	Reduction of overall DNA amounts Size reduction	PCR of overlapping fragments of short length
Oxidative lesions	Damage to bases Damage to deoxyribose residues	Base fragmentation Sugar fragmentation	PCR of overlapping fragments of short length
		Nucleotide modification	Multiple independent PCRs Cloning and sequencing of several clones
DNA crosslinks	Reactions between DNAs as well as DNA and other biomolecules	e.g., Maillard products	PTB (N-phenylacyl thiazolium bromide)
Hydrolytic lesions	Loss of amino groups 1. adenine ⇒ hypoxanthine 2. cytosine ⇒ uracil 3. 5-methyl-cytosine ⇒ thymine 4. guanine ⇒ xanthine	Change of coding potential	Multiple independent PCRs Cloning and sequencing of several clones

Figure 4: A summary of the different types of damage that are commonly observed in ancient DNA (Pääbo et al. 647, Table 1).

Confirming the Ancient Origins of DNA Samples

In addition to the challenge of obtaining a relatively undamaged sample of prehistoric DNA, scientists must verify that the PCR-amplified samples are actually from an ancient species. The nature of the DNA extraction process leaves it particularly susceptible to contamination from material either on or within the fossilized bone. If contaminants are not adequately removed from the fossil's surface, then the PCR procedure may replicate fungal, microbial, or even human DNA. It is impossible to perform a sequence comparison analysis (and thereby identify the sequence's origins) before extraction and PCR; therefore, the threat of sample contamination "remains the single most serious concern" in the study of ancient DNA to date (Handt et al. 1994; Wandeler et al. 2003).

A few embarrassing instances in the past have resulted in healthy skepticism around claims that PCR-amplified samples are from an ancient species. For example, in 1994 Woodward et al. announced the successful extraction and PCR amplification of a dinosaur DNA sequence from cretaceous bone fragments in Utah. However, when multiple independent laboratories ran analyses of Woodward et al.'s sequence to verify his claim, they discovered that the alleged "dinosaur DNA" was almost certainly of human origin[19] (Hedges et al. 1995).

Using the BLAST gene database, Hedges et al. performed a BLAST[20] sequence comparison of Woodward's dinosaur sequence and compared the results with a separate

[19] See Hedges pp. 1191 for a phylogenetic tree that suggests the replicated fragments are human DNA.

[20] An online genetic database that contains billions of verified DNA sequences, and also gives information on their species of origin.

phylogenetic analysis. The BLAST sequence analysis revealed that Woodward's dinosaur sequence showed little similarity to those of its closest evolutionary relatives: birds and crocodiles. Furthermore, the phylogenetic analysis clustered the dinosaur DNA with the human genome, and not with birds and crocodiles as expected (Hedges et al. 1191). Since over 100 million years of evolution occurred on the lineage of dinosaurs and birds after their lineage split from mammals, it is unlikely that a dinosaur sequence would be extensively similar to a mammalian sequence (Benton 1990). Although Woodward countered that the DNA's degraded sequence could be coincidentally similar to mammalian sequences[21], four more independent laboratories published reports confirming that Woodward et al.'s results were erroneous, and the result of sample contamination (Hedges et al. 1995).

Another incident where fossil sample contamination de-railed a study of dinosaur DNA occurred when Peking University researchers claimed the successful cloning and sequencing of "six pieces of 18S rDNAs[22] and another piece of 191-bp DNA" from a fossilized Cretaceous dinosaur egg[23] (An et al. 1995, Li et al. 1995). While their claims attracted a fair amount of press, the researchers refused to publish conclusive evidence to support the sequences' dinosaur origins. Consequently, skepticism over the true identity of the alleged Cretaceous egg DNA encouraged independent analyses of the Peking University sequences by multiple laboratories.

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[21] Woodward on pp 1194 of (Hedges et al. 1995) stated that 80 million years of damage could result in changes that may cause the sample to resemble other evolutionary lineages.

[22] "rDNA" stands for ribosomal DNA; this type of DNA carries the code for ribosomes.

[23] The egg was discovered in the Xixia Basin in China.

A Genbank[24] similarity alignment analysis of the "Cretaceous dinosaur egg" sequences revealed that they shared "striking homology of more than 85% with…fungi and flowering plants". Furthermore, phylogenetic analyses revealed that the samples were "highly divergent from duck, human, alligator, and other animal rDNAs" (Wang et al. 589). As in the case of Woodward et al., the probability that dinosaur DNA would share overwhelming similarities with fungi or mammals is exceedingly low. Experts concluded that the researchers had mistakenly amplified DNA from fungi on the surface of the fossil.

Cases[25] such as those of Woodward and the Peking University researchers demonstrate that sample contamination is a significant problem, and can result in a wasted DNA-extraction procedure. Since a DNA sample's origins cannot be confirmed prior to the amplification and sequencing process, preventing sample contamination is paramount. Concern over contamination is so widespread that it led to Pääbo et al.'s formulation of the criteria of authenticity[26], a set of procedural standards to prevent contamination for researchers working with ancient DNA. Fortunately, as scientists grew more cautious and began following standardized techniques, successful ancient DNA isolations increased.

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[24] Genbank is an online database in which genetic sequences can be compared to others to look for similarities that indicate evolutionary relationships.

[25] Béraud-Colomb et al 1995; in this experiment the purportedly dinosaur sequence was actually quite similar to that of the paper's senior author (Stoneking 1260).

[26] Refer to Pääbo et al. pp 655 for an outline of the criteria of authenticity.

The Utility and Limitations of Ancient DNA

Although paleontologists and geneticists face significant challenges in obtaining and identifying genetic samples from fossils, successful DNA extractions are incredibly useful for evaluating phylogenetic relationships. For example, the verified discovery of DNA sequences from extinct Pleistocene mammals allowed the "direct assessment of the genetic relationships of these extinct animals [both] to each other and to extant animals" (Pääbo et al. 660). This study suggests that ancient DNA sequences can confirm theoretical evolutionary relationships by facilitating sequence similarity alignment analyses. Furthermore, if the prehistoric fragment codes for a protein, comparing the ancient and modern versions of that sequence can elucidate how the protein has evolved over millennia.

While ancient DNA is a valuable foundation, it is quite limited in its utility. Ancient DNA sequences can confirm broad evolutionary relationships; however, since most samples are fragmented and somewhat degraded, they currently cannot provide enough information to support specific claims about the structure, function, or ecology of prehistoric species (Pääbo et al 654). One ancient DNA fragment may only provide half or less of the information needed to ascertain the structure of a protein.

Furthermore, even access to an animal's complete genome would only be a piece of the puzzle. When DNA is transcribed into pre-mRNA, the spliceosome complex edits the molecule extensively before it becomes mature mRNA. Through a process called "alternative splicing", one mRNA molecule could be alternatively edited to code for many different proteins (Pierce 2013). Without direct knowledge of which segments of an ancient gene are kept as exons or discarded as introns, scientists have only a vague notion of the final mRNA sequence, and the subsequently produced protein. Then after the mRNA is translated by a ribosome into an amino acid sequence, the new polypeptide[27] may require a specific cytoplasmic environment to properly fold into its tertiary structure[28] (Pierce 2013). Therefore, while ancient DNA holds the necessary genetic information, scientists may be incapable of using it outside of the species' characteristic intracellular environment.

Lastly, while an ancient DNA fragment is quite informative about an individual, one sequence fails to fully capture genetic diversity of a population. Currently, "the bulk of ancient DNA work…deals with single individuals" which could be "from widely separated geographic locations or from time periods spanning hundreds to thousands of years" (Stoneking 1995). For example, *T. rex* evolved over millions of years in the late Cretaceous period and occupied enormous territorial ranges in both Asia and North America. Consequently, Stoneking's comment demonstrates that a *T. rex* DNA fragment cannot adequately represent the entirety of evolution and diversification that occurred in the *T. rex* population over millions of years.

Ancient DNA is invaluable evidence for confirming theoretical evolutionary relationships and providing general information about an extinct species' proteins. However, "the chemical properties of DNA probably restrict the survival of any molecules to this side of a million years", indicating that most prehistoric genetic sequences are badly damaged by the time paleontologists can extract them from fossils

[27] A polypeptide is a polymer of amino acids that are linked together by covalent peptide bonds.

[28] A protein's primary structure is its amino acid sequence; its secondary structure consists of alpha helices and beta pleated sheets; its tertiary structure is its complete shape once it has folded properly, and is often its functional form.

(Pääbo et al. 661). The current literature indicates that ancient DNA samples cannot—within the limitations of modern technology—provide enough information to accurately characterize an ancient animal. Nevertheless, paleontologists and molecular geneticists continue to search for dinosaur DNA.

Promising Future Developments

Despite unfavorable odds, the directions for future research stated by Pääbo et al. create an optimistic outlook for the fields of molecular genetics and paleontology. Pääbo et al. argue that "our knowledge of damage in ancient DNA and of misincorporations caused by such damage is still limited", and "many ancient samples contain no endogenous DNA detectable with current techniques" (Pääbo et al 652, 654). This statement suggests that an increased understanding of ancient DNA damage could help develop technology to remedy or circumvent these destructive processes. Moreover, if DNA detection and extraction techniques were improved, it is likely that the amount of available genetic information on ancient species would increase drastically.

This optimistic outlook is reinforced by the recent discovery of soft tissue from a *Tyrannosaurus rex* skeleton that was excavated from the Hell Creek Formation in Montana (Stokstad 2005). Schweitzer, chief paleontologist on the project, initially believed that she had found blood vessels and osteocytes, although precedent mandates that soft tissues degrade after one million years[29]. Despite receiving criticism from the scientific community, subsequent analyses in 2007 revealed that *T. rex* collagen fibers were marvelously preserved. Encouraged by this incredible discovery, Schweitzer and

[29] Microbes normally degrade soft tissues within weeks, and chemical decomposition processes normally destroy proteins within one million years (Pappas 2013).

colleagues examined other fossils and found that dinosaur soft tissue "was present in about half of their samples going back to the Jurassic Period" (Pappas 2013).

In both *T. rex* and another dinosaur, *Brachylophosaurus Canadensis,* Schweitzer found that the preserved soft tissue was closely associated with iron nanoparticles. As demonstrated by Keil and Meyer in 2014, DNA and proteins can escape degradation over millennia by undergoing adsorption into a mineral matrix (Keil et al 2014). In this case, iron nanoparticles released from the dinosaur's blood after death acted as a mineral matrix, thereby helping prevent molecular decomposition, and generated free radicals in the surrounding tissues (Pappas 2013). The free radicals catalyzed the formation of molecular cross-links, or Maillard products, and further aided in preserving the proteins from structural decay (Vasan et al. 1996).

Furthermore, researchers determined that the conditions of fossilization contributed to the preservation of soft tissue in these fossils as well. For example, the fossil bones of each specimen were articulated (not scattered about), suggesting that they were buried rapidly after the *T. rex* died (Pappas 2013; Pruitt 2015). The fossils were also excavated from sandstone, "which is porous and may wick away bacteria and reactive enzymes that would otherwise degrade the bone" (Pappas 2013). These findings are consistent with Pääbo et al.'s conclusion that organic molecules may be preserved over millennia if the tissue is desiccated after death, as porous sandstone could dehydrate the dinosaur's tissue (Pääbo et al. 646; Willerslev et al. 2004).

The verified analysis of dinosaur proteins from a *T. rex* fossil prompted similar experiments by other paleontologists and scientists. In June of 2015, researchers at the Imperial College in London analyzed bones from the late Cretaceous using an electron microscope and found what appeared to be red blood cells (erythrocytes) and collagen fibers (Bertazzo et al. 2015). Additionally, Bertazzo noted that once "they sliced through one of the red blood cells and saw what looked like a nucleus…", they were confident that the blood was not human contamination, since human and mammalian erythrocytes lack nuclei (Pruitt 2015). Most recently, Schweitzer has identified chemicals that could be fragments of *T. rex* DNA, but is withholding a definitive statement until further tests can be conducted (Pappas 2013). Combined with available data from DNA fragments, promising future studies of soft tissue from fossils could provide invaluable insight into the physiology, genetics, and evolution of dinosaurs.

Back to the Park

When the first discoveries of ancient DNA from fossils were published in the 1980s, public and professional enthusiasm ran wild with dreams of resurrecting species from local museums. One reporter announced that the military had a secret plan to resurrect dinosaurs for the purposes of national security and defense (Clifton 1984). Comparatively, thirty-one years later, the film *Jurassic World* portrayed highly-intelligent *velociraptors* that could be trained for tactical operations. Crichton's original *Jurassic Park* novel generated so much public enthusiasm for dinosaurs that it inspired articles in professional journals, and Spielberg's unforgettable film *Jurassic Park* is often mentioned alongside a breakthrough in dinosaur science (Weishampel 1991; Pruitt 2015).

The *Jurassic Park* series creates a world where dinosaurs once again roam the Earth, albeit as theme park attractions on a billionaire's private island. In *Jurassic Park*'s laboratory, patrons watch as a technician extracts dinosaur DNA from mosquitoes preserved in amber[30], and listen as they explain how the DNA is replicated, supplemented with genes from other organisms, and injected into a manufactured embryo (*Jurassic Park* 1993). Although this process seems logical in the film, this chapter demonstrates that the challenges of working with ancient dinosaur DNA would severely complicate a Jurassic Park-like process. Despite the incredible advances in fields such as electron microscopy and molecular genetics, modern technology still lags behind the *Jurassic Park* ideal.

The astonishing discovery of soft tissue from a *Tyrannosaurus rex* indicates that resurrecting a dinosaur may be possible in a quite distant future. Not only are there clues that DNA may have been preserved, but these soft tissue samples could also provide invaluable insight into *T. rex*'s physiology and cellular biology. Genetic data from ancient DNA and new information on dinosaur soft tissue may give future scientists a rudimentary foundation for ascertaining and replicating dinosaur biology. If researchers ever attempt to re-create a dinosaur, the most logical candidate would be the species on which we have the most information; therefore, the ideal species for dinosaur resurrection would be *Tyrannosaurus rex*.

[30] One researcher questioned how DNA could remain intact in amber, after tests revealed that other organic molecules, such as chitin in the insect's exoskeleton, did not survive decomposition over time (Stankiewicz et al. 1998).

CHAPTER TWO

Tyrannosaurus rex

"This isn't some species that was obliterated by deforestation of the building of a dam. Dinosaurs had their shot and Nature selected them for extinction!" – Jeff Goldblum as Dr. Ian Malcom in *Jurassic Park*

A Pop-Culture Phenomenon

This chapter gives a brief summary of the current state of *Tyrannosaurus rex* research. Initially described by H. Osborn in 1905, *Tyrannosaurus rex* is one of the most iconic dinosaurs in all of history (Osborn 1905). Due to the abundance and well-preserved quality of its fossils, *T. rex* is arguably the most well-understood dinosaur in the fossil record, and is an exemplary species used to study themes in vertebrate paleontology (Brusatte et al. 1481). The breadth of information available on *T. rex* anatomy, biomechanics[31], and paleoecology is unparalleled in paleontology, and contributes to this species' enduring status as an archetypal dinosaur (Schweitzer 2005). Additionally, *T. rex* grew to a magnificent size (over 5000 kg), was capable of crushing bite forces, and had tiny, useless arms (Erickson 772). These characteristic features make *T. rex* an intriguing animal to study, and also make the dinosaur instantly recognizable. When Michael

[31] In 2005, Schweitzer et al. discovered soft tissue in *T. rex* fossils from the late Cretaceous period, and later identified that tissue as collagen fibers (Stokstad 2005; Pruitt 2015).

Crichton chose *T. rex* as the central dinosaur in *Jurassic Park*, the dinosaur's existing paleontological fame had already primed it to become a pop-culture phenomenon. The novel *Jurassic Park* was published in 1990, and three years later Steven Spielberg's film-adaptation of the same name was released (Crichton 1990; *Jurassic Park* 1993). In both works, a giant *T. rex* played a central role as a terrifying predator, and the dinosaur park's main attraction (*Jurassic Park* 1990). An noted spike in interest in dinosaurs generally, and in *T. rex* specifically after 1990 shows how *Jurassic Park* catapulted *T. rex* to its current status as an icon.

Jurassic Park[32] was released during the Internet's infancy[33]; therefore, measuring interest in *T. rex* via non-internet media will create the most accurate trend. Google's Ngram tracks the usage of specified terms in print over a designated length of time (Andrews 2013). As shown in Figure 5, a Google Ngram tracking[34] the usage of "T. rex" and "Tyrannosaurus Rex" from 1980 to 2008 observed a spike in usage between 1990 and 1994, and a subsequent increase in popularity until 2002 ("Tyrannosaurus rex" 2016). *Jurassic Park* played a role in popularizing *T. rex* scientific literature and in popular culture and in scientific literature.

A core idea of the *Jurassic Park* franchise is the human fascination with resurrecting dinosaurs in the modern world. This is impossible within the limits of modern technology; but the evolving state of dinosaur research indicates that the *Jurassic Park* idea may be feasible in the distant future. For example, recent discoveries of *T. rex*

[32] For simplicity, *Jurassic Park* will be used to describe both the film and the novel; unless a distinction is necessary apart from the citation.

[33] The World Wide Web was invented in 1990, in the same year that Michael Crichton published *Jurassic Park*.

[34] The Google Ngram only searched English-language print sources.

soft tissue along with developing technological approaches are helping to elucidate this dinosaur's biology and evolution in unprecedented detail (Stokstad 2005, Pruitt 2015). These soft tissue samples may allow researchers to isolate DNA sequences, and provide a greater understanding of dinosaur cellular physiology. With this information, scientists could supplement their data on *T. rex* obtained through traditional paleontology.

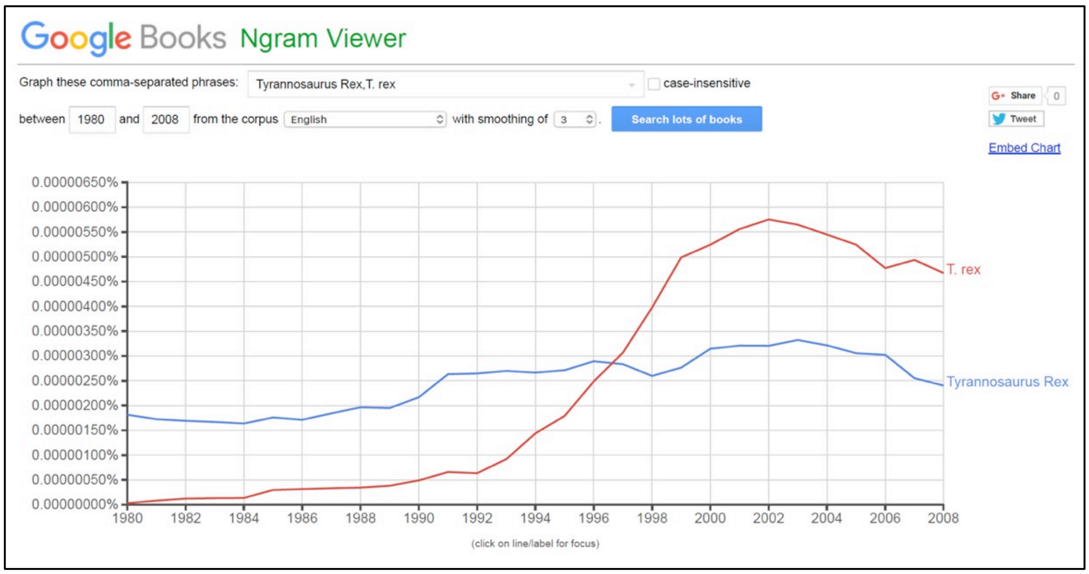

Figure 5. Ngram tracking "T. rex" and "Tyrannosaurus Rex" in print, 1980 – 2008. Note "T. rex" spike in popularity at 1992; "Tyrannosaurus Rex" usage increases gradually. "T. rex" is shorter, more recognizable, and used as an abbreviation (Google 2016).

Foundations of Dinosaur Paleontology

Dinosaur paleontology began in 1818 when a Connecticut farmer accidentally unearthed bones of an *Anchisaurus* while digging a well in his backyard ("Dinosaur" 2016). Although this is the first verified dinosaur bone, it is likely that dinosaur fossils were discovered long before the 1820s, but were misidentified or simply left alone. In the 1830s, the fossilized jawbone of a *Megalosaurus* and teeth from an *Iguanadon* were found in ancient sandstone sediments in Europe ("Dinosaur 2016).

Unable to ascertain a complete structure from the fragmented fossils, paleontologists initially classified dinosaurs as giant versions of modern reptiles. In 1842, Richard Owen recognized that these fossils belonged in their own group of species, which he called "dinosauria" (Paul 9). The first complete dinosaur fossils were excavated in Europe during the 1860s, verifying Owen's decision to place them in a class of their own, and concurrent with the publication of Darwin's *On the Origin of Species* (Paul 9).

Prior to Darwin's theories, many paleontologists struggled to analyze dinosaur fossils within the context of the biblical creation narrative According to the predominantly Roman Catholic view of Europe in the late 1800s, the earth was merely thousands of years old, and the dinosaurs are absent from the biblical account of creation in Genesis ((Engels et al. 2008; *The Holy Bible* 1989). In some ways, Darwin's theory of an ancient earth provided a different explanation[35], and allowed paleontologists to analyze dinosaurs as one step on a vast geologic time scale, instead of as an extra-biblical mystery.

Within the context of evolution and natural selection, paleontologists could analyze dinosaurs as prehistoric relatives of modern species (*Origin of Species* 1859). Morphological studies of fossils determined that dinosaurs were the evolutionary ancestors of modern reptiles and birds, and paleontologists began to form theories about dinosaur behavior and paleoecology by observing the dinosaurs' closest living relatives. Over time, information from fossils accumulated to form the fossil record of the Earth's

[35] Although Darwinian theories of evolution and natural selection continue to generate skepticism, at the time they seemed a reasonable explanation for the discovery of ancient fossils that were unlike most other species in the modern world.

evolutionary history, and the vast period of time in which dinosaurs dominated the planet became known as the Mesozoic Era.

The Mesozoic Era

According to the fossil record, the lush ocean-world of the Paleozoic era (541 Ma to 252.2 Ma[36] ago) culminated in the development of amphibians, reptiles and vertebrates, but ended abruptly in one of the largest mass extinctions in Earth's history (Thornberry-Ehrlich 2014). The cause remains a mystery, but some asteroid, volcano, or climate change triggered the extinction of over 90 percent of Earth's species ("Triassic Period" 2016). In the wake of this desolation, surviving species repopulated the planet and diversified into the first dinosaurs, and the Mesozoic Era began ("Triassic Period" 2016).

The Mesozoic Era lasted for approximately 180 Ma (from 252.2 to 66.0 Ma ago), and is divided into the Triassic, Jurassic, and Cretaceous periods. Dinosaurs first appeared in the Triassic Period (252.2 to 201.3 Ma ago) and continued to thrive as the Pangea supercontinent fractured in the Jurassic Period (201.3 to 145.0 Ma ago) and the Cretaceous period (145.0 to 66.0 Ma ago) (Thornberry-Ehrlich 2014).

The Cretaceous period was populated with some of the largest predators to ever walk the planet: *Spinosaurus* grew to 18 meters long, and weighed up to 13 tons; *T. rex* grew to 12.3 meters long, and weighed over 9 tons (Castro 2016). The age of dinosaurs ended in another mysterious mass extinction that destroyed all non-avian dinosaurs, and over half of the plant and animal species ("Triassic Period" 2016). Free of the predation risks from the giant carnivores of the late Cretaceous, the surviving mammals dominated

[36] Ma stands for Megaannum, equivalent to one million years.

the Cenozoic Era and evolved into modern humans (66 Ma to present day). See Figure 6 for a representation of the Earth's geological history.

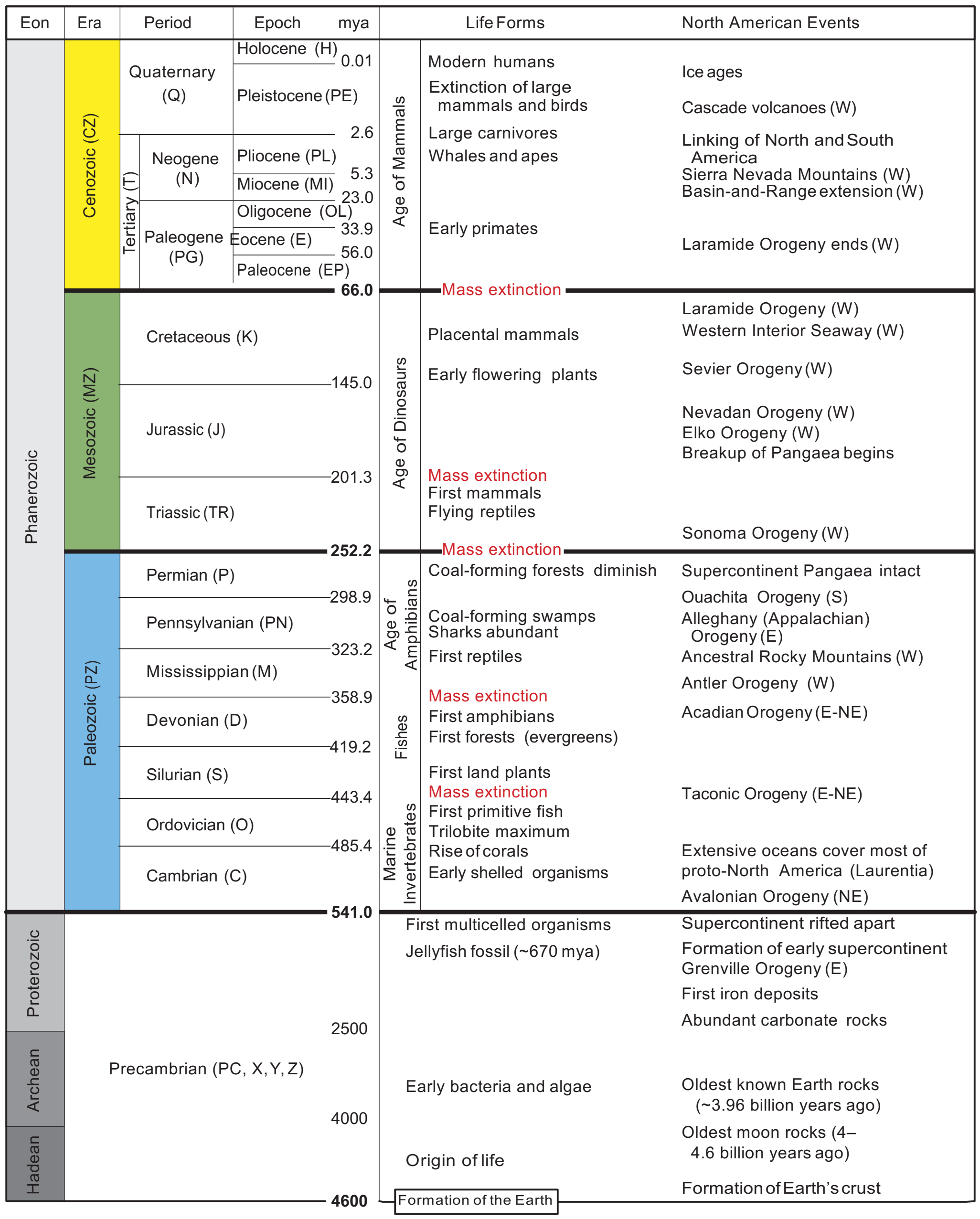

Figure 6. Divisions of the geologic time scale. Boundary ages are in millions of years ago (Ma). Note the Mesozoic Era, the age of the dinosaurs, from 252.2 to 66.0 Ma. The Mesozoic Era is divided into the Triassic, Jurassic, and Cretaceous periods. Mass extinctions are in red (Thornberry-Ehrlich 2014).

Distinguishing Dinosaurs

Before mammals reigned in the Cenozoic Era, the dinosaurs controlled the Earth for approximately 180 million years in the Mesozoic Era. Richard Owen theorized that the dinosaur phylogenetic clade began with one common ancestor of all dinosaurs, birds, and reptiles, which split from the mammalian clade over 200 million years ago, possibly after the mass extinction that began the Triassic period (Hedges et al. 1191). With the support of morphological fossil studies and gene sequencing data, the majority of modern researchers agree that dinosaurs are monophyletic[37], and that their closest living relatives are reptiles and birds (Hedges et al. 1191).

Anatomically, dinosaurs are distinguishable by their characteristic hip sockets, in which "the head of the femur is a cylinder, turned in at a right angle this skeletal morphology orients a dinosaur's legs "in the nearly vertical plane…with the feet directly beneath the body" (Paul 13). This type of hip socket exists only in hind-limb-dominant organisms, and indicates that a dinosaur's legs served as the primary weight-bearing structures. Studies of dinosaur hip structure[38] provided the rationale for the *Velociraptor*'s bird-like walk in the *Jurassic Park* film (*Jurassic Park* 1993).

All dinosaurs possessed extensive and complex sinuses and nasal passages, indicating that they had a strong and important sense of smell. Also, contrary to a popular rumor, dinosaurs had well-developed eyes, and their vision was not dependent upon motion (Stevens et al. 2006). Predatory theropod dinosaurs, such as *T. rex,* had some of

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[37] The term monophyletic indicates that all dinosaurs share a common ancestor.

[38] Hutchinson et al. in 2002 determined that *T. rex* did not assume an increasingly crouched posture, but stood up a little straighter than modern birds, and thereby maximized its mechanical advantage (Hutchinson et al. 1018).

the best eyesight of all dinosaurs (Stevens et al. 2006). Since dinosaurs presented with distinct anatomical morphology and sensory capabilities, it is useful to differentiate true dinosaurs from other species to more accurately classify and theorize about their paleoecology and behavior. In this thesis, any dinosaurs mentioned are non-avian species that existed from 252.2 to 66.0 Ma ago in the Mesozoic Era[39].

Classifying T. rex

The Tyrannosaurs

Millions of years of dinosaur evolution culminated in the appearance of the sophisticated and powerful tyrannosaurs. This group of theropods[40] includes some of the largest carnivores to ever walk the planet, and they roamed Asia and North America for approximately 15 Ma in the Late Cretaceous period (51 Ma to 66.0 Ma ago) (Paul 22). The tyrannosaurs themselves were sub-divided into basal and giant varieties, and displayed a variety of skeletal and cranial morphology. (Brusatte et al. 1481).

Basal tyrannosaurs were sleek, bird-like, and smaller, and had longer arms. In contrast, the specific sub clade of the giant tyrannosaurs, called Tyrannosauridae, includes the "multi-ton, deep-skulled behemoths from the terminal Cretaceous", such as *Tyrannosaurus Rex* (Brusatte et al. 1481). As shown in Figure 8, the formulaic body plan for these enormous tyrannosaurs includes a large and deep skull, robust teeth, small arms, long hind limbs, and a long tail (Brusatte et al. 1481). Only in the Late Cretaceous did

[39] When dinosaurs are portrayed in popular culture, this term is often used to describe species that are not truly dinosaurs. For example, *Jurassic World's* version of Shamu, a giant Mosasaurus, is actually a marine reptile (*Jurassic World* 2016). With the exception of the semi-aquatic predator *Spinosaurus aegypticus*[39], dinosaurs were persistently terrestrial (Milius 2014).

[40] A theropod is a predatory dinosaur.

T. rex and its relatives grow to enormous sizes, and reach lengths of up to 13 m, and masses between 5 and 8 tons (Erickson 772).

The Giant Tyrannosauridae

The giant Tyrannosauridae of the Late Cretaceous period have multiple characteristics that distinguish them from other dinosaurs. All Tyrannosauridae are bipedal predators, and all present anatomically with large "D-shaped…teeth, fused nasals, extreme pneumaticity in the skull roof and lower jaws, a pronounced muscle attachment ridge on the ilium, and an elevated femoral head" (Brochu 2003; Brusatte et al. 1482). Extreme pneumaticity refers to a high concentration of air pockets in these bones, and indicates that Tyrannosauridae possessed relatively light skulls.

Additionally, their skulls are large and deep, with reinforced sutures between skull bones for added strength. Their powerful jaw muscles, signature skull structure, and general body structure are considered "adaptations for a hypercarnivore to function at a large size" (Brusatte et al. 1482). The term hypercarnivore denotes the dominant status of *T. rex* and its relatives, such as *Alioramus* in Figure 8, at the top of their respective ecosystems' food webs. *T. rex* occupied the top spot in the Tyrannosauridae group, and was the most successful large predator in the Late Cretaceous period. These skeletal adaptations, along with specific features of its skull morphology, are considered essential to *T. rex*'s evolutionary success. The amount of data on *T. rex* is unparalleled, making it the most well-understood dinosaur in modern paleontology (Brusatte et al. 1481).

Anatomical Adaptations of T. rex

Structural Adaptations of T. rex's Skull

Because of the conditions of their fossilization, many *T. rex* skulls are remarkably well-preserved, and have been thoroughly examined for decades (Brusatte et al. 1483). Modern digital imaging techniques have provided new insight into these skulls, and consequently, into the capabilities of these dinosaurs as well. CT scanning revealed that *T. rex*'s neuroanatomy enabled it to function as a successful predator. Their "encephalization quotient—an estimation of relative brain size—varies between 2.0 and 2.4, larger than in basal theropods but lower than that of birds" (Witmer et al. 2009, Brusatte et al. 1483).

CT scans of *T. rex* brain cavities also revealed the presence of large olfactory lobes, the region of the brain where smell-related neural activity is concentrated, and this well-developed region indicates a strong sense of smell (Witmer et al. 2009). These enlarged olfactory lobes are an adaptation for predation that allowed *T. rex* to track mobile and dispersed prey (Erickson 1996).

Additionally, the skulls presented with long cochlear and semicircular canals, which are essential structures for balance and hearing in the inner ear. These indicate that *T. rex* was capable of "elevated sensitivity to low-frequency sound and [of] highly coordinated head and eye movements" (Brusatte et al. 1483). Increased sensitivity to low-frequency sounds allowed *T. rex* to detect large prey at great distances, and highly-coordinated head and eye movements are considered necessary adaptations for a predatory animal.

T. rex bite marks have been found on the skeletons of a wide variety of species, including other tyrannosaurs. Also, discoveries of bite traces from a *T. rex* indicate that this species crunched through bone easily. To produce and withstand such a massive bite force, supportive adaptations evolved in *T. rex's* skull structure (Depalma et al. 12562). As seen in Figure 7, results of finite element analysis indicate that *T. rex* had a skull that was "optimized to endure strong bites, as various sutures absorbed [musculoskeletal] stress and the fused nasals strengthened the snout" (Brusatte et al. 1484). In light of these features, biomechanical analyses estimate that *T. rex* "generated bite forces of at least 13,400 N" (Brusatte et al. 1484).

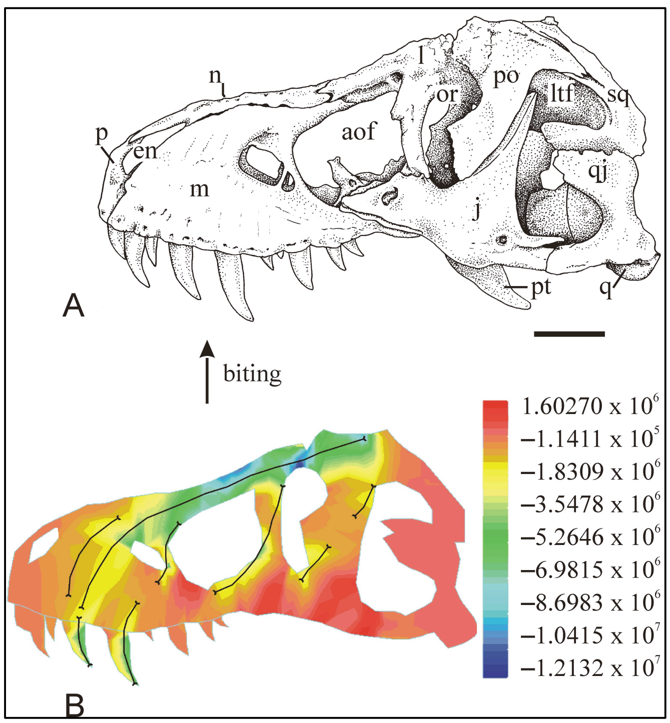

Figure 7. Finite Element Analysis of *T. rex* skull during a bite. Lines denote patterns of pressure induced; red areas absorb the most pressure (Benton 2010).

Implications of T. rex's Skull Anatomy

With crushing bite strength, acute senses, and the brain of a top Cretaceous predator, a mature *T. rex* was capable of hunting smaller dinosaurs and giant armored herbivores alike. However, analyses of juvenile *T. rex* skulls indicate that *T. rex'*s preference for stronger, larger prey developed concurrent to its ontogeny[41]. As seen in Figure 8, juvenile *T. rex'*s skull (E) underwent a complete overhaul[42] as it matured into an adult (F). The juvenile *T. rex's* jaws shortened and deepened, its sutural surfaces developed, its teeth grew larger and more robust, and its pneumatic bones inflated as the skull's overall shape changed (Horner et al. 2011).

These structural changes that shape a juvenile *T. rex* skull into its adult form indicate that a *T. rex'*s ecological role also changed throughout its development. The longer and slightly more delicate juvenile *T. rex* skull lacked the necessary skeletal reinforcements to produce or withstand an adult's crushing bite force. For this reason, it has been suggested that juvenile *T. rex* hunted much smaller prey than an adult *T. rex*, which frequently tackled the larger and often armored herbivores (Horner et al. 2011). Consequently, a juvenile *T. rex* occupies a lower spot on the food web than an adult, and shifts gradually to the top ecological niche throughout its development.

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[41] Ontogeny is the origination and development of an organism's bones and skeletal structure (Horner et al. 2011).

[42] In some cases, juvenile *T. rex* skulls were so different from adults that they were misclassified as different species (Horner et al. 2011).

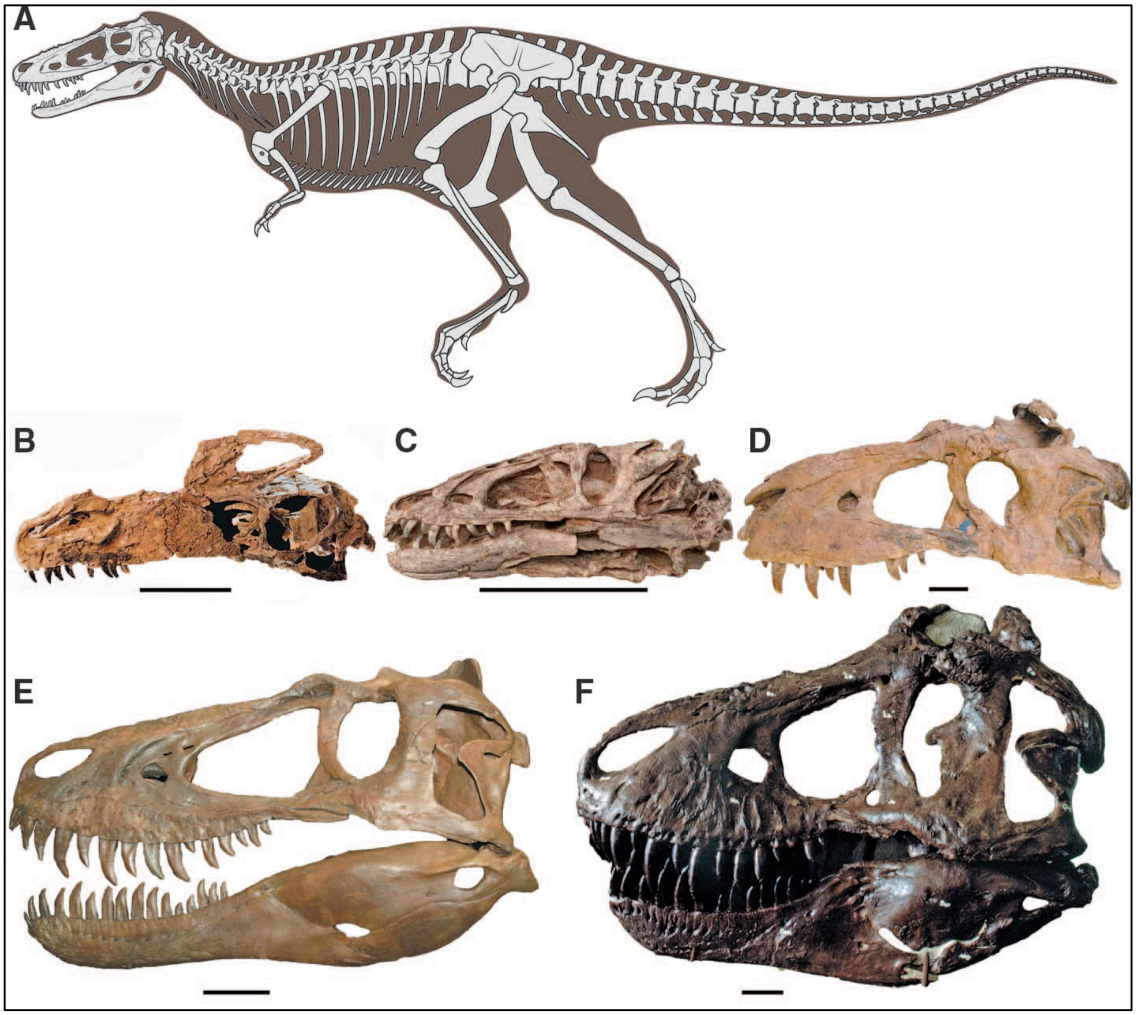

Figure 8. (A) skeletal reconstruction of Alioramus, demonstrating general Tyrannosaurid morphology. (B –D) are different Tyrannosaurid species. (E) is the skull of a juvenile *T. rex*, while (F) is the skull of a mature adult *T. rex*. Note the structural differences between (E) and (F) (Brusatte et al. 1481).

T. Rex: An Ecological Opportunist

Scavenger or Predator?

Equipped with a powerful skull and a capable brain, a mature *T. rex*'s options for food were limited only by its size and speed. However, although the fossil record provided evidence for predatory behavior in *T. rex*, researchers[43] have argued that *T. rex* was an obligate scavenger. In 1994, J. Horner argued that *T. rex* was too large and slow to pursue the prey items in its ecosystems, and that large theropods like *T. rex* procured their food primarily by scavenging instead of hunting (Horner 1994). This proposition exemplifies the debate that lasted for decades over *T. rex'*s ecological role, and whether it was an obligate scavenger, obligate predator, or ecological generalist. To find proof of *T. rex'*s paleoecology and behavior, paleontologists turned to biomechanical analyses, studies of *T. rex*'s morphology, and data from the fossil record.

In 2003, an analysis of *T. rex'*s skeletal biomechanics estimated that the dinosaur could reach ambulatory speeds between 20 and 40 kilometers per hour, and proved that it had the capability to pursue and kill prey species (Ruxton et al. 2003). Additionally, studies by DePalma et al. revealed that an adult *T. rex*'s main food source consisted of large, lumbering herbivores such as *Ankylosaurus*, *Alamosaurus*, and *Hadrosaurus* (DePalma et al. 2013). None of the aforementioned herbivores are capable of quick movements, so a *T. rex* traveling between 20 and 40 kph would be able to apprehend them easily.

Studies of *T. rex'*s jaw and tooth morphology also contributed to the scavenger vs. predator debate and confusion (Brusatte et al. 1482). Ruxton et al. argued that *T. rex'*s bone-crunching bite pressure and resilient jaw structure were analogous to those of modern scavenging species, who are similarly adapted for consuming bone and tissue while scavenging carcasses (Jacobsen 1998, Ruxton et al. 2003). Due to these adaptations and the *T. rex*'s large size, Ruxton et al. suggested that it would be more energetically

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[43] Horner 1994; Horner, Goodwin, and Myhrvold 2011; Ruxton et al. 2003.

favorable for an adult *T. rex* to scavenge rather than engage in predatory behavior, because it would expend less energy overall (Ruxton et al. 732). Ruxton et al. calculated that a *T. rex* could survive purely by scavenging, but only if a number of conditions were met in the environment (Ruxton et al. 731).

Conclusive Evidence of Predation

Paleontologists lacked conclusive evidence for predatory behavior in *T. rex* until a discovery in the Hell Creek fossil formation in South Dakota. In 2013, DePalma et al. discovered a *T. rex* tooth crown embedded in a hadrosaurid[44] vertebrae. There was evidence of extensive infection (osteomyelitis) in the bone around the embedded tooth, and bone overgrowth had fused the damaged hadrosaurid vertebrae together in the healing process (DePalma et al. 12561). This evidence documents an unsuccessful predatory attack by a *T. rex*, since the hadrosaurid lived long enough afterwards to permit bone growth. Therefore, this discovery served as conclusive evidence that *T. rex* was an active predator[45] (DePalma et al. 12561).

Consensus: An Ecological Opportunist

DePalma et al. posits that *T. rex* may have scavenged carcasses, just as modern large predators, such as lions and coyotes, scavenge food in their environments (Stuart et al. 1997; DePalma et al. 12561). This suggestion that *T. rex* was neither an obligate predator or an obligate scavenger fits the dinosaur's distinct skeletal adaptations, its

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[44] A large herbivore recognized as a main food source for *T. rex*.

[45] A counter-argument would be that T. rex was a poorly-skilled predator, so this evidence is unimportant, but DePalma et al. note that modern terrestrial predators fail or abort over half of their attacks (DePalma et al. 12563; Kingdon 1997).

tendency to shift ecological behavior throughout its development, and studies of its paleoenvironment (Horner et al. 2011). Consequently, *T. rex* is currently classified as an ecological opportunist and generalist; a top carnivore that acts as scavenger and apex predator in a wide range of ecosystems (Horner et al. 2011).

The fossil record shows that *T. rex* was an active predator, and no specific evidence refutes the argument that *T. rex* scavenged occasionally. Therefore, paleontologists classify *T. rex* as an ecological opportunist. In addition to the hadrosaurid discovery in 2013, *T. rex*'s neurological, sensory, and skeletal adaptations contribute to its success and strength as a predatory theropod. DePalma et al. suggest that *T. rex* functioned mostly as a top predator, but scavenged given the opportunity (DePalma et al 2013). Additionally, this conclusion suggests that *T. rex'*s proclivity for scavenging or predation may have shifted over its lifetime, as its prey preferences changed during its skeletal development.

Paleoenvironments of T. rex

Analysis of the fossil record revealed that *T. rex* roamed over vast portions of western North American and central Asia during the Cretaceous period (145.0 to 66.0 Ma) (Sampson et al. 471). Paleontologists can ascertain which environments were ideal for a *T. rex* by studying the sedimentology and taphonomy[46] of the fossil's excavation environment. By combining data from both studies, paleontologists can construct the ancient ecosystem and the original ecological interactions between the fossilized species

[46] A sedimentological analysis provides information on the physical characteristics of where the dinosaur lived and died, and taphonomic study of the organic material gives information on the biotic biological processes during that time period (Behrensmeyer 15).

(Behrensmeyer 15). Using sedimentological data, taphonomic studies, and documentation of fossil excavations, Sampson et al. reconstructed the ancient ecology of *T. rex* and three herbivores in western North America. Figure 9 maps the environments and dinosaur distribution of this area in the Late Cretaceous period, from 80 to 66.0 Ma ago (Sampson et al. 471).

T. rex's Ideal Environments

T. rex's large territorial range spans a variety of paleoenvironments, and its successful expansion confirms its status as a top carnivore. However, *T. rex*'s level of ecological adaptability was an anomaly in the Late Cretaceous. As shown in Figure 9, North American large herbivores lived in one type of environment, while *T. rex* lived in three (Sampson et al. 471). The fossil record indicates that many of the large herbivores[47] in western North America were confined to specific latitudes (Sampson et al. 471). *T. rex* is the only known dinosaur that could live in such a wide range of environments (Sampson et al. 472).

Intermontane basins. Intermontane basins are semi-arid and upland environments, and are often found in valleys between mountain ranges. In Late Cretaceous North America, *T. rex* lived in the intermontane basins in the lower region of what is now known as the Rocky Mountains. In this environment, most precipitation falls on the surrounding mountains, and the basin floor often experiences the least moisture, and the most extreme temperature fluctuations. Additionally, trees are often absent at the basin

[47] *Alamosaurus, Triceratops,* and *Leptoceratops gracilis* were main food sources for *T. rex* when it occupied their paleoenvironments.

floor (Barnosky 296). Figure 9 shows that *Alamosaurus* lived in this type of ecosystem as well.

Coastal plains. Coastal plain environments are warm, humid and vast (Sampson et al. 471). The Late Cretaceous coastal plains occupied by *T. rex* were generally flat or at low elevations, and had relatively stable temperatures. As shown in Figure 9, what is now the Great Plains region was seaway during the Late Cretaceous period. Consequently, this area received adequate rainfall, and supported a wide variety of flora and fauna such as *Leptoceratops gracilis* (Sampson et al. 470 - 471).

Alluvial plains. Also called floodplains, alluvial plains are relatively cool, semi-arid and flat, and are formed by sedimentary deposits from rivers and streams ("floodplain" 2016). Large terrestrial life tends to stick to the elevated areas, and depending on the course of the river that formed the alluvial plain, may move into lower elevations if flooding is no longer a concern (Lubinski 2). These environments often support rich and diverse ecosystems, and supported larger dinosaurs such as *Triceratops horridus* (Figure 9, Lubinski 10).

Unprecedented Adaptability

T. rex's enormous range of environments reveals its adaptability and flexibility as a predator, and also supports the argument for its role as an ecological opportunist. A top carnivore that can act as a scavenger and apex predator could survive to a larger range of environments, and sustain itself in a variety of ecosystems (Horner et al. 7). *T. rex*'s potential to oscillate between predation and scavenging indicates it could adapt to different ecosystems as needed (DePalma et al. 2013).

These studies demonstrate that *Tyrannosaurus rex,* the central dinosaur of *Jurassic Park*, was one of the most successful large carnivores in the Mesozoic Era, and dominated its territory across Asia and North America in the Late Cretaceous. Its distinctive anatomical adaptations and structure exemplify most of the features associated with being a top carnivore (Brusatte et al. 1483). The scientific debate over *T. rex*'s ability to engage in predatory behavior ended in 2013 with DePalma et. al's evidence of active *T. rex* predation (DePalma et al. 2013). Current studies classify *T. rex* as a hypercarnivore with unusual ecological flexibility.

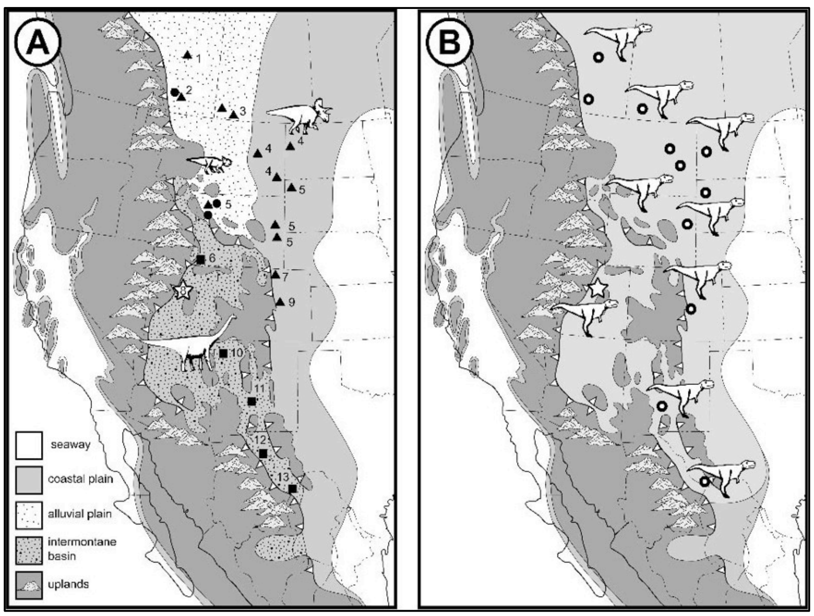

Figure 9. Paleoecology of *Alamosaurus* (square), *Leptoceratops gracilis* (circle), *Triceratops horridus* (triangle); and *T. rex* in western N. America (Sampson et al. 471).

Resurrecting T. rex

The amount of research on *T. rex* is unparalleled, making it the most well-understood dinosaur in modern paleontology (Brusatte et al. 1481). Consequently, researchers with a desire to see dinosaurs in a modern environment have ample reasons to view *T. rex* as a productive avenue to "bringing back the dinosaurs".

Biological Information

Schweitzer et al. believe that the *T. rex* soft tissue samples will yield complete DNA sequences with the help of future technology; while examining other soft tissue samples from Cretaceous fossils, London researchers gained new insight into dinosaur physiology and molecular cellular biology[48] (Pappas 2013, Bertazzo et al. 2015). Knowledge of the dinosaur's cellular processes will complement the species' genetic data by demonstrating how the dinosaur's genes were expressed as proteins[49]. These discoveries are a rudimentary foundation for ascertaining *T. rex*'s cellular biology, and will facilitate a greater understanding of the species' physiology.

Ecological Flexibility

The genetics of the species notwithstanding, due to its ecological flexibility, *T. rex* is one of the most likely of all dinosaurs to be able to adapt in the modern world and climate (Brusatte et al. 1483). As shown in Figure 9, the three paleoenvironments favored by *T. rex* (alluvial plain, intermontane basin, and coastal plain) in the Late Cretaceous display a range of fluctuating temperatures, and vary widely in their species composition and vegetation distribution (Horner et al. 2011; Sampson et al. 417). Since *T. rex* adapted

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[48] When examining the claw of a predatory theropod using an electron microscope, Bertazzo et al. found that dinosaur red blood cells had been preserved. These red blood cells were nucleated, unlike mammalian red blood cells. Further analysis may answer the question of whether dinosaurs cold-blooded like modern reptiles, warm-blooded like modern birds, or somewhere in-between (Pruitt 2015, Bertazzo et al. 2015).

[49] The complex process of editing mRNA in indicates that the RNA transcription of a eukaryotic (not microbial) gene sequence may be a shortened, alternate version by the time it is translated into protein by the ribosome.

successfully to this range of environments in the ancient world, it may be capable of adapting to modern ecosystems with similar climates and species compositions.

T. rex in the Modern World

If scientists resurrected a *T. rex* (as in Crichton's *Jurassic Park* novel), the literature and the fossil record suggest that it would operate as an ecological opportunist and top predator if placed within a suitable environment (Brusatte et al. 1482 – 1483). The practice of rewilding is the modern analog to bringing back the dinosaurs. In this conservation practice, extirpated species, such as large carnivores and top predators, are restored to their former ecosystems. Therefore, analyses of contemporary rewilding initiatives will help predict how a *T. rex* would impact a modern ecosystem if restored to the natural world.

CHAPTER THREE

Rewilding

"If there's one thing the history of evolution has taught us, it's that life will not be contained. Life breaks free. Life expands to new territories. Painfully, perhaps even dangerously. But life finds a way." – Jeff Goldblum as Dr. Malcom in *Jurassic Park*

Rewilding a T. rex

In a distant future, some corporation or scientific enterprise may develop the methodology to resurrect a *Tyrannosaurus rex*. This would include scientific analyses of its ecology and physiology to ascertain if it would survive in a modern environment. Studies indicate that *T. rex* was a top predator and ecological opportunist, and dominated a range of ecosystems across North America and Asia in the Late Cretaceous period (Brusatte et al. 1484). *T. rex* lived in a range of climates, and its ecological opportunism granted it a high level of ecological adaptability (Sampson et al. 471).

With this knowledge of *T. rex* paleoecology, examining cases of rewilding with large predators may reveal how a *T. rex* would interact an ecosystem if it were reintroduced to the modern world. Rewilding offers a framework for understanding essential ecological interactions, and two of its central theories, the keystone species theory and the top-down hypothesis, further elucidate how top predators influence their ecosystems (Donlan et al. 664). This chapter examines rewilding initiatives using recently-extirpated megafauna or extant conspecifics to indicate how *T. rex,* an ancient top predator, might influence a contemporary ecosystem, and what problems it may encounter throughout its reintroduction into the modern world.

Ecological Theory Behind Rewilding

Large predators are often eliminated from their ecosystems by over-hunting, intentional extirpation, or habitat degradation due to pollution or industrialization[50] (Soulé et al. 24, Foreman 2004). Studies show that eliminating a large carnivore or predator species[51] from an ecosystem will trigger changes across all trophic levels[52] that result in habitat loss and extirpation of other species in the food web (Terborgh et al. 1999, Paine 1966, Soulé et al. 22). Consequently, rewilding is the practice of repairing ecosystems by restoring an extirpated species into its former environment (Soulé et al. 22). If the species' rewilding succeeds, most of the disrupted trophic interactions will return to their fully-functioning levels, and the damaged ecosystem will begin to heal (Soulé et al. 24).

Since its development in the 1980s, rewilding has become a conservation practice for restoring many ecosystems around the world (Zimov 2005, Donlan et al. 2006, Foreman 2004). Rewilding for conservation typically uses species that were extirpated from their ecosystems within a few hundred years, which is a relatively small interval in the context of evolutionary history. Ecologists estimate that few evolutionary changes have occurred in the species or its former ecosystem in such a short amount of time

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[50] Rewilding is often viewed as a fulfillment of human responsibility (Soulé et al. 22).

[51] In this thesis, the terms "large carnivore", "top predator", and "large predator" are treated as equivalent terms, and "large predator" will be used as a summation of the three.

[52] Trophic levels describe the intricate interactions in an ecosystem's food web, with large predators and carnivores generally at the top, and microorganisms and plants at the bottom.

(Rubenstein et al. 232). Barring any ecological upheaval or natural disaster, the rewilded species is believed to be able to fill the vacant niche and adapt to its former environment.

There are many ecological variables that could change even within a few hundred years, however, which explains why rewilding efforts require extensive research to evaluate how a species' restoration will impact an ecosystem. The "top-down" hypothesis and keystone theory help determine how the species interacted with its environment in the past, and how it might do so after reintroduction. The keystone species theory suggests that "keystone" species are pillars of biodiversity in their ecosystems (Paine 1966, Terborgh et al. 1999). Similarly, the "top down" hypothesis argues that large predators and carnivores prevent loss of biodiversity by managing prey species' population levels.

The Keystone Species Theory

Predatory starfish. R. Paine formulated the keystone species hypothesis[53] while studying marine ecosystems in the 1960s (Soulé et al. 23). When his team removed a predatory starfish (*Pisaster ochracues*) from its environment, the starfish's disappearance triggered extensive changes in the population densities of all other species in the community ("Keystone Species" 1996). This particular starfish, the first identified keystone species, governs the biodiversity of an entire intertidal zone (Mills et al. 1993). As a result of his study, Paine concluded that keystone species are essential regulators of biodiversity and stability (Soulé et al. 22, "Keystone Species" 1996).

[53] A keystone species is a focal species whose impact on an ecosystem is disproportionate to its biomass, or its numerical population (Paine 1966).

Following Paine's experiment, other studies have identified more keystone species that transform landscapes, such as elephants; provide critical prey resources, such as prairie dogs; and regulate herbivores and mesopredators, such as wolves (Soulé et al. 22). Verified by the results of other studies, Paine's keystone species theory demonstrated to conservationists that the ecological impact of a single species matters (Terborgh et al. 1999, "Keystone Species" 1996). Due to a keystone species' profound ecological influence, Soulé et al theorized that rewilding an extirpated keystone species could repair a damaged ecosystem's structure (Soulé et al. 26).

The "Top-Down" Hypothesis

According to the "top-down" hypothesis, large carnivores initiate top-down trophic interactions through predatory behavior and regulate an ecosystem's structure, biodiversity, and population levels (Terborgh 1988). As evidence for this concept, studies in the United States and Venezuela show that when large carnivores disappear, ecosystems undergo drastic changes triggered by exploding herbivore and mesopredator populations (Mills et al. 1993).

Venezuela's Lego Guri Reservoir. The creation of Lago Guri reservoir rapidly flooded a large area and created small rainforest islands that were inaccessible to large predators (Terborgh et al. 1999). The sudden absence of large predators on the new islands (e.g., jaguar, puma) resulted in the superabundance of herbivores (e.g., monkeys, rodents) (Terborgh et al. 1999). Consequently, unchecked herbivore populations have severely hindered the reproduction and growth of tree species on the islands, and caused a rapid loss of biodiversity (Terborgh et al. 1999).

Coyotes in California. Another documented consequence of disappearing large predators is the corresponding hyperabundance of smaller mesopredators (e.g., small cats, foxes, and opossums); this imbalance causes sharp declines in populations of small prey animals (e.g., small birds, rodents) (Crooks et al. 1997). Studies in Southern California revealed that coyotes, the dominant large carnivore in their area, push mesopredators such as small cats out of the coyotes' scrub habitats, and thereby prevent the cats from causing overkill of small songbirds (Crooks et al. 1997).

Ungulates in North America. In North America, large carnivores have often been intentionally removed from big wilderness areas (Soulé et al. 22). The disappearance of large predators such as bears, cougars, and wolves from parts of the United States has allowed moose or elk populations[54] to explode in the absence of natural population control, triggering negative ecological consequences and that result in loss of biodiversity (McShea et al. 1977).

Complementary Theories

Studies in Venezuela and in North America demonstrate that the removal or disappearance of a large predator disrupts essential "top-down" regulation interactions, leading to competition among former prey for food and space, and resulting in habitat loss and elimination of other species (Soulé et al. 23; Terborgh et al. 1999). The "top down" hypothesis demonstrates that restoring large predators could manage populations of herbivores and mesopredators, and regulate a damaged ecosystem (Terborgh et al. 1999).

[54] Examples of problematic ungulates in North America are deer, moose, and elk.

The examples of Paine's predatory starfish and the mini islands in Venezuela are evidence that the "top down" hypothesis and keystone species theory are complementary concepts (Terborgh et al. 1999, Crooks et al. 1999). Many top predators[55] (e.g. wolves) are are also keystone species because they influence their ecosystems in a manner that is disproportionate to their relative biomass (Paine 1966). These keystone predators initiate top-down interactions in the food web via predation, and help maintain an ecosystem's structural integrity and biodiversity (Mills et al. 1993; Soulé et al. 22).

Contemporary Rewilding in North America

Using data on an extirpated species' behavior, diet and ecology, conservationists can evaluate how the reintroduction of an extirpated large predator species will impact its former ecosystem. The keystone species theory and the "top down" hypothesis suggest that rewilding a large predator or keystone carnivore could preserve an ecosystem's biodiversity, prevent habitat loss, and stabilize its species' population densities (Soulé et al 23; Terborgh et al. 1999). These theories demonstrate that rewilding a large predator can effectively regulate an environment that is being rapidly degraded by unchecked populations of mesopredators and herbivores.

Evaluating an Environment

To ensure that the target environment will facilitate a successful reintroduction, conservationists apply several types of environmental and scientific knowledge. To determine where the rewilded species might congregate after its release, ecologists gather

[55] In this thesis, the terms "large carnivore", "top carnivore", "top predator", and "large predator" will be used interchangeably, and will be treated as equivalent.

information on the distribution of vegetation and fresh water, the frequency and location of environmental disturbances[56], and the population distributions of other species in the system (Soulé et al. 24). Additionally, the analysis may include phenomena such as migration patterns, breeding areas, towns, and endemism[57] hotspots (Noss 1996).

Next conservationists must ensure that the habitat is protected and prepared to support the large predator species. Characteristics of an ideal rewilding environment generally include (1) large, strictly-protected wilderness reserves, (2) corridors among wildlife reserves to facilitate connectivity, and (3) verified analyses of its food web and ecological structure (Soulé et al. 22). Studies by Soulé et al. indicate that large predator species require extensive space for foraging, seasonal movement, and territorial considerations (Soulé et al. 22). As a result, rewilding large predators requires huge areas of protected land, and creating corridors between wildlife reserves to increase the amount of space available to the species.

Implementation

After identifying the best habitats for rewilding, conservationists calculate the number of animals needed for the initial reintroduction, and prepare the animals for life in the wilderness[58]. Prior to their release into the ecosystem[59], the selected species may spend time in a protected acclimatization area. Once the species adjusts to the new habitat, small populations (analogous to colonists) are released at pre-determined

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[56] Such as floods and earthquakes.

[57] Endemism: where species are concentrated in an environment.

[58] Some species may have been raised in captivity, and would need to be taught survival skills.

[59] Przewalski's horses spent a year acclimating to Mongolia prior to release (Moehlman 2002).

locations. Local wildlife authorities monitor the new population extensively at first, especially if the first population fails to grow[60]. In an ideal situation, the rewilded species requires less assistance over time because it achieves a stable population growth rate, and the ecosystem begins to self-regulate (Soulé et al. 24).

Rewilding Wolves in Yellowstone National Park

Large carnivores and top predators exert strong influence over their ecosystems, and their removal leaves the environment susceptible to damage from exploding herbivore and mesopredator populations. An analysis of rewilding with large predators in Yellowstone National Park exemplifies how the rewilding of a large predator can impact an ecosystem's biodiversity, resilience, and ecological structure (Foreman 2004; Mills et al. 1993).

Concept and Rationale

Following the extirpation[61] of wolves (*Canis lupus*) from the Yellowstone National Park area in the late 1940s, the park's population of northern elk (*Cervus elaphus*) increased dramatically in the relative absence of primary predators until it grew to 19,000 elk by the 1990s (White et al. 942; Laundré et al. 1401). As the elk population rapidly[62] approached the estimated carrying capacity[63], ecologists feared that the

[60] As seen with the Onager in Israel, which required multiple introductions over decades until population growth was finally achieved in the 1990s (Rubenstein et al. 238).

[61] Extirpation is a local extinction in which a species ceases to exist in a specific area, but still exists elsewhere.

[62] Prior to wolf restoration, adult female elk survival was around 0.99, indicating the probability of near-constant increasing population, with no foreseen dip in population growth (White et al. 942, 957).

[63] White et al. 942 state that Yellowstone National Park's elk carrying capacity is between 20,000 and 25,000 elk.

increasing elk density would eventually overwhelm the park's diverse ecosystem[64] (White et al. 953).

In an effort to curb population growth, the authorities issued hunting permits and strategically removed individual animals from the park; however, these measures were insufficient to substantially reduce the elk population (White et al. 950). Elk over-grazing of riparian vegetation severely depleted the primary food source of local beavers, driving the beavers away from large valleys in the park, causing beaver ponds and riparian habitats to diminish (Soulé et al. 23). Additionally, inter-species competition resulted in dwindling resources for elk and other ungulates in the region (White et al. 951). Since previous measures had failed, local wildlife authorities looked to rewilding with wolves as a potential solution.

Although bears roam the park freely, wolves, a keystone species, were absent for over fifty years (Laundré et al. 1401). According to the keystone species hypothesis, a strategic rewilding of Yellowstone National Park with wolves could potentially regulate the ecosystem by initiating "top-down" trophic interactions, and preventing the hyperabundance of elk via predation (Terborgh et al. 1999). Additionally, since wolf packs often prey on old or sick animals, re-introducing wolves could "conceivably contribute to higher reproductive and survival rates for [healthy] elk" by removing weaker elk from the gene pool (White and Garrot 943).

[64] The Yellowstone National Park area supports 5 large predators and 7 ungulate species other than wolves and elk.

Implementation

Between 1994 and 1995, authorities released twenty wolves into the Lamar Valley area of the park (Laundré et al. 1401). As shown in Figure 10, the wolves quickly formed several packs[65], and dispersed throughout the area (Laundré et al. 1403). This type of cooperative predatory behavior signifies the successful formation of a social community structure, and indicates that the wolves were adjusting well to the environment. Once the wolves fully adapted to life in Yellowstone Park, Crête predicted that "elk numbers would decrease to a [desirable] equilibrium level of 4,000-5,000 [animals]" (Crête 1999).

Ecological Impact

Although data is still being collected, the literature indicate that the effort to rewild Yellowstone National Park with wolves was largely successful in regulating the elk population (Soulé et al. 1998). In 2001, Laundré et al. found that the wolves were managing population levels of both bison and elk in Yellowstone; and in 2005, White et al. confirmed that the wolves had significantly decreased elk populations in a relatively short period of time (Laundré et al. 1402; White et al. 942). As a result, since the wolves' reintroduction into the park many of the "habitat-creating" species, e.g. beavers, have been returning as their food sources recover from elk over-grazing (Soulé et al. 24).

However, the wolves' reintroduction also had unanticipated effects. In 2000 Laundré et al. determined that the wolves' sudden reappearance[66] induced a negative

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[65] This type of cooperative predatory behavior is well-documented in wolves, and is particularly effective against adult elk. It also indicates successful acclimatization to the new environment (Laundré et al. 1402).

[66] Most of the elk in 1994-1995 had never encountered wolves.

behavioral change[67] in the elk population, resulting in a state of hyper-vigilance which he called a "landscape of fear" (Laundré et al 1409). While this term is a sort of anthropomorphism, Laundré et al. observations confirm that the state of constant vigilance increases physiological stress in adult elk, and decreases their survival rate[68]. Transmission of this behavior from parent to offspring will reduce survival rates for Yellowstone's future elk population as well. If this situation is left unmonitored, it may result in a constant decline in the elk population. If the wolf population constantly increases[69], the negative behavior change and resulting decreased elk survival rates could undermine a long-term balance between wolf and elk populations in the park.

In addition to the elks' behavioral change, other consequences include the unexpected movement of wolf packs in close proximity to towns and tourist sites, which increases the likelihood of human-predator interaction, and necessitates constant monitoring to prevent negative encounters (White et al. 942). Additionally, the wolves preyed more on elk, and less on other ungulates, than what was statistically predicted (White et al. 942). This data led White et al. to believe that wolf-imposed population control may be too efficient over long periods of time, and without strategic intervention from local authorities, unchecked wolf predation and with seasonal hunting could decimate the elk population (White et al. 942). These challenges notwithstanding,

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[67] Elk spent less time feeding and foraging, and more time on guard for predators.

[68] Through "lower body masses…of females; lower survival rates…during stress periods such as winter; and lower birth masses of calves in the spring" (Laundré et al 1409).

[69] Recent assessments by Wilkinson 2016 indicate that wolf populations are stable and increasing gradually.

preliminary data suggest that the wolves are reassuming the ecological role they filled in Yellowstone park for millennia (Donlan 76).

Yellowstone Park Consensus: Success

Although the Yellowstone Park rewilding initiative is relatively new[70], a recent *National Geographic* study suggests that the rewilded wolves are tipping the ecosystem back into balance (Wilkinson 2016). The scientific literature[71] confirm that the wolves are positively influencing the park's biodiversity by reducing the inflated ungulate populations via predation[72] (Soulé et al. 23, Wilkinson 2016). Their reintroduction seems to have reversed the negative trophic cascade[73] triggered by their absence (Donlan 76). The wolf population is small but self-sustaining, and their profound influence on the park's ecosystem indicates they are a keystone species (Paine 1966). Additional studies will determine if the park can support its self-sustaining wolf population, and if the ecosystem will achieve a long-term balance between wolf and ungulate populations.

[70] The wolves were reintroduced to the park in 1994 and 1995 (Laundré et al 1401).

[71] Laundré et al 2001; White et al 2005; Terborgh et al 1999; Soulé et al 1998.

[72] Since the wolves impact the ecosystem in a manner that is disproportionate to their biomass, they are a keystone species (Paine 1966).

[73] Donlan writes that the wolves' disappearance "propagated a trophic cascade from predators to herbivores to plants to birds and beavers", but these negative reactions are being gradually reversed (Donlan 76).

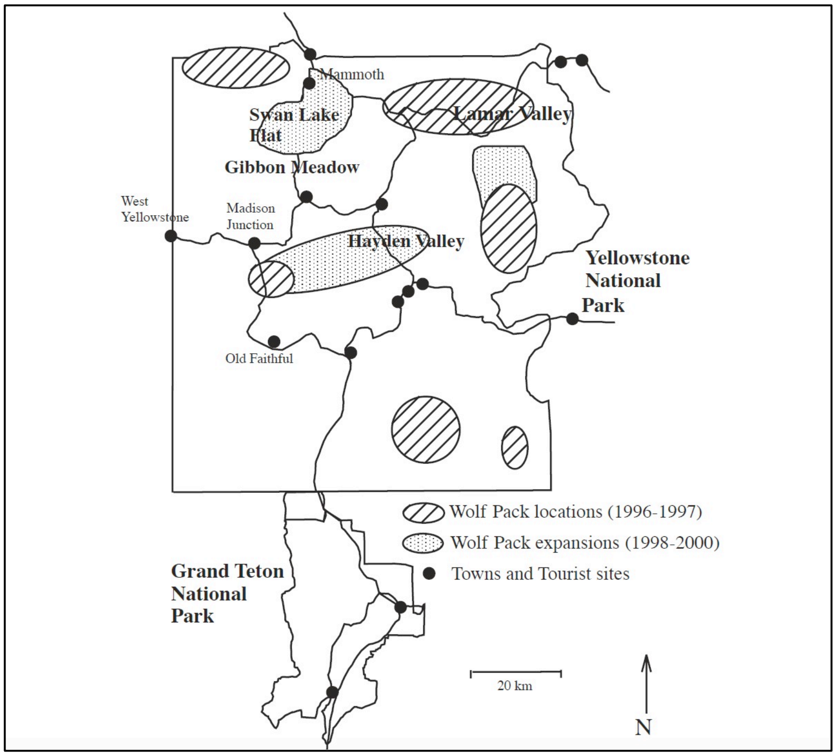

Figure 10. Locations of wolf packs after reintroduction in to Lamar Valley in 1994-1995, and their subsequent territories by 1998-2000 (Laundré et al. 1403).

Pleistocene Rewilding in North America

Contemporary rewilding focuses on restoring populations of recently-extirpated species into their ecosystems. Working with recently-extirpated species (absent for less than 200 years) allows insufficient time for evolution to change the extirpated species or its environment[74] (Rubenstein et al 234). However, in 2004, David Foreman proposed a plan to rewild North America with large mammals (megafauna) whose ancestors went extinct on the continent after the Pleistocene Epoch[75] (2.6 to 0.01 Ma) (Foreman 2004).

[74] And consequently, decreases the probability that the extirpated species would no longer fit into its former ecosystem.

[75] The Pleistocene epoch: refers to a geological time period that preceded the Holocene epoch. Pleistocene ecosystems were dominated by megafauna. See Figure 6.

His “Pleistocene rewilding” plan would reintroduce (1) extant substitutes for extinct Pleistocene species, or (2) evolutionary descendants of Pleistocene species, into modern North American systems to recreate a semblance of the Pleistocene ecosystems from 13,000 years ago (Callicott 2002).

North American ecosystems during the Pleistocene Epoch were dominated by megafauna and reached their pinnacle approximately 13,000 years ago. Around 12,000 years ago, North America experienced a decline in biodiversity that lasted 2,000 years and resulted in the extirpation of many megafauna species (Soulé et al 22, Soulé 1998). Two major factors are cited as the cause of this mass extirpation, the first being the arrival of humans from Asia, and the second being climate change at the start of the Holocene epoch[76] (Ward 2007, Zimov 796).

After humans migrated to North America, the continent’s megafauna began to disappear due to hunting pressure (Ward 2007). Some species managed to escape the human hunters but died out by the time of the Holocene warming[77]. As a result of the “Pleistocene Overkill” and a rise in global temperatures, most of North America’s megafauna such as ground sloths, mammoths, and saber-toothed tigers, went extinct along with their ecosystems (Zimov 797, Donlan et al. 2005). Foreman’s plan would restore the missing ecological functions of these extinct Pleistocene megafauna using extant substitutes and related taxa (Foreman 2004).

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[76] The Holocene epoch is the age of humans; from 10,000 years ago to present day. See Figure 6.

[77] A rise in global temperatures at the dawn of the Holocene Epoch (Zimov 796).

Ecological Argument

As justification for the radical plan, Donlan et al. argue that Pleistocene rewilding would boost the "ecological potential of some of North America's ecosystems by re-introducing predators", which would restore the "evolutionary potential" of North American species through predation (Donlan et al. 2005). The strategic reintroduction of large predators and herbivores incorporates organisms across several trophic levels, and would restore necessary ecological processes (e.g. grazing) to Great Plains and Southwestern ecosystems (Donlan et al. 2005; Donlan et al. 660, Rubenstein et al. 233). Also, Donlan et al. imply that Pleistocene rewilding redresses the "excesses" of modern humanity's ancestors that catalyzed the decline North American biodiversity thousands of years ago (Ward 2007, Rubenstein et al. 233).

Additionally, Donlan et al. propose that Pleistocene rewilding will provide economic and conservation benefits to the American public. Establishing reserves for African megafauna creates "new, and presumably better protected, populations" in North America and enhances the survival of threatened African species (Donlan et al. 2005). Donlan also suggests the African megafauna will increase the appeal, economic value, and social benefits of private and public parks (Donlan et al. 666). These "Pleistocene wildlife parks" will attract visitors and strengthen public support for conservation worldwide.

Implementation

Following Foreman's proposal, in 2005 Donlan et al. published an ambitious plan to rewild North American ecosystems with African megafauna to serve as substitutes for extinct Pleistocene species (Donlan et al. 2005). Donlan's initiative would translocate

African lions, cheetahs, and elephants to North America to partially restore the ecological processes of grazing and predation systems that lacked them for millennia (Donlan 70). Reintroductions would occur gradually in the Great Plains and the Southwest, primarily on large private or public lands with low human population densities (Donlan 73). For the first stage, Donlan posits that small experimental groups of cheetahs, lions and elephants could be immediately rewilded on private property, but the larger-scale rewilding initiatives of the second stage must wait until adequate land reserves are available (Donlan et al. 670, 674). Despite his publications in *Nature, The American Naturalist*, and *Scientific American,* Donlan's theory remains untested (Donlan et al. 2005, 2006, 2007; Rubenstein et al. 2006).

Pleistocene Rewilding in Siberia

Although these complementary Pleistocene rewilding proposals have been around since the early 2000s[78], Donlan and Foreman's concepts have been stalled by complex challenges and speculation over the project's feasibility (Rubenstein et al. 2006). Many of these difficulties stem from Donlan's inclusion of unpredictable and dangerous megafauna, such as cheetahs and lions, which pose a risk to public safety. Since its final stages often include rewilding of large predators, Pleistocene rewilding theory is largely untested except for one project in the Yakutia region of northern Siberia (Zimov 697). In the late 1980s, Zimov and colleagues designed a Pleistocene rewilding effort to restore substitute species and extant conspecifics of Pleistocene megafauna to northern Siberia. Their goals are to determine the role that Pleistocene mammals played in preserving their

[78] Foreman released his thesis *Rewilding North America* in 2004, and Donlan first proposed his idea in the scientific journal *Nature* in 2005. Additionally, Paul Martin in 1999 released a paper in *Wild Earth* that proposed bringing elephants back to North America (Martin et al. 1999).

own ecosystem, and to recreate vast Pleistocene-like grasslands to mitigate the effects of global warming[79] (Zimov 796).

The initial phase of experiment will gather the surviving Siberian megafauna of the mammoth ecosystem (moose, reindeer, and musk-oxen) and fence them into the grassland areas of the park (Zimov 798). Large predators will be excluded at first to remove the stress of predation (Zimov 798). When the herbivore population levels are sufficient to impact the vegetation and soil, the fenced boundary will be expanded during the second phase. In the third and last phase, Siberian tigers (proxies of Pleistocene cave lions) will be rewilded to act as a natural population control, and complete the partial construct of the Siberian Pleistocene grassland ecosystem (Zimov 796 – 797).

Re-creating Siberian Pleistocene Grasslands

Siberia's vast Pleistocene grasslands were populated by megafauna such as mammoths, bison, reindeer, musk oxen, moose, and cave lions (Zimov 796). About 10,000 years ago at the beginning of the Holocene epoch, this vast grassland ecosystem disappeared, and as replaced by windswept tundra (Zimov 796). Similar to the situation in Pleistocene North America, Siberian Pleistocene megafauna disappeared due to hunting pressure from humans, and rising temperatures during the Holocene warming (Zimov 798, Donlan et al. 660). Moose and reindeer are the only surviving herbivores from that era, and the mammoth grassland ecosystem vanished with its species.

[79] A tremendous amount of carbon is sequestered in Siberian permafrost soil from the former Pleistocene ecosystem. If left unconsumed by vegetation, the amount of carbon that will be released by rising global temperatures surpasses the total carbon content of the planet's rainforests (Zimov 796). Reintroducing Pleistocene-like mammals will begin the process of breaking the permafrost and allow grasses to grow and trap this carbon in the soil.

The modern Siberian tundra is dominated by unproductive mosses and shrubs. These mosses insulate the ground and insulate the underlying permafrost against thawing, thereby sequestering the soil's nutrients in a layer of ice, and preventing them from cycling through the ecosystem (Zimov 797). However, the mosses are vulnerable to physical disturbance, which will rupture the frozen layers and thaw the soil. This process releases nutrients from the permafrost and makes them available to grasses and other plant species. Large herds of herbivores are the best source of this physical disturbance; they trample and destroy the mosses' frozen ecosystem, and increase the availability of nutrients to grasses in the soil, which in turn allows the grass to grow (Zimov 797).

Currently the overall species composition of the Siberian tundra includes several large mammals (e.g., reindeer, moose, horses, musk oxen), many species of smaller mammals, and many predator species (e.g., wolves, bears, lynxes, wolverines, and foxes) (Zimov 798). Strong hunting pressure from the abundant predators have kept herbivore numbers too low to substantially impact the system's vegetation, so tundra vegetation remains the dominant shrubbery and prevents grasses from taking root (Zimov 798). As the first step in the Pleistocene rewilding effort, Siberian authorities reintroduced musk-oxen into grassland remnants in the Yakutia region of Siberia in 1989 (Zimov 2005).

Next Steps and Projected Results

Initial results from Siberia's Pleistocene rewilding indicate that grassland areas are gradually increasing after the rewilding of musk-oxen, but herbivore densities are still too low to impact vegetation, or to support the reintroduction of large predators (Soulé 1990; Martin and Burney 1999). The next steps in the experiment, and the most important reintroductions, are the rewilding of large bison herds translocated from Canada, and the acclimatization of Siberian tigers (Zimov 798).

Bison herds will greatly increase the amount of "physical disturbance" on the Siberian tundra and rapidly break down the moss and permafrost ecosystem. Consequently, Zimov posits that the total amount of grassland area should increase significantly after rewilding the bison herds (Zimov 798). Once the herbivore populations begin to change the ecosystem into productive grassland, and herbivore densities are sufficiently high, Zimov will apply for permission to rewild Siberian tigers, and complete a partial Pleistocene-like grassland system (Zimov 798). The literature consensus indicates the initial results from Zimov's Siberian Pleistocene rewilding experiment are positive, yet more data is necessary to definitively classify it as a success (Zimov 2005, Soulé 1990, Martin and Burney 1999).

Figure 11. An artist's representation of a late Pleistocene grassland ecosystem, supported by the Siberian fossil. Species represented are mammoths, equids, wooly rhinoceroses, and European cave lions (Turner 2004).

Challenges of Contemporary and Pleistocene Rewilding

Defining Success

Contemporary rewilding experiments involving recently-extirpated species demonstrate larger probabilities of success than those projected for Pleistocene rewilding. Measurement of success in rewilding is defined as (1) sustained population growth of the restored species, (2) widespread integration and adjustment into the target environment and ecology, and (3) minimal need for long-term financial investment and human intervention. Most rewilding initiatives require assistance at the beginning, but a successful experiment would achieve near autonomy. As more rewilding efforts reach the implementation stage and current experiments achieve population growth, researchers will be able to ascertain possible complications much more efficiently.

Contemporary Rewilding with Large Predators

Conservationists rewilded wolves in Yellowstone National Park to restore the essential ecological process of predation that had previously been absent for half a century. In the wolves' absence, exploding ungulate populations triggered a cascade of trophic interactions that negatively impacted the park's vegetation, smaller herbivores, and biodiversity (Laundré et al. 1401). According to the keystone theory and top-down hypothesis, rewilding the wolves would naturally regulate ungulate populations (specifically elk) and initiate restorative top-down trophic interactions via predation (Paine 1966, Terborgh et al. 1999). Once elk and ungulate populations are managed, the rest of the park's processes should begin to stabilize (Soulé et al. 23).

Ecological Challenges. Although contemporary rewilding efforts use recently-extirpated species, Rubenstein remarks that "unanticipated biological constraints", such as diseases or abrupt environmental changes, "suggest that even reintroductions of native species to their historical habitats are not assured of succeeding"[80] (Rubenstein et al. 236). Sometimes one rewilding attempt is not enough[81], and a species requires multiple re-introductions before it achieves a stable growth rate (Rubenstein et al. 236). The case of the Onager in Israel, involving a large herbivore, documents that rewilding attempts can fail in a species' ancestral habitat due to unforeseen challenges. In this case, the first group's uneven ratios of male to female offspring prevented the population from reproducing sufficiently, and required multiple reintroductions (Moehlman 2002). As demonstrated by the Yellowstone National Park wolves, the rewilded large predator species may have negative consequences on the prey species in their ecosystem, or encroach upon communities in the surrounding areas[82].

Consensus. Despite these challenges, the most recent data from the Yellowstone National Park rewilding indicates that the wolves are thriving after their reintroduction. In May of 2016, *National Geographic* magazine will release an issue dedicated entirely to this famous rewilding experiment, and documenting the wolves' success in regulating the ecosystem's ungulate populations, fostering biodiversity, and restoring the park's ecosystem to a "primordial state" (Wilkinson 2016). Therefore, the consensus view of the

[80] Efforts to rewild the Grevy's zebra in Eastern Africa failed due to naïveté towards predators and unanticipated changes in the environment (Rubenstein et al. 236).

[81] Attempts to rewild the Onager in the Negev Desert required multiple introductions for decades until population growth was finally achieved at the end of the 1990s (Moehlman 2002).

[82] As demonstrated by the wolves in Yellowstone National Park.

the rewilding of large predators in Yellowstone Park (wolves) was successful, and supported predictions made using the keystone species theory and the "top-down" hypothesis.

Pleistocene Rewilding with Large Predators in North America

Following David Foreman's 2004 proposal in *Rewilding North America*, Donlan et al. formulated a complex plan to rewild areas of Central and Southwestern North America with megafauna translocated from Africa (e.g. cheetahs, lions, and elephants) (Donlan et al. 2006). Using extant conspecifics and related species, Donlan et al. hoped to restore strong interactors to North American food webs, and consequently restore ecological functions (via grazing, predation) and evolutionary potential of North American species (Donlan et al. 660). The plan would begin initially with small reintroductions of the three African species on private property, and eventually move to larger wildlife reserves as it gains momentum (Donlan et al. 670). In addition to conservation benefits for both North American and African megafauna, they believed these "Pleistocene parks" would generate revenue and renew public interest in conservation efforts (Donlan et al. 666).

Ecological Challenges. Donlan believes that rewilding African megafauna will restore North American ecosystem functioning to desirable Pleistocene levels (Donlan 2005). However, Donlan et al. implies that conservationists are not entirely certain what those desirable levels are, since "Pleistocene rewilding offers an experimental framework to better understand the biology of a continent that vanished 13,000 years ago" (Donlan et al. 664). Scientists cannot ascertain the entirety of complex biological interactions of

Pleistocene ecosystems from the fossil record. Before making an investment in Donlan's proposal, ecologists may consider that current knowledge is insufficient to describe optimum levels of Pleistocene ecosystem function.

Since these ecological interactions are not fully understood, adding competent predators into naïve ecosystems could have disastrous consequences for American megafauna. Behavior changes due to predation[83] and transmission of diseases[84] are examples a few of the problems that could arise when translocating African species to North America. Additionally, exotic megafauna would be translocated into North American "temperate grasslands and shrub-steppe habitats, which are among the most threatened, but least protected, ecosystems in the world" (Hoekstra et al. 2005). Conservationists must weigh the chance of repairing North American ecosystems against the overwhelming probability of losing these ecosystems completely (Rubenstein et al. 234). In the worst case scenario, the Pleistocene rewilding plan to save African megafauna could potentially drive North American species to extinction[85].

Evolutionary Discrepancies. Rubenstein et al. indicate that modern-day elephants, cheetahs and lions have experienced enough genetic drift over 13,000 years to significantly differentiate them from their extinct Pleistocene ancestors, yet Donlan's Pleistocene rewilding proposal lacks sufficient consideration of the evolutionary changes that have occurred in North American ecosystems and African megafauna. (Rubenstein et

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[83] Landscape of Fear cited by Laundré et al. about elk behavior in novel presence of wolves.

[84] Grevy's zebra rewilding efforts were thwarted by unexpected diseases. Rubenstein et al. 232

[85] Rubenstein et al. argues if ecologists are so concerned about North American ecosystems that they are willing to entertain Donlan's drastic measures, conservation efforts should logically invest in direct conservation efforts to protect threatened North American megafauna such as the puma, or mountain lion (Rubenstein et al. 235).

al. 235). Studies of zebra rewilding in eastern Africa[86] demonstrate that rewilding a recently-extirpated species in its ancestral habitat does not guarantee success[87]; yet, Donlan and colleagues give scant attention to the difficulties of translocating African megafauna to an ecosystem where their ancestors were absent for 13,000 years (Moehlman 2002).

Additionally, evolutionary drift may result in the rewilded African conspecifics being received as exotic or invasive species by North American ecosystems. The potentially negative effects of transplanting exotic species to non-native environments are well-documented in Ricciardi et al.'s report regarding how biological invasions of exotic species could result in an ecosystem's collapse (Ricciardi 2000). Transmission of diseases, unpredictable changes, and destructive ecological interactions[88] are some of the negative consequences that are triggered by the introduction of invasive species, and could occur after the introduction of African megafauna to North America as well.

Containment Challenges. An obvious challenge would be the fact that it is nearly impossible to test Pleistocene rewilding on a small and financially feasible scale in North America due to the habits of the species involved. Donlan et al. proposes that cheetahs, elephants and lions be first introduced on small pieces of private property to facilitate a safe, controlled reintroduction (Donlan et al. 670). However, cheetahs and elephants require enormous roaming ranges, and and have behaviors that are difficult to predict and

[86] Attempts to rewild Grevy's zebra in Eastern Africa were thwarted by disease, naïveté to predators, and unexpected environmental changes (Moehlman 2002).

[87] Another example is the case of the Onager in Israel, which required multiple reintroductions and constant monitoring over decades before achieving stable population growth in the 1990s (Rubenstein et al. 234).

[88] See Footnote 37.

control. Elephants are especially notorious for smashing fences and straying outside the protection of park boundaries (Dublin 1997). Consequently, it seems naïve to assume that these species could be successfully contained on a small scale prior to release in a larger wildlife reserve, and similarly improbable that a wildlife reserve would always contain them securely.

Additionally, as the unanticipated territorial expansions of the wolves in Yellowstone demonstrate[89], the re-wilded African megafauna could expand far beyond the intended ecosystem and wreak havoc on surrounding communities and damage local infrastructure. Elephants are notorious for flattening fences, destroying crops, and escaping wildlife preservations (Dublin 1997). Rubenstein et al. remarks that Donlan's plan if implemented could result in "anti-conservation backlash… [from North American] farmers coping with crop destruction by herds of elephants, or lions and cheetahs attacking cattle, or even children" (Rubenstein et al. 237).

Public Safety Concerns. In contrast to the Siberian Pleistocene rewilding effort that occurred in a sparsely-populated area, Donlan's plan would introduce large, formidable predators into developed regions with relatively high population densities, such as Texas[90] (Donlan et al. 2005, Rubenstein et al. 234). In 1999, Lauber et al. report that North American communities resist rewilding with native predator species, and even with relatively harmless ungulates (such as the moose) (Lauber and Knuth 1999). With local conservation efforts to preserve native species already meeting resistance in the

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[89] See Figure 10.

[90] A map of Donlan's "Pleistocene Park", published in *Nature*, includes much of Texas and the central United States.

United States, it follows that a plan to translocate modern proxies of extinct Pleistocene predators from another continent will face aggressive opposition as well (Shay 2005). One can only imagine the Texan reaction to such a policy.

Public safety concerns also hinge on the fear of predators generally[91], and on the possibility that a translocated African predators will prey upon humans. In Kenya, instances of lions actively preying upon humans and livestock, such as the "Tsavo Man-Eaters" that killed dozens of railroad workers in the 1890s[92], are well-documented and provide reason for concern (Rafaele 2010). More recently, deadly lion attacks in Tanzania highlight the danger of living in close proximity to predators, even when lions are largely contained in a wildlife reservation ("Tanzania Lions" 2006). In addition to attacks on joggers in North America[93] by endemic mountain lions, North American citizens have ample justification for concern about rewilding the Southwest and Central United states with translocated African lions and cheetahs.

Consensus. The literature consensus[94] is largely against Donlan's version of Pleistocene rewilding in North America, and it is unlikely that Donlan's Pleistocene Park in which giraffes, elephants and lions roam freely across the central United States, will be established in the future. The proposal's inclusion of large, formidable predators was particularly problematic due to the unpredictability of their ecological interactions with

[91] Donlan et al. calls the North American population's fear of predators a post-Columbian phenomenon and bias against the natural process of predation (Donlan et al. 661).

[92] The two lions were killed by Lt. Col. Patterson, and are on display in the Field Museum in Chicago.

[93] "List of Fatal Cougar Attacks in North America" up to March of 2016.

[94] Rubenstein et al 234 lists multiple sources.

North American fauna, and the probability that they would pose a risk to surrounding communities (Rubenstein et al. 234).

Pleistocene Rewilding in Siberia

Preliminary data from the Siberian Pleistocene rewilding experiment is positive, and large herbivores are disrupting moss-permafrost ecosystems and allowing grass to take root in areas of the Siberian tundra (Rubenstein et al. 232). Herbivore population densities are increasing steadily, but are still relatively low, and Zimov and colleagues have yet to secure permission for rewilding a tiger (Lewis 2012).

Ecological Challenges. the Siberian Pleistocene rewilding experiment shows that decades are necessary to ascertain sustainable results from rewilding initiatives (Zimov 2005). The aforementioned rewilding effort involving recently-extirpated species required extended oversight[95]; the Siberian experiment began in 1989 and has yet to reach the second phase of sufficient herbivore population densities (Zimov 2005). Therefore, it is reasonable to assume that both contemporary and Pleistocene rewilding requires prolonged oversight and extended investment of time, research, and resources.

Projected Success. When Siberian authorities are able to translocate herds of bison from Canada and other large herbivores to increase population densities, Zimov suggests the grasslands will grow at an accelerated rate, due to the increased physical disturbance on the frozen moss and soil (Zimov 796). It is probable that the Siberian tundra experiment will continue to produce positive outcomes in the next few years, and

[95] The wolves are yielding definitive data in 2016, but were released in 1995 (Wilkinson 2016).

that Zimov's chances of eventually rewilding tigers in the sparsely-populated Siberian tundra, are greater than the chances of rewilding large cats (e.g. lions, cheetahs) in North America.

Implications for Future Rewilding with Large Predators

Rewilding experiments have been limited, and have produced some successes in recent years (Wilkinson 2016, Rubenstein et al. 2006). This chapter underscores many of the issues that may arise when rewilding recently-extirpated species, or a conspecific of extinct Pleistocene megafauna. Compared to rewilding with large herbivores, rewilding involving large, dangerous predators tends to encounter more challenges throughout the reintroduction process, and to require extensive oversight thereafter[96] (Donlan et al. 670).

The wolves of Yellowstone National Park are a prime example of the barriers that complicate the rewilding of large predators in terms of public interest and outcry, barriers to environmental integration, and possibly adverse effects on their ecosystems (Laundré et al. 1401, White et al. 942). Despite these challenges, an emerging study in *National Geographic*'s 2016 May issue indicates that the wolves have effectively regulated ungulate populations in the park's ecosystem, and are currently maintaining a predator-prey population balance with assistance and monitoring by local wildlife authorities (Wilkinson 2016). Additionally, the wolves of Yellowstone support the keystone species theory and the "top-down" hypothesis by demonstrating the potentially positive outcomes of restoring necessary ecological functions (in this case, predation) to a damaged ecosystem (Terborgh et al. 1999, Paine 1966).

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[96] Przewalski horses were rewilded in the early 2000s in semi-reserves in Mongolia's Gobi desert region; and after a few years, required less monitoring and intervention than the wolves of Yellowstone, which were re-wilded in the 1990s, and still require close observation (Moehlman 2002, Wilkinson 2016).

The wolves were absent from Yellowstone Park for approximately 50 years, and as a result, the species and the Yellowstone ecosystem remained relatively unchanged by evolution (Rubenstein et al. 232). The challenges inherent in Donlan's Pleistocene theory indicate a rewilding experiment's probability of success may be inversely proportional to the length of time between the species' extirpation and reintroduction. Rubenstein et al. remark that rewilding a recently-extirpated species in its ancestral habitat does not guarantee success; yet, Donlan and colleagues do not adequately consider how evolutionary drift may hinder extant conspecifics of Pleistocene species from adapting to their ancestral ecosystems (Rubenstein et al. 234, Donlan et al. 2006).

Additionally, rewilding translocated species for Pleistocene rewilding projects requires extreme caution. The reintroduced exotic species could transmit diseases or trigger other adverse changes and thereby catalyze an ecosystem's collapse (Ricciardi 2000, Rubenstein et al. 232). Ecological interactions of Pleistocene ecosystems are not fully understood[97], and those of modern ecosystems are often unpredictable; therefore, carelessly adding competent predators into naïve ecosystems could have disastrous consequences for American megafauna, and for surrounding communities (Donlan et al. 2006, Rubenstein et al. 237, Rafaele 2010).

Both cases also demonstrate that the rewilding of large predators creates the need for greater focus on security and extended oversight to prevent the species from negative encounters with humans and the surrounding ecosystems, and to help the reintroduced population achieve a stable growth rate. Rewilding Yellowstone wolves poses a risk to

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[97] Donlan et al.'s observe that Pleistocene ecological interactions are not entirely understood, and neither are the intricate ecological interactions of modern ecosystems as well (Donlan et al. 664).

nearby towns and tourist areas, and has required increasing observation as the wolf population grows (See Figure 10, Wilkinson 2016). In comparison, the Siberian Pleistocene experiment has required extended investment and oversight to sustain the rewilded large herbivore populations (Zimov 2005). These instances imply that rewilding requires a collaborative effort between conservationists, ecologists, local authorities, and surrounding communities to invest in the effort's sustainable success, and to maintain the process of extended oversight in the future.

The rewilding of wolves in Yellowstone park may also provide insight into the ecological and experimental challenges of rewilding an ancient large predator species[98], such as *T. rex*. An ancient predator species would face some of the same barriers to environmental integration that were encountered by the Yellowstone wolves; and according to the "top down" hypothesis, could also be expected to influence modern environmental interactions in a manner analogous to extant large predators.

Analyzing the difficulties of Pleistocene rewilding may also benefit future experiments using substitute (or possibly ancient) species of large predators. Examining the criticism surrounding Donlan's proposal helps researchers avoid incorporating fundamental flaws into an experimental design. Also, once the Siberian Pleistocene rewilding gains momentum, its long-term results may direct future Pleistocene rewilding initiatives. Lastly, as scientists learn more about ecological interactions through future Pleistocene rewilding experiments, their observations will help establish a framework for studying an ancient system's paleoecology using evidence from the fossil record.

[98] If the Siberian experiment had progressed to stage 3 and reintroduced Siberian tigers, its results would be valuable for this sort of experiment as well.

Cretaceous Rewilding

Knowledge of the complex challenges encountered by contemporary and Pleistocene rewilding has revealed several problems that may arise if scientists brought a *T. rex* back into the modern world. Some of the public safety and animal containment concerns faced by the Yellowstone wolves are likely to rewilding an ancient predator as well, along with many of the ecological and evolutionary hindrances faced by Pleistocene rewilding efforts. The technical and biological difficulties of resurrecting a *T. rex* aside, rewilding this species would be impeded by seemingly insurmountable challenges.

Ecological Argument

There is no modern analogue for a top predator and ecological opportunist such as *T. rex*. Although the dinosaur's physiology could be mammalian, reptilian, or in-between[99], the only large modern species that exhibit similar predatory and scavenging behavior are mammals. In regard to the scientific consensus on *T. rex's* ecology, the best comparison would be the spotted hyena. This species scavenges carcasses and engages in active predatory behavior, and is an ecological opportunist[100] similar to *T. rex*[101] (Sutcliffe 1110). The Yellowstone wolves also scavenge carcasses, but rarely or in dire circumstances ("Grey Wolf" 2016). However, hyenas have not been rewilded, thus the

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[99] New studies of dinosaur soft tissue may eventually solve the question of whether or not dinosaurs are cold-blooded like modern reptiles, warm-blooded like modern birds and mammals, or somewhere in-between (Pappas 2013, Pruitt 2015).

[100] In the 1970s, studies in Tanzania's Ngorongo crater by A. Sutcliffe demonstrate that wild hyenas are proverbial scavengers, but will chase and kill wildebeest and and zebras (Sutcliffe 1110).

[101] *T. rex* is thought to have oscillated between scavenging and predation (Brusatte et al. 2010, DePalma et al. 2013).

Yellowstone wolves provide the best comparison when examining *T. rex'*s potential reintroduction.

According to the "top down" hypothesis, rewilding large predators, such as *T. rex* and the Yellowstone wolves, in their former ecosystems restores the necessary ecological process of predation, and initiates top-down trophic interactions that regulate an ecosystem's structure, biodiversity, and population levels (Terborgh 1988, Mills et al. 1993). Paine's complementary keystone species theory indicates that keystone species are essential regulators and profoundly influence their ecosystems; thus their reintroduction may stabilize the entire ecosystem (Paine 1966).

As evidenced by the Yellowstone Park wolves, rewilding with a keystone large predator species initiated restorative top-down trophic interactions via predation, and is successfully managing the park's ungulate populations (Terborgh et al. 1999, Wilkinson 2016). Since *T. rex* engaged in active predation, it is likely that a reintroduced *T. rex* would influence a modern environment in a similar manner (DePalma 2013, Sampson et al. 471). *T. rex*'s proclivity for scavenging may also increase its chances of survival, as the dinosaur may be able to sustain itself on carrion[102] until it learns to hunt modern species (Ruxton et al. 731). Based upon the Yellowstone wolves' rewilding, it is possible that rewilding a *T. rex* could restore necessary predation pressure to an ecosystem that lacks this ecological process. However, it is likely that a rewilded *T. rex* would face challenges similar to those encountered by the Yellowstone wolves.

[102] Ruxton et al. calculated that an environment similar to the modern Serengeti would produce enough carrion to support an adult T. rex (Ruxton et al. 731)

Ecological Challenges

The rewilded Yellowstone wolves are well-researched, but their reintroduction still produced unexpected consequences that affected the park's ungulate populations in an unpredictable manner (Laundré et al. 1402, White et al. 942). Although *T. rex* is the most well-understood dinosaur in the entirety of paleontology, the current understanding of its *T. rex's* paleoecology is extrapolated from the fossil record and comparisons to modern species. This leaves ample room for error, especially if the fossil evidence is scarce or misleading[103], and the extant species is poorly researched (Brusatte et al. 2010). In light of these uncertainties, the previous rewilding of Yellowstone wolves indicates that *T. rex'*s behavior and ecological influence are somewhat unpredictable.

T. rex's ecology is also complicated by its tendency to shift its preference for scavenging and predation throughout its development. This characteristic equipped *T. rex* with a high level of ecological adaptability, and allowed it to survive across a range of ecosystems and environments (Sampson et al. 471). *T. rex'*s flexibility would be an advantage in adjusting to the modern world and climate, which is warmer than the Mesozoic Era (Zimov 2005). However, *T. rex's* ecological opportunism also requires a prospective modern ecosystem to support the dinosaur in both ecological roles. Finding a modern ecosystem capable of sustaining such a massive ecological opportunist may be impossible.

[103] Scientists once classified the Apatosaurus and Brontosaurus fossils as two different species for years, until discovering one day that the bones were from one species instead. They kept the name Apatosaurus, and then recently decided that Brontosaurus did exist. This controversy demonstrates that the fossil record can be unclear, and studies of the same fossil could lead to opposite conclusions (Choi 2015).

Additionally, *T. rex* has been extinct since the end of the Mesozoic Era over 66.0 Ma ago. As demonstrated by Donlan's Pleistocene proposal and the Siberian experiment, evolutionary drift can severely hinder a species' ability to adapt to its environment, even if that species is an extant conspecific of the extinct species (Rubenstein et al. 235). Modern cheetahs, lions and elephants have experienced enough genetic drift over 13,000 years to distinguish these species from their Pleistocene ancestors, and these evolutionary changes would hinder them from adapting to North American ecosystems (Rubenstein et al. 235). *T. rex* would be reintroduced into an ecosystem that evolved for 66.0 million years in its absence; therefore, it is reasonable to assume that evolutionary differences between the species and a modern ecosystem would greatly hinder the dinosaur's ability to adapt and survive in the modern world.

Containment and Safety Concerns

Yellowstone Park authorities have adopted careful policies to protect and contain the wolves within the park, and to prevent them from interacting with humans (Wilkinson 2016). Criticism of Donlan's proposal demonstrates that rewilding large, dangerous animals (such as elephants) along with formidable predators (such as cheetahs and lions) poses a serious risk to the public and the surrounding ecosystem. Elephants in particular are notorious for smashing fences and wreaking havoc on surrounding communities (Dublin 1997). African elephants can grow up to 5 meters long and weigh up to 6 tons, and *T. rex* grew up to 12.3 meters long, and weighed over 10 tons[104] (Castro 2016). Thus a mature *T. rex* is equally capable of smashing a fence as an adult elephant, and may be

[104] Yellowstone wolves grow to approximately 1.8 meters in length, and weigh up to 100 pounds ("Wolves in Yellowstone" 2016).

even more difficult to contain. Therefore, if *T. rex* were brought back and placed in a wildlife reservation, a *Jurassic Park*-like situation (in which a *T. rex* breaks through an electric fence and devours the nearest human) could occur (Crichton 1990).

Consensus

These rewilding initiatives provided a framework for understanding how large predators influence ecosystems through top-down ecological interactions, and revealed problems that arise when rewilding dangerous species (Donlan et al. 664). In light of *T. rex's* classification as an ecological opportunist and top predator, it is likely that it would influence an ecosystem in a top-down manner, similar to the Yellowstone park wolves. However, its behavior in the modern world cannot be accurately predicted from the fossil record, and the risk of introducing such a massive and formidable predator cannot be ignored. Therefore, *T. rex* is too dangerous to rewild in any proximity to civilization, and should not be brought back into a modern ecosystem.

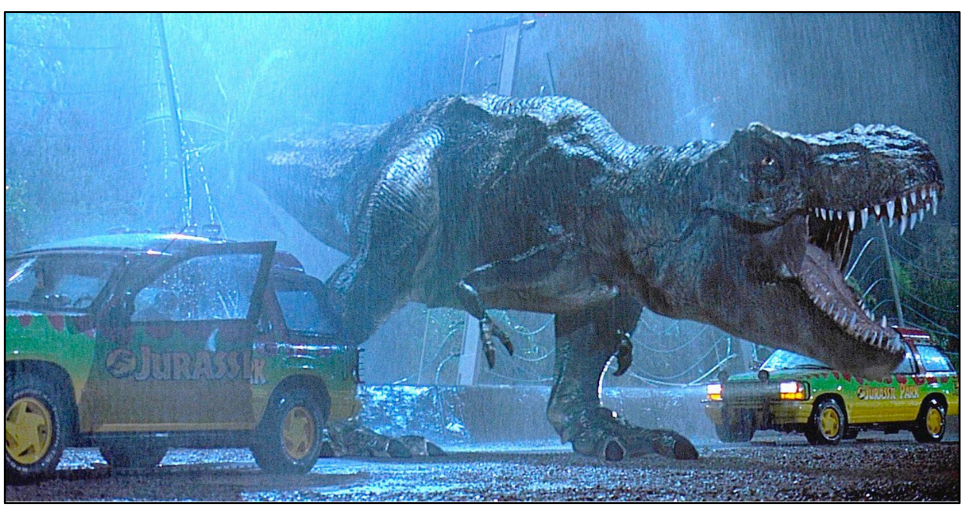

Figure 11. The famous *T. rex* from Spielberg's *Jurassic Park* (*Jurassic Park* 1993), image rights owned by Universal Studios.

CONCLUSION

"Your scientists were so preoccupied with whether they could, they didn't stop to think if they should" – Dr. Ian Malcom in *Jurassic Park*

The *Jurassic Park* series explores the ethics and feasibility of a scientific effort to bring the dinosaurs back. When *Jurassic Park*'s scientists reverse the course of natural selection by reviving extinct species, they are unable to contain or control the newly-created dinosaurs. The park's *T. rex* escapes and triggers a domino effect that results in the eventual collapse of the park's ecosystem and infrastructure, and death for many involved (*Jurassic Park* 1990). By demonstrating the possible destructive consequences of resurrecting dinosaurs, *Jurassic Park* cautions against the rash use of scientific power to reverse evolutionary processes, and suggests that dinosaurs do not have a place in the modern world.

Examining rewilding initiatives with large predators and megafauna demonstrates that resurrected dinosaurs may wreak havoc on modern ecosystems and environments. Using the example of *Tyrannosaurus rex*, the analyses in chapter three predict that a top predator and ecological opportunist such as *T. rex* would dominate modern ecosystems, and initiate top-down trophic interactions similar to modern species of large predators, albeit on a dangerously unstable level (Donlan et al. 664). As discussed in chapter three, the ecological and humanitarian risk of introducing such a massive and capable predator cannot be ignored. There is adequate reason to believe that *T. rex* is too dangerous to rewild in proximity to civilization, and should not be released into a modern ecosystem. A proper place for *T. rex* does not currently exist.

Even if there were a way to safely rewild a *T. rex*, chapters one and two demonstrate that scientists are far from understanding, replicating, or imitating any portion of dinosaur genetics and biology. The current assessment of ancient DNA research indicates that biochemical degradation may have damaged dinosaur DNA fragments beyond repair, rendering the sequencing of dinosaur genomes impossible. Without comprehensive knowledge of a dinosaur's genome, scientists lack the basic instructions for cellular machinery that would provide invaluable insight into an ancient species' proteins, traits and characteristics. As discussed in chapter one, studies indicate that future technology may be incapable of bringing a dinosaur back due to a dearth of salvageable and complete genetic information.

Additional Challenges

In scientific literature and popular press, most of the examinations of *Jurassic Park* and its theoretical dinosaur-creation enterprise center on the discovery, evaluation, and manipulation of dinosaur DNA. However, the literature often glosses over the genetic difficulties of such a project, and attributes a disproportionate amount of importance to the discovery of dinosaur DNA. The successful extraction of a dinosaur genome would be an enormous leap ahead, but the literature analyzed for this thesis emphasized DNA as the sole foundation needed for recreating an entire organism. While DNA is important, focus on that element has led to the neglect of other essential factors such as epigenetics, RNA processing, immunology, and physiology that are equally important as a DNA foundation.

RNA Editing and Epigenetics

A DNA sequence is analogous to a rough draft; without cellular machinery to transcribe, edit and translate the gene into protein, knowledge of ancient DNA sequences is insufficient for resurrecting an extinct species. Each gene sequence of nucleotides in a DNA molecule is transcribed into a complementary RNA sequence, but that sequence is normally edited and spliced (by a spliceosome) in a manner that removes and recombines portions of the molecule (Pierce 2013). One original RNA transcript can be edited to produce many different RNA sequences, and these variations are then translated into proteins by ribosome complexes (Pierce 2013). Due to the RNA editing process, the initial transcription of a DNA gene sequence may result in the production of several different proteins. Without the cellular machinery from an extinct organism, it is difficult for scientists to determine which proteins are made from ancient DNA sequences.

Additionally, without knowledge of an ancient species' cell biology, scientists cannot determine how epigenetic factors control the species' gene expression throughout its lifespan. Epigenetic factors regulate RNA transcription proteins' access to DNA, and thereby regulate gene expression. A cell's DNA is wrapped around histone protein complexes. When an enzyme adds an acetyl group to the histone, DNA's coil around the histone relaxes, making it accessible to RNA transcription proteins. When a methyl group is added to the histone complex, DNA winds tighter and is inaccessible to transcription proteins. In this manner, adding acetyl groups turns genes "on", and adding methyl groups turns genes "off" (Pierce 2013). Epigenetic interactions like these are managed by proteins, and are carefully timed throughout development. Without knowledge of a species' molecular biology and proteins, it is difficult to determine when certain genes should be expressed.

These concepts of epigenetics and RNA editing demonstrate that an ancient species' genome is only a rough draft. Epigenetics controls when genes are expressed over time, and where they are expressed throughout the body. RNA editing and splicing manages how those genes are expressed, and which proteins they produce. Both are necessary for the success of *Jurassic Park*-like experiment, because they are vital to and determine a created dinosaur's survival. Scientists have yet to determine the finer points of dinosaur molecular cell biology, and cannot ascertain this information from fragmentary DNA sequences. Until technology develops the capability to ascertain the cellular processes of extinct species, ancient DNA will remain useful, but insufficient for re-creating an extinct species, such as a dinosaur.

The Act of Creation

Scientists are currently incapable of recreating an entire organism. Genetically-engineered organisms are created by editing existing biological agents and species, and by hijacking extant cellular machinery and genomes. CRISPR, a gene-editing tool that allows scientists to (theoretically) alter genomes at will, could be used to construct the hybrid genomes[105] mentioned in *Jurassic Park* (*Jurassic Park* 1990, Parham 2005). However, most ancient DNA samples are too fragmented and damaged to provide even a small portion of a dinosaur genome; therefore, CRISPR technology cannot be used for a dinosaur experiment until sufficient genomic data exists. Using CRISPR technology to edit genomes of modern animals and thereby supplement dinosaur DNA also raises ethical questions, since the process would require genetic experimentation on modern species.

[105] *Jurassic Park*'s scientists created dinosaurs by supplementing their fragmentary genomes with gene sequences from extant species.

Dinosaur Physiology

In addition to the limitations and scant knowledge of dinosaur epigenetics and molecular biology, paleontologists are divided on the group's physiology as well. Knowledge of dinosaur physiology determines which modern species will be observed to study the extinct species' physiology, and by comparison, reveal information about the ancient species' cellular biology. Fossil evidence has yet to confirm whether dinosaurs are warm-blooded (like modern birds), cold-blooded (such as modern reptiles), or in-between. In 2015, examination of preserved soft tissue from Late Cretaceous fossils revealed that dinosaur erythrocytes are nucleated[106], whereas normal human and mammalian red blood cells are a-nucleated (Bertazzo et al. 2015). Further analysis of other soft tissue samples may reveal that dinosaur blood cells are more similar to reptilian blood cells. Once the warm vs. cold-blooded debate is settled by future studies, scientists will study the appropriate modern species for clues about dinosaur physiology.

Immunology

Even with adequate knowledge of a dinosaur's physiology and genetics, it is possible that certain biological vulnerabilities would prevent any recreated dinosaur from surviving in the modern world. The dinosaurs missed millennia of immunological evolution that selected the individuals of a species with the best antibodies[107] (and disease resistance) for survival. Since the immune system evolves with a species, *T. rex* would be over 60 million years behind schedule, and dinosaurs from the Triassic and

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[106] Erythrocytes are red blood cells, and nucleated implies that they posses a nucleus.

[107] Immune system genes are edited and recombined to produce antibodies, proteins which apprehend foreign material (and sometimes the body's own cells) to facilitate their recognition and destruction by leukocytes. These genes can be passed down through generations (Parham 2005).

Jurassic periods could be 100 million years behind the immunological evolution of modern species (See Figure 6). Without the evolutionary opportunity to develop natural immune system resistance to modern diseases, a re-created dinosaur could die from exposure to modern pathogens that are innocuous to modern organisms.

Successful pathogens are also byproducts of natural selection, and will evolve to match and beat their host's evolving immune system (Parham 2005). As pathogens evolve through natural selection, they become more resistant to the body's immune system. Since modern pathogens have evolved for between 250 to 66 million years, it is plausible that some would be increasingly deadly against extinct species that lack the antibodies and developed immunity against modern disease agents. Consequently, modern pathogens could potentially wipe out any resurrected dinosaur, and it would be highly susceptible to disease transmission from other species if released into the wild. Due to its extreme immunological vulnerability, a recreated dinosaur may require containment in a completely sterile environment throughout its lifetime.

In light of these significant obstacles to a dinosaur's re-creation and survival in the modern world, the success of a *Jurassic Park*-like experiment seems highly improbable without multiple advances in genetic engineering technology and molecular cell biology research. The available evaluations of *Jurassic Park*-like experiments give no mention of the immunological, physiological, and extended genetic difficulties that stand in the way of a dinosaur project. This could be influenced by the franchise's reliance on ancient DNA as the key to bringing back the dinosaurs, and the tendency of scientific literature in the 1980's and 1990's to over-estimate ancient DNA's utility during the advent of genetic technology (*Jurassic Park* novel 1990, film 1993). This thesis' emphasis on the limitations of ancient DNA's usefulness for a *Jurassic Park*-like experiment, and the need for additional studies of ancient and conspecific molecular cellular biology, are unique in the realm of *Jurassic Park* criticism and evaluation.

Ethical Concerns

Since modern science lacks the foundation to actually bring back the dinosaurs, serious discussions on the ethical questions of resurrecting extinct species are quite limited. If technological advances ever make such an experiment possible, more ethical discussions and analysis will be needed. If such a *T. rex* were ever created, analyses of rewilding efforts in chapter three, genetics in chapter one, and immunological evolution suggest that the dinosaur could destroy modern ecosystems, or die outside of a sterile environment. In the *Jurassic Park* film, Dr. Grant remarks, "dinosaurs and man, two species separated by sixty-five million years of evolution, have just been suddenly thrown back into the mix together. How can we possibly have the slightest idea of what to expect?" (*Jurassic Park* 1993). His comment underscores the unpredictable quality of any experiment that would bring a *T. rex* back, and the probability that humanity would be incapable of controlling the re-created dinosaur. These factors combined with the aforementioned ecological and biological barriers suggest that a *Jurassic Park*-like experiment provides limited benefits beyond the satisfaction of human intellectual and scientific curiosity.

Attempting to re-create life, along with investing in a possibly futile enterprise, raises many difficult ethical questions. Rubenstein et al. state that modern ecosystems are experiencing a conservation crisis, and the Center for Biodiversity estimates that the planet is experiencing a wave of mass extinctions at approximately 1,000 times the background rate[108] (Greenwald 2016, Rubenstein et al. 2006). In the midst of such a conservation and biodiversity crisis, it is difficult to justify investing massive amounts of resources and attention in re-creating an extinct species, while allowing modern species to continue on the path toward extinction. Criticism of Pleistocene rewilding indicated that a re-created dinosaur could destroy modern ecosystems, and serve to worsen the global conservation crisis. Investing in conservation strategies for existing species seems more logical as a step to alleviate the biodiversity crisis, than focusing on an extinct Mesozoic predator.

Finally, the fragmentary nature of ancient DNA makes it probable that resurrecting a dinosaur would require extensive editing and manipulation of the genomes of modern species. Creating life at the expense of existing animals is unethical, especially since this procedure would require multiple experimental trials using extant species. The re-created dinosaur would likely be a "chimera", its patchwork genome supplemented with genes from extant species. Public concern over other genetically modified organisms, such as plants, is widespread. It is reasonable to assume that the creation of a *T. rex* with a patchwork genome would generate controversy in the public

[108] The background rate describes the normal rate of species extinction that it expected due to natural causes and processes.

arena. As discussed in chapters one and three, bringing a formidable dinosaur like *T. rex* into the modern world could have extensive negative effects on the environment and the modern world; consequently, recreating a species with such potential for destruction seems unethical and unwise.

Future Research and Conclusion

If a *Jurassic Park*-like experiment is ever to be possible, more research is needed on the types of molecular decay that affect ancient DNA samples, and on possible methodology for reversing these processes, and for ascertaining the original gene sequences. The recent discoveries of dinosaur soft tissue indicate that more fossils may hold some of the same material. More funding is needed to re-examine known fossils, and to begin excavations for new fossils as well. New imaging technology will facilitate future studies of dinosaur soft tissue, and provide new insight into dinosaur physiology, genetics, and molecular cellular biology. In addition to these scientific advancements, renewed investment is needed to sustain current rewilding experiments, and formulate strategic implementation of new rewilding efforts. Increased analysis of the subsequent data will help determine if rewilding is effective, and maximize the concept's efficiency as a conservation strategy by allowing ecologists to learn from previous rewilding initiatives.

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[109] Norris 2015; demonstrates the intense amount of public concern over genetically-modified foods, which are largely deemed safe for consumption by the FDA.

In conclusion, the study of dinosaurs and ancient DNA has the allure of time travel into the Earth's ancient history, and the possibility of restoring magnificent and formidable animals to natural world. While attempts to recreate life and reverse the process of natural selection, such as those in Jurassic Park, are captivating and novel, they are ethically and ecologically questionable. Humanity remains insufficiently informed of our own species' developmental processes and molecular biology, and knows even less about these areas in extant species. Bringing a *T. rex* back could result in the destruction of modern systems, pose a danger to surrounding communities, or end in its quick re-extinction due to biological complications. There are matters of conservation and humanitarian interest that merit more immediate attention than an effort to resurrect a *Tyrannosaurus rex*. Therefore, researchers should revisit the question of resurrecting dinosaurs after a long period of time, when technology and science are capable of sustaining this type of experiment, and if the world is ever ready to once again support a *T. rex*.

BIBLIOGRAPHY

Andrews, Evan. "Who Invented the Internet?" *History.com*. A&E Television Networks, 18 Dec. 2013. Web.

Bakker, RT. *The Dinosaur Heresies*. William and Morrow: New York 1986. Print.

Barnosky, A.D. *Biodiversity Response to Climate Change in the Middle Pleistocene: The Porcupine Cave Fauna from Colorado*. Berkeley: University of California, 2004. Print.

Behrensmeyer, A.K. *Terrestrial Ecosystems through Time: Evolutionary Paleoecology of Terrestrial Plants and Animals*. Chicago: University of Chicago, 1992. Print.

Benton, M,J. "Studying Function and Behavior in the Fossil Record." *PLOS One Biology* 8.3 (2010). Web.

Benton, M.J. "Phylogeny of the Major Tetrapod Groups: Morphological Data and Divergence Dates." *Journal of Molecular Evolution* 30 (1990): 409. Web.

Bertazzo, S., Susannah, C. R., Maidment, C.K., Fearn, S., Stevens, M., Xie, Hai-Nan. "Fibres and Cellular Structures Preserved in 75-million–year-old Dinosaur Specimens." *Nature Communications* 6.7352 (2015). Web.

"Box Office History for Jurassic Park Movies." *The Numbers*. Nash Information Services, LLC, Dec. 2015. Web. <http://www.the-numbers.com/movies/franchise/Jurassic-Park#tab=summary>.

Brochu, C.A. "Principles of Locomotion: Functional Morphology and Artistic Reconstructions." *Society of Vertebrate Paleontology.* Department of Geological Sciences, Indiana University: 2003. Print.

Brusatte, S. L., Norell, M. A., Carr, T. D., Erickson, G. M., Hutchinson, J. R., Balanoff, A. M., Bever, G. S., Choiniere, J. N., Makovicky, P. J., Xu, X. "Tyrannosaur Paleobiology: New Research on Ancient Exemplar Organisms." *Science* 329.5998 (2010): 1481-485. Web.

Burger, J., Rosendahl, W., Loreille, O., Hemmer, H., Eriksson, T. "Molecular Phylogeny of the Extinct Cave Lion *Panthera leo spelaea*." *Molecular Phylogenetics of Evolution*. 30. (2004): 841–49. Web.

Callaway, Ewen. "Second Chinese Team Reports Gene Editing in Human Embryos." *Nature.com*. Nature Publishing Group, 08 Apr. 2016. Web.

Callicott, J.B.. “Choosing appropriate temporal and spatial challenges in the coming decades.” *Conservation Biology* 4, *Ecology Letters* 8 (2002): 23–29. Web.

Castro, Joseph. "Spinosaurus: The Largest Carnivorous Dinosaur." *LiveScience*. TechMedia Network, 18 Mar. 2016. Web.

Choi, Charles. "The Brontosaurus Is Back." *Scientific American*. 7 Apr. 2015. Web.

Clifton L. “Mad Scientists are Cloning Dinosaurs as Weapons of the Future.” *National Examiner*. August 7, (1984): 31. Web.

Crete, M. “Ecological correlates of regional variation in life history of the Moose *Alces Alces*.” *The Ecological Society of America*. 77.5 (1998): 1493-1500. Web.

Crichton, Michael. *Jurassic Park: A Novel*. New York: Knopf, 1990. Print.

Darwin, Charles. *On the Origin of Species*. Ed. David Quammen. New York: Sterling, 2008. Reprint.

Debruyne, R., Barriel, V., Tassy, P. “Mitochondrial Cytochrome b of the Lyakhov Mammoth (Proboscidea, Mammalia): New Data and Phylogenetic Analyses of Elephantidae.” *Molecular Phylogenetics of Evolution.* 26.2 (2003): 421–34. Web.

Depalma, R. A., D. A. Burnham, L. D. Martin, B. M. Rothschild, and P. L. Larson. "Physical Evidence of Predatory Behavior in Tyrannosaurus Rex." *Proceedings of the National Academy of Sciences* 110.31 (2013): 12560-12564. Web.

"dinosaur". Encyclopædia Britannica. Encyclopædia Britannica Online. Encyclopædia Britannica Inc., 2016. Web. <http://www.britannica.com/animal/dinosaur>.

Donlan, J., Berger, J., Bock, C., Bock, J., Burney, D., Estes, J., Foreman, D., Martin, P., Roemer, G., Smith, F., Soulé, M., Greene, H. "Pleistocene Rewilding: An Optimistic Agenda for Twenty-First Century Conservation." *The American Naturalist* 168.5 (2006): 660-81. Web.

Donlan, C. Josh. "Restoring America's Big, Wild Animals." *Scientific American* 296.6 (2007): 70-77. Web.

Donlan, Josh. "Re-wilding North America." *Nature* 436.7053 (2005): 913-14. Web.

Dublin, Holly. "Saving Africa's Elephants: No Easy Answers." *African Wildlife Foundation*. African Wildlife Foundation, 1 May 1997. Web.

Eglinton, G., Logan, G.A. “Molecular Preservation.” *Philosophical Transactions of the Royal Society of London Ser. Biological Sciences* 333. (1991): 315–27; discussion 27–28. Web.

Engels, E.M., Glick, T., Shaffer, E.S. *The Reception of Charles Darwin in Europe*. London: Continuum, 2008. Print.

Erickson, et al. “Gigantism and comparative life-history parameters of tyrannosaurid dinosaurs” *Nature* (2004) 430: 772. Print.

Erickson, G.M et al. “Bite force estimation for *Tyrannosaurus rex* from tooth-marked bones”. *Nature* (1996) 382: 706-708. Print.

F.A., Soule´, M.E. “Re-wilding North America.” *Nature* 436: 2005. Print.

Figure 1. “Jurassic World AR- Life Size Dinosaur Scaling Chart” <https://s-media-cacheak0.pinimg.com/736x/bc/c6/41/bcc64118742ed90b9733048887913144.jpg

Figure 3. By Thomas Shafee - Own work, CC BY-SA 4.0, <https://commons.wikimedia.org/w/index.php?curid=39630174>

"floodplain". *Encyclopædia Britannica*. Encyclopædia Britannica Online. Encyclopædia Britannica Inc., 2016. Web. <http://www.britannica.com/science/floodplain>.

Foreman, D. *Rewilding North America: A Vision for Conservation in the 21st Century*. Island Press, Washington: 2004. Print.

"Gene Expression." *Nature.com*. Nature Education Publishing Group, 1 Jan. 2014. Web. <http://www.nature.com/scitable/topicpage/gene-expression-14121669>.

"Google Ngram Viewer." *Google Ngram Viewer*. Google, Web. 16 Apr. 2016.

Greenwood, A.D., Castresana, J., Feldmaier-Fuchs, G., Pääbo, S. “A Molecular Phylogeny of Two Extinct Sloths.” *Molecular Phylogenetics of Evolution*. 18. (2001): 94–103. Web.

Greenwald, Noah. "The Extinction Crisis." *The Extinction Crisis*. Center for Biological Diversity, 2016. Web.

"Grey Wolf Conservation and Management." *Frequently Asked Questions About Wolves in Washington.* Washington Department of Fish and Wildlife, Feb. 2016. Web.

Grunblatt, J., Said, M.Y., Nutira, J.K. “Livestock and wildlife summary 1987 – 1988 for Kenya Rangelands.” Department of Resource Surveys and Remote Sensing, Ministry of Planning and National Development, Nairobi, Kenya. 1989. Print

Handt, O., Richards, M., Trommsdorf, M., Kilger, C., Simanainen, J. “Molecular Genetic Analyses of the Tyrolean Ice Man.” *Science* 264. (1994): 1775–78. Web.

Hedges, S. B. et al. “Detecting Dinosaur DNA”. *Science* 268.5214 (1995): 1191–1194.

Web.

Hoekstra, J.M., Boucher, T.M., Ricketts, T.H., Roberts, C. “Confronting a biome crisis: global disparities of habitat loss and protection.” *Ecology Letters,* 2005.

Horner, J. *Dino Fest Paleontological Society Special Publication*. Eds Rosenberg G, Wolberg, D. Indiana University Press, Bloomington, IN: 1994. 157-164.

Horner, J.R., Goodwin, M.B., Myhrvold, N. "Dinosaur Census Reveals Abundant Tyrannosaurus and Rare Ontogenetic Stages in the Upper Cretaceous Hell Creek Formation (Maastrichtian), Montana, USA." *PLOS ONE* 6.2 (2011). Web.

Hutchinson, J, Garcia, M. “Tyrannosaurus was not a fast runner.” *Nature* 415, (2002): 1018. Web.

Ice Age. Dir. Carlos Saldanha. Prod. Lori Forte. By Peter Gaulke. Perf. Ray Romano, John Leguizamo, and Denis Leary. 20th Century Fox, 2006. DVD.

Jacobsen, A.R. “Feeding behavior of carnivorous dinosaurs as determined by tooth marks on dinosaur bones”. *Historical Biology* 13 (1998): 17. Print.

Jurassic Park. Dir. Stephen Spielberg. Perf. Sam Neill, Laura Dern and Jeff Goldblum. Universal Pictures, 1993. Film.

Jurassic World. Dir. Colin Trevorrow. Perf. Chris Pratt and Bryce Dallas. Universal Pictures, 2015. Film.

Karlstro O, Lindahl T. “Heat-Induced Depyrimidination of Deoxyribonucleic Acid in Neutral Solution.” *Biochemistry* 12. (1973): 5151–54. Web.

Keil, RG, Mayer, LM. "Mineral Matrices and Organic Matter." Organic Geochemistry, *Treatise on Geochemistry*. Vol. 12. Elsevier, 2014. 337-59. Print.

"Keystone Species Hypothesis." *Keystone Species Hypothesis*. University of Washington, 1 Nov. 1996. Web.

Kingdon, J. *The Kingdon Field Guide to African Mammals*. Princeton University Press, Princeton, NJ: 1997. Print.

Kondratas, Ramona. "Smithsonian Institution Archives." *Smithsonian Institution Archives*. Smithsonian Institution, 1992. Web.

Lauber, T.B., Knuth, B.A. “Measuring fairness in citizen participation: a case study of moose management.” *Society and Natural Resources* 12, (1999) 19–37. Web.

Laundré, J.W., Hernández, L., Altendorf, K. "Wolves, Elk, and Bison: Reestablishing the "landscape of Fear" in Yellowstone National Park, U.S.A." *Canadian Journal of Zoology* 79.8 (2001): 1401-409. Web.

Lewis, Martin. "Pleistocene Park: The Regeneration of the Mammoth Steppe?" *GeoCurrents*. 12 Apr. 2012. Web. 19 Apr. 2016.

Lindahl, T. "Instability and Decay of the Primary Structure of DNA." *Nature* 362 (1993): 709–15. Web.

"List of Fatal Cougar Attacks in North America." *Wikipedia*. Wikimedia Foundation, 21 Mar. 2016. Web. <https://en.wikipedia.org/wiki/List_of_fatal_cougar_attacks_in_North_America>.

Lubinski, Kenneth. "Floodplain River Ecology and the Concept of River Ecological Health" United States Geological Survey. *Ecological Status and Trends of the UMRS*. (1998): 1-12. Web

Martin, P.S., Burney, D. "Gone But Not Forgotten: Bring Back North American Elephants." *ScienceDaily*. ScienceDaily, 8 June 1999. Web. <www.sciencedaily.com/releases/1999/06/990607154315.htm>.

McShea, W.J., Underwood, H.B., Rappole, J.H. *The Science of Overabundance: Deer Ecology and Population Management.* Washington, DC: Smithsonian Institution Press, 1997. Print.

Mendelson, Will. "Bryce Dallas Howard: No Heels for Jurassic World 2." *Us Weekly*. Us Magazine, 22 Oct. 2015. Web. <http://www.usmagazine.com/celebrity-news/news/bryce-dallas-howard-swears-off-heels-for-jurassic-world-2-20152210>.

Michael, Soulé, and Noss, Reed. "Rewilding and Biodiversity: Complementary Goals for Conservation." *Wild Earth* 8.3 (1998): 19-28. Web.

Milius, Susan. "Life & Evolution: Lost-and-found Dinosaur Led a Semiaquatic Life: Fossil Reconstruction Revises View of Spinosaurus." *Science News* 186.8 (2014): 10. Web.

Mills, L.S., Soulé, M.E., Doak, D.F.. "The History and Current Status of the Keystone Species Concept". *Bioscience* (1993) 43:219-224.

Moehlman, Patricia. *Equids--zebras, Asses, and Horses: Status Survey and Conservation Action Plan*. Gland, Switzerland: IUCN-the World Conservation Union, 2002. Print.

Norr, R.F., Quigley, H.B., Hornnrktr, M.G., Merrill, T., Paqurr, PC. "Conservation biology and carnivore conservation in the Rocky Mountains." *Conservation Biology* (1996) 10:949-963. Web.

Norris, Megan. "Will GMOs Hurt My Body? The Public's Concerns and How Scientists Have Addressed Them." *Science in the News*. Harvard University Graduate School of Arts and Sciences, 10 Aug. 2015. Web.

Osborn, H.F. *Bulletin of the American Museum of Natural History*. 21: 259. 1905.

Pääbo, Svante, Poinar, H., Serre, D., Jaenicke-Després, V., Hebler, J., Rohland, N., Kuch, M., Krause, J., Vigilant, L., Hofreiter, M. "Genetic Analyses from Ancient DNA." *Annual Review of Genetics* 38.1 (2004): 645-79. Web.

Pääbo S, Hofreiter M, Serre D, Poinar H.N., Kuch M. "Ancient DNA". *Nature Review of Genetics* 2.1 (2001): 353–59. Web.

Pappas, Stephanie. "Controversial T. Rex Soft Tissue Find Finally Explained." *LiveScience*. TechMedia Network, 26 Nov. 2013. Web.

Parham, Peter. *The Immune System*. New York: Garland Science, 2005. Print.

Paul, Gregory. "History of Discovery and Research". *The Princeton Field Guide to Dinosaurs*. Princeton University Press, 2010. 9–65. Web.

"PCR." *Learn.Genetics*. University of Utah: Health Sciences, 1 Jan. 2016. Web. <http://learn.genetics.utah.edu/content/labs/pcr/>.

Pierce, Benjamin A. *Genetics: A Conceptual Approach*. W H Freeman, 2013. Print.

Poinar H, Höss M, Bada J, Pääbo S. "Amino Acid Racemization and the Preservation of Ancient DNA." *Science* 272.5263 (1996): 864-866. Web.

Pruitt, Sarah. "Scientists Find Soft Tissue in 75-Million-Year-Old Dinosaur Bones." *History.com*. A&E Television Networks, 16 June 2015. Web.

Rafaele, Paul. "Man-Eaters of Tsavo." *Smithsonian*. Smithsonian Magazine, Jan. 2010. Web. <http://www.smithsonianmag.com/science-nature/man-eaters-of-tsavo-11614317/?no-ist.>.

Ricciardi, A., William W., Steiner, M., Richard N., Simberloff, D. "Toward a Global Information System for Invasive Species." *BioScience* 50.3 (2000): 239. Web.

Rubenstein, D., Rubenstein, D., Sherman, P., Gavin, T. "Pleistocene Park: Does Re-wilding North America Represent Sound Conservation for the 21st Century?"

Biological Conservation 132 (2006): 232-238. *ScienceDirect*. Elsevier Ltd., 5 June 2006. Web.

Ruxton, G. D., Houston, D.C.. "Could *Tyrannosaurus rex* Have Been a Scavenger Rather than a Predator? An Energetics Approach." *Proceedings of the Royal Society B: Biological Sciences* 270.1516 (2003): 731-33. Web.

Sampson, Scott D., Loewen, Mark A. "Tyrannosaurus Rex from the Upper Cretaceous (Maastrichtian) North Horn Formation of Utah: Biogeographic and Paleoecologic Implications." *Journal of Vertebrate Paleontology* 25.2 (2005): 469-72. Web.

Schweitzer, M.H., Wittmeyer, J., Horner, J.R., Toporski, J. "Soft tissue vessels and cellular preservation in *Tyranosaurus rex". Science* 307, 1952 (2005).

Shay, Steven. "Re-wilding: Don't Overlook Humans Living on the Plains." *Nature* 437.7058 (2005): 476. Web.

Soule, Michael E. "The Onslaught of Alien Species, and Other Challenges in the Coming Decades." *Conservation Biology* 4.3 (1990): 233-40. Web.

Stankiewicz B, Poinar H, Briggs D, Evershed R, Poinar G. "Chemical Preservation of Plants and Insects in Natural Resins." *Proceedings of the Royal Society of London Ser. B* 265 (1998): 641-47. Print.

Stevens, Kent A. "Binocular Vision in Theropod Dinosaurs." *Journal of Vertebrate Paleontology* 26.2 (2006): 321-30. Web.

Stokstad, Erik. "*Tyrannosaurus rex* Soft Tissue Raises Tantalizing Prospects." *Science*, 307.5717 (2005): 1852. Web.

Stoneking, Mark. "Ancient DNA: How do you know when you have it, and what you can do with it?" *American Journal of Human Genetics* 57.6 (1995): 1259-262. *National Center for Biotechnology Information*. U.S. National Library of Medicine. Web.

Stuart, CT., Stuart, MD. *The Larger Mammals of Africa*. Struik Publishers, Cape Town, South Africa: 1997. Web.

Sutcliffe, Antony J. "Spotted Hyaena: Crusher, Gnawer, Digester and Collector of Bones." *Nature* 227.5263 (1970): 1110-113. Web.

"Tanzania Lions - National Geographic Adventure Magazine." *National Geographic Adventure Magazine*. National Geographic, Oct. 2006. Web. 10 Apr. 2016. <http://www.nationalgeographic.com/adventure/photography/africa/tanzania/lions

-maneating-5.html>.

Terborgh, J., Estes, P., Paquer, K., Ralls, D., Boyd, B., Miller, and R. Noss. “The role of top carnivores in regulating terrestrial ecosystems.” *Continental Conservation: Design and Management Principles for Long-term Regional Conservation Networks*. Covelo, CA and Washington, DC: Island Press, 1999.

Terborgh, J. 1988. “The Big Things that Run the Word—a Sequel to E.O. Wilson”. *Conservation Biology* (1998) 2:402-403. Web.

The Holy Bible: Containing the Old and New Testaments with the Apocryphal /Deuterocanonical Books: New Revised Standard Version. New York: Oxford UP, 1989. Print.

Thornberry-Ehrlich, Trista, and Rebecca Port. "Geologic Time Scale." *Earth Science Concepts*. National Park Service, United States Deparment of the Interior. 13 May 2014. Web. <https://www.nature.nps.gov/geology/education/concepts/Geo%20Time%20Scale%20FY13.pdf>.

"Triassic Period, Mesozoic Era Information, Dinosaur Facts -- National Geographic." *National Geographic Science*. National Geographic, 2016. Web.

Turner, Alan. *National Geographic Prehistoric Mammals*, Washington, D.C.: National Geographic, 2004. Print.

“Tyrannosaurus Rex and T. rex, in English-Language Print from 1980 to 2008”. *Google Ngram Viewer*. Google Books Library Project, 4 Mar. 2016. Web. <https://books.google.com/ngrams/graph?content=Tyrannosaurus+Rex%2CT.+rex&year_start=1980&year_end=2008&corpus=15&smoothing=3&share=&direct_url=t1%3B%2CTyrannosaurus%20Rex%3B%2Cc0%3B.t1%3B%2CT.%20rex%3B%2Cc0>.

Vasan S., Zhang X., Kapurniotu A., Bernhagen J., Teichberg S., et al. “An Agent Cleaving Glucose-Derived Protein Crosslinks in vitro and in vivo.” *Nature.* 382 (1996): 275–78. Web.

Voet, D., Voet, J., Pratt, C. *Fundamentals of Biochemistry*. New York: 2003. Print.

Wandeler P., Smith S., Morin P., Pettifor R., Funk S. “Patterns of Nuclear DNA Degeneration Over Time—a Case Study in Historic Teeth Samples.” *Molecular Ecology* 12. (2003): 1087–93. Web.

Ward, P.D. *The Call of Distant Mammoths*. New York: 1997. Print.

Weishampel, D. B. “Jurassic Park. By Michael Crichton.” *American Journal of Physical Anthropology* 85.2 (1991): 239-240. Web.

"What Is Paleontology?" University of California Museum of Paleontology, 2006. Web.

White, P. J., and Robert A. Garrott. "Northern Yellowstone Elk after Wolf Restoration." *Wildlife Society Bulletin* 33.3 (2005): 942-55. Web.

Wilkinson, Todd. "Yellowstone National Park: Threatened Species Are Thriving in Yellowstone. Now What?" *National Geographic Magazine*, 07 Apr. 2016. Web. 16 Apr. 2016.

Willerslev, Eske, and Alan Cooper. "Ancient DNA." *Proceedings of the Royal Society* 272 (2005): 3-16. Department of Zoology, University of Oxford, 20 Dec. 2004. Web. 30 Mar. 2016.

Witmer, Lawrence, and Ryan Ridley. "New Insights into the Brain, Braincase, and Ear Region of Tyrannosaurs (Dinosauria, Theropoda), with Implications for Sensory Organization and Behavior." *The Anatomical Record* 292 (2009): 1266-296. *Wiley Interscience*. Web.

"Wolves in Yellowstone." *National Parks Service*. United States Department of the Interior. Web. 20 Apr. 2016.

Zimmer, Carl. "Is Dinosaur 'Soft Tissue' Really Slime?" *Science* 321.5889 (2008): 623. Web.

Zimov, S. A. "Essays on Science and Society: Pleistocene Park: Return of the Mammoth's Ecosystem." *Science* 308.5723 (2005): 796-98. Web.

Zischler, H., Hoss, O., Handt, A., Von Haeseler, A., Van Der Kuyl, and J. Goudsmit. "Detecting Dinosaur DNA." *Science* 268.5214 (1995): 1192-193. Web.