

Tissue Variability Effects on Saw Mark Evidence in Bone

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Submitted to the Graduate Faculty of the
Dietrich School of Arts and Sciences in partial fulfillment
of the requirements for the degree of
Doctor of Philosophy

University of Pittsburgh

2020

UNIVERSITY OF PITTSBURGH

DIETRICH SCHOOL OF ARTS AND SCIENCES

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To be scientifically valid under the “*Daubert* standards,” scientific testimony must be tested, subjected to peer review and publication, have a known or potential error rate, have maintained standards for its proper operation, and be widely accepted within the relevant scientific community (*Daubert v. Merrell Dow Pharmaceuticals, Inc.* 1993). Forensic research has demonstrated that tooth hop (**TH**) is a valuable measurement from saw-cut bones as it can be used to indicate the teeth-per-inch (**TPI**) of a saw in postmortem dismemberment cases; however, error rates of TPI estimation are still in infancy and our knowledge of how bone tissue affects TH measurements is unclear. The purpose of this research is to investigate the effects of tissue variability (through use of different taxa of known sex and age) on the accuracy and precision of TH measurements in bone to estimate TPI of the saw blade. This will further aid in the creation of error rates associated with TH measurements while also assessing the validity of nonhuman proxies in saw mark research. This researcher measured TH from human (280), pig (797), and deer (689) long bones cut by two saw blades of different tooth type; human remains are from one individual, while pig and deer are from multiple. 50 distance-between-teeth measurements before and after sawing were collected from each saw blade for comparison. ANOVA and F-tests were used to compare mean TH measurements and variance, respectively, by saw-species, species, sex, and number of TH in a chain (versus isolated cases of TH), with significance determined at the $p < 0.05$ level. It is concluded that significant differences in TH (mm) do not reflect significant differences in associated TPI ranges of suspect blades. With this knowledge, fresh deer and pig

proxies may be used in TH research, although deer is less advisable. Forensic case reports should report mean TPI \pm 1 TPI (narrow) and mean TPI \pm 2 TPI (wide) intervals with a sample size indicating number of tooth hops measured. Tooth hops in longer chains did not greatly affect results, so cases of isolated tooth hops may be used to estimate blade TPI.

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Preface

This research would not have been possible without the help of those around me. First, I must thank my advisor, Dr. Michael Siegel, for his guidance throughout this project, use of laboratory materials and office space, as well as his constant support when challenges arose. This truly helped me gain independence and self-confidence as a researcher. I am also grateful for Dr. Margaret Judd, who not only let me use her laboratory for (some would say smelly) research, but who also has provided me with many opportunities to help further my career. I must also thank the other members of my committee, Dr. Mark Mooney and Dr. Elizabeth Arkush, for their generous donations of time and invaluable input, as well as the Anthropology Department at the University of Pittsburgh for the freezer and use of laboratory space.

I am indebted to Dr. Dennis Dirkmaat for access to the Hirtzel Forensic Anthropology Laboratory and donated human tissue. You were my guide and mentor throughout both my undergraduate and master's programs, providing me with amazing opportunities to work on forensic anthropological cases, experiences that have opened so many doors in my life. I can only hope to one day pay forward that same level of investment into my future students. Along those lines, I must also thank Dr. Steven Symes for his guidance and training in microscopic saw mark analysis and his trust and confidence in me as an undergraduate student. His imparted passion for skeletal trauma research has led me down a similar path; I can only hope to achieve as much in the field of skeletal trauma.

I must also thank Mr. Joe Ott for his gracious donation of deer limbs (which he cleared of most surrounding soft tissue) to a Ph.D. student free of charge. I would also like to thank the support team behind the scenes of *mypetcarnivore.com*; this was the only facility to not only

provide freshly frozen pig bones, but who also responded to my inquiries of pig age, sex, and diet. Without either of these bone samples, this research would have been impossible. Finally, I must thank Theresa De Cree for assisting me during my time in the Hirtzel Forensic Anthropology Laboratory and for prepping the materials in advance.

I must also thank my supportive network of friends and family. To Nick, my fiancé, you have had to put up with a rollercoaster of emotions tied to this project and endless education. You have gone through so much, some of which I am sure you never hope to experience again (I can't make any promises). To my gym group, Ljiljana, Deb, Junyang, Peiyu, Erin, Alysha, and now, Petra, without morning gym time, I would not be the same person. We have hit so many PRs, both in and out of the gym. I can only hope to have gym buddies like you wherever I end up next. And to Sharon, you are also an invaluable friend, a rock of support, an ear to listen, and an excellent baker. Special thanks are due to my parents and brothers for their endless support and encouragement, as well as engineering advice. You too have put up with weird things that only forensic anthropologists do, like macerate outside and do decomposition studies of the local, deceased fauna.

I would like to dedicate this dissertation to my grandparents, Jeannette Donnelly, Thomas Donnelly, and Rody Grosso, who all passed away in the final years of my Ph.D. Each has left a lasting impression on my life and given me greater appreciation for whatever time I do get to spend with family and friends. And to Grandpa Grosso, without you, our trips to the science museums, and your perfectly picked gifts throughout my childhood, I may never have discovered my passion for anatomy and science.

1.0 Introduction

1.1 Background

In 2009, the National Academy of Sciences reported that “the adversarial process relating to the admission and exclusion of scientific evidence is not suited to the task of finding ‘scientific truth’” (NAS 2009, 110). The judicial system is not composed of scientists; nor are the judges, lawyers, and jury expected to have expertise in all areas of forensic evidence when they evaluate and weigh what is presented to or by them in the courtroom. Specific issues regarding evidence admissibility, validity, and reliability arise on a case-by-case basis, so an entire field of evidence, i.e., toolmark analysis, will not be comprehensively evaluated through systematic research methods during any given trial. This process would also be extremely time consuming and costly. “Given these realities, there is a tremendous need for the forensic science community to improve. Judicial review, by itself, will not cure the infirmities of the forensic science community” (Mnookin 2008). Together, the judicial system and the forensic sciences seek to rightfully convict those that commit crimes and protect those that are innocent of the crimes in which they are accused. It is not a perfect system and foundations like the Innocence Project (founded 1992) seek to exonerate those that have been wrongfully convicted. But, it is ultimately up to the forensic science community to be proactive in the evaluation of the accuracy and reliability of the methods that we use. Justice guards both life and liberty. These legal decisions are made based on the evidence that we collect, analyze, and present. How confident are we in these methods and our resultant conclusions?

There are three primary Supreme Court decisions that set the admissibility criteria for expert testimony at the federal level in the United States: *Daubert v. Merrell Dow Pharmaceuticals, Inc.* (1993), *General Electric, Co. v. Joiner* (1997), and *Kumho Tire, Co. v. Carmichael* (1999). Prior to 1975, expert witness admissibility was interpreted through *Frye v. United States* (1923) until the Federal Rules of Evidence (FRE) were established; however, it was not clear at that point in time which, *Frye* or the FRE, the federal judiciary should follow until the *Daubert* decision. Both the *General Electric, Co. v. Joiner* (1997) and *Kumho Tire, Co. v. Carmichael* (1999) decisions further elucidate the language of the *Daubert* standards. These decisions directly affect the federal judiciary, which is separate from the state courts. Today, most states adhere to the *Daubert* standards while some states follow *Frye*. Meanwhile, several states have established their own interpretation of these decisions based on relevant court cases that have been handled at their state level. It is important to examine each of the aforementioned court cases in more detail to gain understanding for the implications of each and what the difference is between *Daubert* and *Frye*, if a meaningful difference truly exists.

Frye was convicted of 2nd degree murder in 1922. A “deception test” (polygraph) had been requested by the defense to aid in demonstrating his innocence of the crime. The Supreme Court doubted admissibility of the polygraph evidence; primarily it was concerned with the methodology behind the test, stating: “...the thing from which the deduction is made must be sufficiently established to have gained general acceptance in the particular field in which it belongs” (*Frye v. United States* 1923). This type of evaluation process can be difficult when new methodology is introduced, a common occurrence in the forensic sciences when unique circumstances of a crime make a research question suddenly relevant; thus, not enough time has passed for proper evaluation and acceptance by fellow practitioners (this same detriment befalls the *Daubert* standards, as will

be seen). However, “general acceptance” alone is quite vague, which is problematic. We can hope that good science, by the very nature of science and scientific research, through hypothesis testing and peer review, would still occur, ultimately leading to this “general acceptance” in a relevant field (NAS 2009). This takes time. Science, ideally, is self-correcting, and encourages continued questioning and critical evaluation. But would this effort alone be enough to encourage the pursuit of validation studies of current forensic methods and enough to encourage the appropriate funding for such pursuits? In Western education and scientific publication, we often strive to be new and innovative, placing higher value on ground-breaking, flashy technological designs or processes. The cost here is that this type of mentality discourages the simple validation of someone else’s work and publishing those results.

Approximately 50 years after the *Frye* decision, the Federal Rules of Evidence (1975) were established as guidelines for criminal and civil litigation in federal courts. Of these, most attention has been paid to rules 702 and 703, regarding the admissibility of expert witness testimony and bases of an expert. Originally, rule 702 stated:

“If scientific, technical, or other specialized knowledge will assist the trier of fact to understand the evidence or to determine a fact in issue, a witness qualified as an expert by knowledge, skill, experience, training, or education, may testify thereto in the form of an opinion or otherwise” (FRE 702, 1975).

This does not indicate the need for “general acceptance” of a method for the presentation of expert witness testimony in court. It was up to the judge, as gatekeeper, to determine whether testimony would be admissible. And as stated previously, it became unclear as to where the federal courts stood regarding the admission of expert testimony, to follow *Frye* (1923) or the FRE (1975). This would later be decided during the *Daubert* trials.

In the early 1990s, Daubert argued that Bendectin, marketed as an anti-nausea drug by Merrell Dow Pharmaceuticals, Inc., caused severe birth defects in infants whose mothers took the drug during pregnancy. Prosecution provided eight experts who testified that Bendectin did cause birth defects in these infants; they referenced case studies finding birth defects in animal trials. The court decided that the testimony from these experts was not admissible as their evaluation methods were not supported by the scientific community (following *Frye's* general acceptance test) because their reports, establishing that Bendectin would have similar effects on human development as it did in the animal proxies, were not published and thus lacked peer review from the relevant scientific community. The case was appealed and when reached Supreme Court level, the Court ruled that the FRE, not *Frye*, dictated expert witness testimony in a federal trial. The FRE did not specify the need for general acceptance. Because Rule 702 specified “scientific knowledge,” the Court deemed “scientific” as a something grounded in science’s methods and procedures. (*Daubert v. Merrell Dow Pharmaceuticals, Inc.* 1993).

To be scientifically valid under the “*Daubert standards*,” scientific testimony must be tested, subjected to peer review and publication, have a known or potential error rate, have maintained standards for its proper operation, and be widely accepted within the relevant scientific community (*Daubert v. Merrell Dow Pharmaceuticals, Inc.* 1993). Scientific conclusions by witnesses are not the main concern here; what is of primary interest is the methodology from which these conclusions are drawn. The judiciary does not seek a largescale understanding for how something like toolmark analysis works; they are resolving one legal dispute at a time (NAS 2009). This role belongs to the scientific community—to be proactive in establishing validity and reliability of our methods as they arise, to test the underlying principles these methods are based

upon, and to be transparent in how analyses are performed so that they become less reliant on practitioner experience alone. (*Daubert v. Merrell Dow Pharmaceuticals, Inc.* 1993).

Post-*Daubert*, Joiner, who had worked for the General Electric company for many years, claimed that through frequent oral and ocular exposure to dielectric fluid he had developed lung cancer, or that it had at least exacerbated the development of this cancer. Joiner was known to have smoked for at minimum eight years and had a family history of lung cancer. It had been discovered in laboratory tests that this fluid was indeed harmful, but no peer reviewed human studies had been performed. The judge ruled that the testimony agreeing that the fluid was indeed carcinogenic in humans was inadmissible as the evidence did not rise beyond speculation. The case was eventually elevated to the Supreme Court level, there concluding that “abuse of discretion” (a common standard to review evidentiary rulings where there is a firm belief that a lower court erred in judgement) is the correct standard to review district court decisions on the admission or exclusion of expert witness scientific testimony. Abuse of discretion is a common way to gain appellate review, where the admittance of evidence at the original trial is under question. With *Joiner*, the Court is emphasizing the role of the trial judge as gatekeeper, and that unless there is a firm belief that the trial judge made a huge mistake in evidence admittance for the trial, the appellate court should not reverse the ruling. (*General Electric, Co. v. Joiner* 1997).

Another important case in this “*Daubert* trilogy” is *Kuhmo Tire, Co. v. Carmichael* (1999). Carmichael experienced a rear tire blow out while driving his minivan, resulting in the death of one of his passengers, while injuring the other occupants. The Carmichael family sued Kuhmo Tire, Co., the manufacturer of the tires, claiming that the tire was defective, causing the accident. A tire failure expert was placed on the stand and concluded that the tire was defective, based on his experience and observations, and was thus responsible for the accident. It was deemed that this

testimony was not “scientifically valid,” so it was excluded for not meeting the *Daubert* standards. It was unclear, then, whether *Daubert* applied to non-scientific expert testimony (i.e., testimony based on technical or other specialized knowledge). The Supreme Court determined that it should not be the concern of the courts to distinguish what is “scientific” and what is “technical.” As mentioned earlier, the average judge or juror is not expected to be an expert in what is scientific, or even what is technical. The *Daubert* standards, while substantially more descriptive than *Frye*, remain quite flexible and expand beyond scientific expert testimony. Other factors, not outlined as criteria in *Daubert*, can also be taken into consideration, depending on the nature of the testimony and what would help show its foundation in validity and reliability. That decision is up to the judge. (*Kuhmo Tire, Co. v. Carmichael* 1999).

Since the *Daubert* (1993), *Joiner* (1997), and *Carmichael* (1999) decisions, the FRE have since been amended. Rule 702 was specifically amended to reflect the aftermath of this “*Daubert* trilogy” in 2000. As of 2011, FRE rule 702 currently states:

“A witness who is qualified as an expert by knowledge, skill, experience, training, or education may testify in the form of an opinion or otherwise if: (a) the expert’s scientific, technical, or other specialized knowledge will help the trier of fact to understand the evidence or to determine a fact in issue; (b) the testimony is based on sufficient facts or data; (c) the testimony is the product of reliable principles and methods; and (d) the expert has reliably applied the principles and methods to the facts of the case” (FRE 702, 2011).

So regardless of whether a state complies with *Daubert*, *Frye*, or something in-between, it remains the task of the scientific community to uphold proper science. Through continued hypothesis testing and peer review we validate our methods and continue to pursue these scientific

truths. As Dirkmaat *et al.* 2008 summarized, “because of the focus on methods, *Daubert* reinforced the view that forensic anthropologists should be scientists first and professionals second.” This is true of all scientists, not just forensic anthropologists. Scientific validity takes precedence over personal experience and opinion. It is not to say that experience has no value. But a conclusion is only worth so much given the foundation on which it is set. Is the method scientifically grounded? To do this, forensic scientists must perform and publish validation studies.

One of the broader goals of this dissertation is to continue the discussion on the purposes of validation studies in forensic anthropology, while simultaneously performing one for microscopic saw mark analysis in bone, specifically on measuring saw blade tooth size through tooth hop measurements from bone. While courts often cite the previous and since thereafter continued admission of evidence (i.e., fingerprints or toolmarks) as grounds for admissibility of that evidence in the courtroom, this reasoning is invalid (NAS 2009). We cannot and should not underestimate how vital validation studies are, particularly in the forensic sciences. This may mean redistributing funds and time for a more thorough review of our methods and potentially reviewing evidence from past convictions that relied on conclusions from significantly flawed methods, so as to better pursue justice (this latter topic being an entirely separate ethical debate of its own).

1.2 Validation studies

Validation studies allow scientists to evaluate the validity and reliability of methodology. They are used for the observation, documentation, and interpretation of variation in data generated under specific laboratory conditions (Butler 2014). *Validity* is a measure of accuracy; how correct

or close are the results to reality? *Reliability* is an assessment of consistency or precision; when we repeat an analysis several times, to what degree will the results be the same? An unreliable method cannot be a valid method. And as Christensen and Crowder (2009) state, “it is also important to understand that the point of developing methods under the rubric of evidentiary examination is not to completely quantify the field, and that subjectivity does not necessarily equal unreliability.” It is often assumed that all subjectivity is bad and must be eliminated, that anything qualitative must become quantitative if it is to be “good” science. Humans are biological beings; we are highly variable. We vary by age, sex, ancestral background, etc., and these variables are not categorical, but more/less continuous. For example, one female may have higher levels of estrogen or dietary calcium than another; this in turn can lead to differing levels of bone quality between them. Methods evaluating bone trauma would be subjected to this variability. If impacted tissue varies in a bone trauma study, this may impact a fracture’s initiation and propagation. Hormonal and dietary impacts are just two factors that can affect bone quality. Normal (and abnormal) human variation creates bias and because we cannot account for every unique circumstance in an individual’s life that ultimately went into the creation of a fracture, some bias will always remain. Again, validation studies and the *Daubert* standards do not require complete objectivity. What is the accuracy rate? What is the precision level? As reliability of a method increases, the error decreases. The goal here is for a method to be both highly accurate and reliable. With more validation studies, we can push the system to failure and better understand the limitations of a method (Butler 2015).

1.3 A brief history of toolmark and saw mark analysis

As aforementioned, this dissertation will also serve as a validation study of using tooth hop measurements to estimate saw blade tooth size, a component of microscopic saw mark analysis. Saw marks fall under firearm and toolmark evidence, which has long been established in the historical record. In 1930, a legal precedent was set for the use of toolmarks in United States courts, declaring that “the edge on one blade differs as greatly from the edge of another blade as do the lines on one human hand differ from the lines on another. [...] The scientific means afforded should be used to apprehend the criminal” (*Washington v. Clark* 1930). Toolmark evidence used in police investigations pre-dates examination of firearm evidence, although the first instance recognizing the value of toolmarks in an investigation is vague. In *State v. Fasick* (1928), knife cuts in freshly cut wood that had been used to conceal a murder victim at the scene were compared to experimental marks produced in a laboratory on similar wood to examine the consistency between them. Several publications on tool mark evidence appear shortly after, though out the 1930s, i.e., May 1930 and Koehler 1937; however, recorded history of the firearm and toolmark discipline remains biased towards the analysis of ballistics evidence. Burd and Greene (1948) report that toolmark analysis had been well-regulated by police laboratories for “many years,” while also stating that most early cases involving toolmark comparisons were those involving forcible entry (Burd and Greene 1948).

The Association of Firearm and Tool Mark Examiners (AFTE) was founded in 1969 and offers training, conferences, and a peer-reviewed journal; this association has also allowed for the creation of standard protocols and overall methodological improvement across the field as further research develops. The 2009 NAS report that assessed the current state of and needs of forensic science disciplines (outside of DNA analysis) made thirteen recommendations, several of which

applied to firearm and toolmark analysis. Granted, to review and comment on all the forensic disciplines could only mean a shallow assessment of each. Regardless, one concern with toolmark analysis focused on the notion of “sufficient agreement,” a phrase that appears in the guidelines produced by the AFTE Criteria for Identification Committee (established in 1985), that there needs to be “sufficient agreement” in the pattern of two sets of marks (AFTE 1992). The Criteria for Identification Committee elaborates that sufficient agreement is related to the “significant duplication of random toolmarks,” with a significant agreement occurring when “it exceeds the best agreement demonstrated between two toolmarks known to have been produced by different tools and is consistent with agreement demonstrated by toolmarks known to have been produced by the same tool” (AFTE 1992). This analysis is thus subjective, by the nature of comparison and the inability to realistically check every potential tool on the market.

The AFTE critically responded to all relevant NAS (2009) recommendations, reporting that many of their recommendations had already been addressed by the field, such as a glossary of terms and standards (created in 1980), training programs (established by AFTE since 1982), a certification program (established by AFTE through NIJ funding since 1999), etc. Thus, the NAS report, while extensive, was an incomplete picture of firearm and toolmark analysis (and likely for other disciplines as well), so its conclusions and recommendations should be taken with a grain of salt. Toolmark analysis has indeed been well-established and continues to pursue research and publication in numerous subareas to further advance the field and ensure validity and reliability of various utilized methods.

Saw mark analysis is one such component of toolmark analysis and typically, saw mark evidence in bone is evaluated by a forensic anthropologist and not a traditional firearm and toolmark examiner. However, similar conclusions are drawn by forensic anthropologists, for

example, reporting that suspect tools are in “sufficient agreement” with marks found in bone or that marks found would be “consistent with” a listed set or subset of tools. Regarding saw mark evidence and its appearance in bone, Bonte (1975) was the first to publish on saw marks in bone and their potential use in forensic investigations. Andahl (1978) likewise described saw marks in bone, positing their potential use in human dismemberment cases. However, saw cut characters were not well described or able to be understood in either publication; it would take some time before the issue was revisited in the literature. It was not until 1992, with the dissertation of Symes, that saw mark analysis in bone received proper attention and is likely the most cited reference for saw mark analysis to this day. For those interested in pursuing an in-depth look at microscopic saw mark analysis and class characteristics of saw blades in bone, other vital works from Symes and colleagues include: Symes *et al.* 1989; Symes 1992; Symes *et al.* 1998; Symes *et al.* 2002; Symes *et al.* 2005; Symes *et al.* 2012.

There have been a number of large and small-scale validation studies for saw mark analysis in bone over the years (Saville, Hainsworth, and Ruthy 2007; Freas 2010; Bailey *et al.* 2011; Love *et al.* 2015; Nogueira *et al.* 2016; Berger 2017; Berger *et al.* 2018; Hughes 2018), although each has had its own unique addition to the method, which as mentioned earlier can be a challenge for effective or complete validation if an analysis does not truly replicate the methods set by the original study (Christensen and Crowder 2009). In each of these studies, the tissue selected between studies varied in some way (i.e., animal proxy, bone selected), with no clear purpose in mind or reason beyond availability of tissue. We lack an understanding of the effects this tissue selection process has on the validation success of selected saw traits collected during the study to aid in identification of saw blade class or individual characteristics.

When not utilizing human bone, researchers often select bones from pig or deer. Saw mark validation studies are not the only instances where animal proxies have been substituted for human bone; they have also been included in other skeletal trauma analysis studies involving sharp force trauma, blunt force trauma, and/or burning. The work by researchers at Michigan State University (see Haut and Wei 2017 for a review) is a prime example of using a porcine model to develop an understanding of pediatric cranial fractures, with clear presentation of sample and methods used as well as a thorough evaluation of their porcine model and its usefulness as compared to human tissue. But in other areas of bone trauma research, we as readers are often not presented with the life or medical history of the selected tissues (i.e., age, sex, diet, pathological conditions, general bone strength and mechanical properties, if or how long the tissue had been frozen, and other relevant information of individuals in the study). Or, single individuals are presented in a study as representing an entire population, which can lead to over-interpretation of the results, as their remains are only representing intra-individual tissue variation. **This leads to another goal of this current research, further explained below, to investigate the effects of tissue variability (through use of different taxa) on the accuracy and precision of tooth hop measurements in bone to estimate tooth size of the saw blade.** This goal is broken up into specific research questions in section 1.5.

1.4 Statement of the problem

Previous saw mark validation research performed by this author (Grosso 2014, 2019) utilized two saw blades of varying teeth-per-inch (**TPI**) and tooth type (a 6 TPI rip and an 8 TPI crosscut saw) to cut long bones from two species (white-tailed deer and human). Sample sizes of

individual groups were as follows: n = 32 human crosscut, n = 32 human rip, n = 62 deer crosscut, and n = 55 deer rip cuts. The human tissue used had consisted of two femora from one adult male with metastatic breast cancer, and several lesions appearing within the femora. The deer tissue used consisted of a mixed sample of long bones (femora, tibiae, and metapodials), with no known sex or time since death. Only one side of each saw cut was analyzed. Overall, there were 59 instances of tooth hop in the deer with 104 individual hops; whereas, the human sample had 27 instances of tooth hop with 49 individual hops. **Tooth hops (TH)** are wavy striations that occur on a cut surface, potentially from stopping and starting or skittering of a blade through a cut, that are indicative of the tooth size of a blade. An image illustrating tooth hops can be seen in chapter 3. Thirty measurements were also randomly collected between adjacent teeth along each blade to compare the variability of distance-between-teeth (**DBT**) from the actual blades to the tooth hop measured from bone. Welch two sample t-tests were performed to check for significant differences ($p < 0.05$) between the DBT measurements of saws and species. Mean tooth hop (or DBT) and standard deviation for each group was 3.09 ± 0.03 mm for deer crosscut, 4.03 ± 0.04 mm for deer rip, 3.31 ± 0.19 mm for human crosscut, 3.93 ± 0.15 mm for human rip, 3.06 ± 0.04 mm for the crosscut blade, and 4.12 ± 0.04 mm for the rip blade.

From this earlier research (Grosso 2014, 2019), deer bone more precisely reflected the standard deviation of the DBT measurements of the saw blade than did the human tissue, although the tissue property(s) responsible could not be isolated as the deer sample combined bones of different age, sex, and bone type. Nogueira *et al.* 2018 also detected a similar phenomenon when collecting saw mark data from pig bones, concluding that we should be cautious using nonhuman tissue in experimental studies. The evidence here also makes questionable the idea of creating vast databases of saw types as we are tempted to do with biological populations. Saw blades are man-

made, produced in batches where quality control standards of the manufacturers may impact how similar two “otherwise identical” blades are from the same or distinct manufacturing facility. Blades also degrade through use and wear, increasing in individual variability over time depending on care and maintenance. Overall, it may instead be worthwhile to know *how* blades can vary and focus on *how* variations in bone tissue impact measurements of these characters rather than test every known saw blade or type in existence. The latter option is not feasible or necessary given the “consistent with” conclusion-level we more often obtain in saw mark cases as data derived from bone do not typically perform beyond that level of accuracy and precision in classification.

1.5 Research purpose and testable questions

The purpose of this current research is therefore to investigate the effects of tissue variability (through use of different taxa of known sex and age) on the accuracy and precision of tooth hop measurements in bone to estimate tooth size of the saw blade. This will aid in the creation of error rates associated with tooth hop measurements and their ability to reflect TPI ranges for suspect blades. This type of research, examining the effects of tissue variability on our interpretation of skeletal trauma evidence, is a necessity before further validation of methods associated with microscopic saw mark analysis can be completed. Likewise, this type of research will also serve as an indicator of the ability to compare validation studies that utilize different species in the evaluation of saw mark features, such as tooth hop, to estimate blade TPI.

As previously mentioned, many researchers are quick to use more readily available deer or pig models as human bone tissue proxies in toolmark research, so both species are included here for an assessment of tooth hop, to be compared to tooth hop measured from human bone, as well

as compared to DBT measurements from the blades. *It is hypothesized, given previous research, that there will be significant differences not in mean tooth hop between species, but that the standard deviations of tooth hop will significantly differ between species with bones of varied hardness (material hardness dictating the presence of toolmarks on bone).* Saville, Hainsworth, and Rutty (2007) show that the average hardness for a deer tibia was 54.8 kg mm^{-1} (external surface hardness) and 66.8 kg mm^{-1} (hardness across cortex), while the hardness for a 74-year-old human male femur was much less (39.5 and 39.4 kg mm^{-1}). The pig femur showed the most similarity in hardness to the human bone (26.0 and 37.1 kg mm^{-1}) compared to other species included in the sample (deer, sheep, and cow). *It is then also hypothesized that this similarity between pig and human bone will be reflected in tooth hop error, with variation in tooth hop measurements from pig bone being the most like those from human and the harder deer bone being more reflective of the true variability of blade DBT.*

This researcher tests the following questions:

1. How variable will tooth hop measurements from a single instrument be comparing multiple usages in human bone?
2. How variable will tooth hop measurements be from the same instrument when using animal proxies (deer/pig) to model human bone?
3. What are the properties of bone that affect tooth hop variability?
4. What are the properties of the instrument that affect tooth hop variability?
5. Does having more than one tooth hop in a row improve the accuracy and precision in estimating blade TPI?

Question components addressed statistically are assessed with the basic null hypothesis that there was no difference amongst the relevant groups addressed by each question.

1.6 Significance of the proposed research

Since the NAS reports' release in 2009, the forensic sciences have taken great steps to rectify and improve, but the road is long and unending. Time and funding are limited resources and how much of these can be invested into our methods is highly dependent on how frequent that type of evidence and associated analytical methods have appeared in the courtroom. DNA analysis is one such area where research and testing has flourished since 2009 as it is relied on quite frequently as a means of individual identification and can weigh heavily in exoneration or conviction of individuals.

Testimony on microscopic saw mark analysis in bone has been admitted in multiple criminal trials. In a recent case, *Digirolamo v. New Jersey* (2018), forensic anthropologists Chris Rainwater and Ben Figura, working for the Office of Chief Medical Examiner in New York City, testified on the saw mark evidence from the case. Experimental cuts with the suspect saw were made using cow bones for comparison, with the conclusion that the saw marks from the cow bones were consistent with the saw marks from human bones in the case. Through testing from the defense expert, Peter DeForest, a forensic consultant in Ardsley, New York had noted metal burrs on edges of the saw teeth and when comparing them to new blades from the store, concluded that the suspect blade was new and could not have been used to saw the human bones found during the investigation. It should be prefaced that the presentation of this example case is not meant to say the conclusions drawn by the forensic anthropologists or defense expert were incorrect.

The above case is just one recent instance where nonhuman bone had been used as a proxy for human tissue during an investigation involving saw mark evidence in bone; it is not unique. But it has yet to be thoroughly investigated as to how variation in bone tissue properties (as illustrated by human versus nonhuman proxies) affects our conclusions on potential tooth size as

measured from human bone, for example, and what we should include in our presented error range of “consistent with” before we begin to exclude suspect blades. Through assessment of how tissue proxies affect the variability of measurements, this study will “push the system” and assess potential limitations of microscopic saw mark analysis so that when testimony on experimental marks are provided in the courtroom, there is a better sense of accuracy and precision. Again, legal decisions are made based on the evidence that we collect, analyze, and present, so how confident are we in our methods and consequential conclusions?

2.0 Material and Mechanical Properties of Human and Nonhuman Bone

Bone is a vital component of the human body, shaped and tested by natural selection throughout the evolutionary history of vertebrates. It serves numerous functions, the most obvious being its contribution to body shape and structural support, providing an attachment site for the skeletal muscles that allow us to move. Throughout our lives bone grows and changes form, adapting to and reflecting the demands under which it is stressed. Thus, depending on the anatomical region studied, bone may have different microstructural properties and therefore different biomechanical properties depending on that region's function. Thus, the shape and structure of a bone reveals its function and helps determine where it will fail when the forces impacting bone are too great to be elastically absorbed. Understanding fracture mechanics is key to the interpretation of trauma by forensic anthropologists, but also to health practitioners helping patients recover from a musculoskeletal injury as well as bioengineers who create bone alternatives for tissue implants. These intrinsic, structural features of bone and its relationship to surrounding tissue are what will be reviewed in this chapter to understand why bone fractures the way it does, how this relates specifically to sawing and saw mark research, and will conclude with how human bone differs from that of common animal bone proxies (pig and deer).

In order to understand how bone fails under various injury mechanisms, it is vital to understand what makes bone "strong" in the first place. This requires knowledge of bone's basic components and structural organization, which can be viewed from three, structural, hierarchical levels (Rho, Kuhn-Spearing, Zioupos 1998). Mechanical properties of the lower levels will be incorporated into this discussion when appropriate. The smallest level, nanostructure, illustrates how bone may be both strong and stiff due to the underlying chemical bonds between molecules

of bone's major components, collagen and hydroxyapatite. On the next level, microstructure, strength and stiffness can be analyzed from bone cells, their organization, and their communication networks to the rest of the body. Finally, on the macroscopic level, bone's obvious organization, cortical and trabecular bone, shows how adaptive and effective bone is at distributing forces through the skeleton (and associated tissues), preventing constant injury daily. Due to bone's hierarchical structure, failure can occur at any of these hierarchical levels, which cannot be said for most industrial fibrous composite materials (Currey 2002), making bone a difficult tissue to model and fully understand the intricate relationships between each of these levels.

2.1 Organization of bone at different structural levels

2.1.1 Nanostructure

At the molecular level, bone is a composite material, composed of a fibrous protein, type-1 collagen, which is encased in calcium phosphate crystals or hydroxyapatite. Composite materials are generally stronger than either of the individual components. And unlike homogenous, brittle materials (i.e., glass), any crack that spreads cannot easily travel in one direction. Instead, cracks must deviate at different angles around the various components of the composite, thus making composite materials more effective at preventing further crack propagation. The inorganic mineral component of bone accounts for approximately 60% of a bone's weight, with the remaining percentage coming primarily from collagenous protein and water. Bone being primarily mineral has major implications for its mechanical behavior, making bone ultimately more rigid than elastic (Frankel and Nordin 2001). As just stated, bone also contains large amounts of water; a feature

whose importance is often underrepresented in fracture biomechanics studies (unless injury timing is under question) but plays a vital role in shaping a bone's mechanical behavior when tissue is hydrated and otherwise fresh or in a "green-state" (Symes *et al.* 2012).

Collagen is the most abundant protein found in animals, its mineralization proving unique to vertebrates. Approximately 85-90% of the protein in bone is collagen. Molecularly, it is shaped in a triple helix structure, strengthened by hydrogen bonds (Rho, Kuhn-Spearing, Zioupos 1998). Microfibrils of collagen are laid staggered together, overlapping loose ends, to form thicker fibrils. While it can be described as an overall "rigid" material, in comparison to the hydroxyapatite component of bone, collagen is elastic. The resulting elastic quality of bone allows its exposure to small, daily, repetitive forces without catastrophic failure. Microfractures may still occur (Frost 1960), but these are more readily repaired through continuous remodeling of biological tissue.

The hydroxyapatite component of bone is quite a difficult material to get in isolation due to the nature of its growth amongst collagen fibrils. This mineral component of bone is also impure, sometimes taking the appearance of carbonate apatite (dahllite), which is more often found near vascular channels and marrow spaces. The influence of the mineral component on bone stiffness is obvious; however, its overall individual strength is problematic to measure in isolation, so estimates are usually provided. The location of the mineral, in relation to the collagen fibrils, has been contended over the years. It was once thought that the mineral was only located at the ends of connecting collagen fibrils. Now researchers understand that there is mineral between and within fibrils, although the proportional distribution of each is uncertain. It likely also varies by location on the bone and in the body (Currey 1992).

Furthermore, the crystalline structure of bone mineral led some to investigate the piezoelectric nature of bone (Bassett 1965), which means that when mechanically deformed, the

electric current generated within the tissue shifts to reflect or counter the applied force. Yasuda (1953) was the first to demonstrate this piezoelectric quality of bone and in 1957, Fukada and Yasuda demonstrated that dry collagen also exhibits a piezoelectric property (Fukada and Yasuda 1957). But when bone is tested pre- and post-mineralization of the tissue, the overall electricity generated diminished, so both collagen and mineral have bioelectrical properties (Bassett 1965).

2.1.2 Microstructure

At the microscopic level, bone contains three main cell types: osteoblasts, osteocytes, and osteoclasts. There are also bone-lining cells that make up the periosteum and endosteum. Together, these cells allow for the growth and repair of bone tissue throughout our lives. Channels are formed through the produced bone, surrounding blood vessels (Volkmann's canals), that allow for the exchange of nutrients and waste, as well as cellular communication networks (canaliculi) between bone cells. The bony product of these cells can also be organized in several ways, with differences between mammalian groups. For humans, bone can take the form of lamellar (with primary osteons and secondary osteons/Haversian systems) or woven bone. Bone may also take the form of fibrolamellar bone, also referred to as laminar or plexiform bone, although this type is mostly found in large mammals (including pig and deer) at anatomical sites requiring rapid expansion in diameter (Currey 2002; Hall 2005). Histologically, plexiform bone is a network of tightly packed, symmetrically placed, rectangular vascular canals (Mulhern and Ubelaker 2001). A species and even a specific bone may be composed of both lamellar and plexiform bone. Plexiform bone will be further reviewed with nonhuman bone models at the end of this chapter; focus in this section will be on woven and lamellar bone.

Bone-lining cells coat all surfaces of bone, the exterior, interior, vascular and communication channels, etc. This coating is a strong collagenous sheath, the outer layer of which is referred to as the periosteum while the inner layer, within a bone, is referred to as the endosteum. The periosteum acts as a transducer and when stimulated, by muscles and tendons for example, releases osteoblasts into the area to build bone. The endosteum, however, is assumed to be under more biomechanical strain or greater cytokine exposure from the bone marrow, so instead of building bone, more bone here is typically resorbed (Hall 1978; Clarke 2008). Thus, the marrow cavity normally expands with age.

Osteoblasts are derived from bone-lining cells of the periosteum and endosteum (although they are directly derived from mesenchymal stem cells) and when functioning together, build bone at the surface. Initially, osteoid or unmineralized collagenous matrix is laid down. Eventually this matrix will become mineralized and modeled into actual bone.

Osteocytes are mature osteoblasts that become encased in bone and connect to other osteocytes imprisoned in the matrix, as well as with the bone lining cells via canaliculi. It was often thought that the osteocyte had no function once it became encased in bone; however, actions of biomechanical forces on bone can be sensed by the osteocyte syncytium (mechanosensation) within bone via this canalicular network and gap junctions between cells. Overall, osteocytes are thought to support bone structure and metabolism (Hall 1978, 2005; Clarke 2008). The shape of the lacuna that the osteocyte sits in is that of an oblate spheroid, with an equatorial length approximately five times longer than its polar axis (Currey 2002). It is important that the lacuna, and any hole or channel traveling through bone, not impact the integrity of bone to any significant degree. Empty lacunae suggest that osteocytes undergo apoptosis, likely due to breakdown of

communication channels with adjacent osteocytes. With less osteocytes, bones cannot easily maintain themselves, so overall bone quality decreases in these types of cases.

Osteoclasts break down old or broken bone. These are the largest of the bone cells, derived from cells in the bloodstream, mononuclear precursor cells of the monocyte-macrophage lineage (although still ultimately derived from mesenchymal stem cells), rather than having local origins (Hall 1978; Reddi 1981). They organize on bone's surface and essentially digest the protein and mineral components of bone. The ruffled edges of osteoclasts allow for increased amount of surface area in contact with the bits of bone that need to be resorbed (Hall 1978, 2005). The pits that form and in which osteoclasts can be viewed are named Howship's lacunae.

As aforementioned, in humans, the bone produced by these cells can be arranged as woven or lamellar bone. The first type of bone tissue created by these bone cells during development is called woven bone, found in both fetal and infantile bone, as well as in bony calluses of healing, otherwise mature bone. It is laid down at very rapid rates, typically producing more than 4 mm of bone a day (Currey 2002). The name "woven" is a misnomer, as there are no true "woven" biological structures; however, it does provide a sense of understanding of the disorganized appearance of woven bone. Because of its rapid growth, woven bone is unorganized and highly porous (Boyde 1980). The fibers are not parallel within each layer like typical mature, lamellar bone. "Woven bone is adapted for speed of construction, not mechanical excellence" (Currey 2002). When immediate structural support is needed in a short amount of time, such as at a fracture site, woven bone is ideal. It is particularly brittle because it has a higher mineral content than mature bone. Ultimately, it will be remodeled into mature lamellar bone. If bone tissue had adapted to be isotropic, rather than anisotropic, then woven bone would have been a more ideal choice than

having a “grain” to bone, oriented on the principal loading axis. But, the price to pay for this versatility of woven bone is a reduction in overall stiffness (Su *et al.* 2003).

Lamellar bone in humans appears in mature and remodeled bone. It is far more precisely arranged and oriented than young, immature woven bone. The overall degree of mineralization is also less than woven bone, making it less brittle (in a healthy, mature skeleton). Collagen fibrils and surrounding hydroxyapatite are organized along the long axis of bone in parallel sheets. The collagen fibrils rarely pass between sheets. Giraud-Guille (1988) have shown strong evidence that in various lamellae the collagen fibrils are, like the collagen itself, arranged in a helicoidal structure, also composed of layers, with some alternating direction between layers, as can be seen with the lamellar sheets below. This helicoidal arrangement of fibers is quite common in biological tissues (Neville 1993). The sheets may also vary in thickness, like the rings of a tree trunk. In actuality, collagen fibrils within the lamellar sheets are parallel within that sheet and the fibrils in the next sheet may be perpendicular to these. This alternating pattern of collagen fibrils in lamellar sheets of bone has important mechanical implications, as these alternating sheets allows for a stronger, more adaptable bone overall that can resist forces from multiple directions. These fibrils generally run longitudinally along the bones, which leaves bone strongest in resisting compressive forces.

Where blood vessels pass through bone, primary osteons form around them, arranged in rings of lamellar sheets. Primary lamellar bone (circumferential lamellar bone or parallel-fibered bone) is the intermediate stage of lamellar bone between woven and secondary lamellar/Haversian organization. It is more mineralized than secondary lamellar bone; however, the fibrils have more organization to them than woven bone, hence the name “parallel-fibered” bone. (Ascenzi *et al.* 1967; Enlow 1969). Secondary osteons or Haversian systems, by name form secondary to primary

osteons and still take a ring formation but are further surrounded by cement sheaths. Secondary osteons are formed through pre-existing bone via osteoclastic activity and general bone remodeling, so they appear to be drilled randomly into the concentric sheaths of the original primary osteons, with no concern for pre-existing structure. The cement sheath that surrounds secondary osteons is merely the line at which the cutting cone or remodeling action has stopped and before any new bone is laid down in that region. The mineral and mechanical properties of the cement sheath are controversial; however, it is generally agreed upon that there is less collagen than surrounding bone (Frasca 1981; Schaffler *et al.* 1987). Cement sheaths are well designed for toughness, possibly serving to prevent crack propagation (Currey 2002).

2.1.3 Macrostructure

At the macroscopic level, bone tissue can be distributed in two different ways, as cortical bone or trabecular bone (although they have the same molecular and microscopic origin). Both cortical and trabecular bone are laid down in a lamellar pattern with collagen fibrils altering directions (Eriksen, Axelrod, Melsen 1994). The central shaft of long bones and diploë spaces in the flat bones also contain bone marrow and blood that may also help absorb forces and help internally stabilize hollow spaces (Currey 2002).

Cortical bone, also known as compact bone, is non-porous and dense (with the general exception of canaliculi, lacunae, and Volkmann's canals), making it less forgiving or absorbent of external forces as its spongy counterpart, trabecular bone. It typically makes up the shafts or diaphyses of long bones, providing a rigid structure for support (Hall 1978). Cortical bone tissue is the most frequent type of bone tissue studied. Osteons and their Haversian canals are parallel along the main axis of bone and clearly visible histologically, as compared to trabecular bone. As

previously mentioned, the cement lines surrounding Haversian systems are thought to represent structurally weak places between new bone and old bone; however, this region may also help prevent crack propagation and fatigue, so it is certainly not undesirable (Reilly and Burnstein 1974). The only real porosities are for blood vessels, lacunae, canaliculi, and of course, the marrow space.

Trabecular bone (also known as spongy or cancellous bone) is highly porous, composed of a spongy, rod and plate network of bone. It is located within the epiphyses of long bones, as well as within short bones, flat bones, underneath tendon insertion sites, and within vertebral bodies. The amount of spongy bone present in any specific anatomical site may also vary. For example, the vertebrae have proportionally the most trabecular bone (~60-90%), the intertrochanteric region of the femur has ~50%, the femoral neck ~25%, the distal radius ~25%, the mid radius ~1%, and the femoral midshaft ~5% (Bilezikian, Raiz, and Rodan 1992), although proportions will vary between individuals, especially by age and sex.

Trabecular bone varies in its degree of fine-scale structure (i.e., lamellae orientation), large-scale structure (i.e., strut shapes and thicknesses), and overall porosity. Where longer plates form, between struts, they may preferentially orient along the main axis of mechanical stress, as seen in the femoral neck region (this will have implications for the degree of anisotropy, as discussed below). The amount of porosity of trabecular bone relates to the volume of non-bony tissue, such as marrow in mammals or gas in birds (Hall 2005). Trabecular bone may be classified as either coarse or fine. Coarse trabecular bone makes up most of the trabecular bone in a healthy adult mammalian skeleton. Fine trabecular bone is found more often in the fetal skeleton and early fracture callus sites where bone is healing. Despite its drastically different appearance from

compact bone, it still has osteons. Osteons in trabecular bone are often referred to as packets. These packets are semilunar in shape and still composed of relatively concentric lamellae (Currey 2002).

2.1.4 Summary of bone tissue organization

It is clear that bone is a complex tissue arranged in a variety of ways (i.e., woven or lamellar, cortical or trabecular) and able to be viewed from multiple structural, hierarchical levels. Each perspective and arrangement of the tissue can result in a different understanding of bone material and resultant mechanical properties. How trabecular bone reacts to sawing or blunt force trauma will be unlike that of cortical bone. Similarly, the organization of lamellar bone and disorganization of woven bone will impact the appearance of fractures because these two tissues are so dissimilar in arrangement. It is vital that we take these intrinsic, structural viewpoints with us when considering how varied extrinsic impacts might affect the bones and bone tissue.

2.2 Basic mechanical concepts

Before further discussing macroscopic bone structural organization and its resultant mechanical properties, it is important to review several key concepts. Put simply, ***strength*** is the load a bone may bear before breaking and ***stiffness*** is the rigidity of a bone (Currey 2002). Stiffness is the extent to which a bone resists deformation in response to an applied force, and not necessarily plastic (permanent) deformation. Stiffness is specific to a cross-section of material. Another concept, ***toughness***, describes the amount of work or energy required to yield or fracture bone (Currey 2002). For example, a rubber tube has a low stiffness and can easily bend; however, it is

difficult to break so has been described as “tough.” The opposite would be true of a glass filament which is stiff, not allowing for any deformation, and brittle, breaking easily as it is not very tough or strong (Tencer 2006). Material *hardness* is a correlate of stiffness; how resistant is the material to plastic deformation or penetration (Zysset *et al.* 1999; Currey 2002). A “soft” material would deform more easily than a “hard” material. There are several methods to test material hardness, including the Rockwell, Brinell, and Vickers hardness tests; the type of test used will depend on features, such as the material structure you want to measure and the size of the sample. Overall, the strength and stiffness qualities of bone depend not only on the structure of the material components, as discussed in the earlier sections of this chapter, but also on the shape of the resultant whole bone. Bone cannot be both “really stiff” and “really tough” because in order to increase stiffness, the mineral components must be packed tighter and tighter, in turn making the passage of cracks through bone a lot easier (less tough) (Currey 2002).

These various properties of bone can be gleaned from a stress-strain curve (**figure 2-1**), which can be used to examine the behavior of bone under a given stress and allow for a comparison between bone and different materials. When a bone is loaded under some force, this force produces a stress on the bone. *Stress* is the intensity of a force acting on a specific plane; the load per unit area of a bone (Wescott 2013). *Strain* refers to the response of the bone to that applied stress; the dimensional change in loaded bone (Wescott 2013). To an extent, bone is elastic because of its collagenous protein component. If strained and upon removal of that strain, bone returns to its original shape, then the bone is still within the *elastic* or pre-yield region. This linear portion of the stress-strain curve, where stress and strain are proportional to each other, reflects only bone (material) elasticity, so is also referred to as Young’s Modulus of Elasticity. Young’s modulus is the most straightforward model of this region; however, there are other, more complicated moduli

that could be used here (Currey 2002). Once the strain on a bone becomes too great, the bone will yield and enter the *plastic* region or post-yield region of the stress-strain curve. This means that a bone's shape is permanently deformed (Hildebrand and Goslow 2001; Wescott 2013).

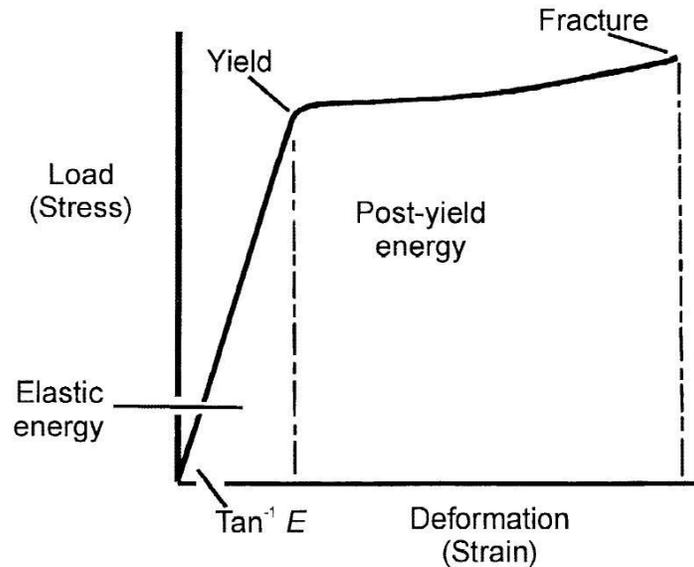


Figure 2-1 A stress-strain curve showing regions of elastic and plastic deformation for a bone loaded in tension. (Figure from Currey 2001; obtained with permission).

Not all materials (or even bone when loaded at a high enough velocity) may show plastic deformation (Symes *et al.* 2012). The less plastic deformation, the more *brittle* the material is behaving (Wescott 2013). If the strain is not removed and the force continues, the bone will eventually fracture. If the region between yield and fracture is quite lengthy, the material can generally be described as more tough. The area under the completed stress-strain curve is proportional to the amount of energy absorbed by the bone. Fracture morphology reflects the forces and resultant stress generated by the specific mechanism of injury, as well as the ability of the bone and its surrounding tissues to resist these forces (Pierce *et al.* 2004). Overall, this model is

overly simplistic and does not fully apply to many biological tissues. For example, cartilage does not have a “linear” region of the stress-strain curve (Currey 2002).

Bone has two other broad descriptors that can be used when discussing its overall mechanical properties. Along with being a composite material, it is also both anisotropic and viscoelastic (Wescott 2013). A material can be described as *anisotropic* when its mechanical properties differ depending on the direction in which it is loaded (Currey 2002; Bankoff 2012). If the material acted consistently, irrelevant of direction of the load, the material would be described as isotropic (Downey and Siegel 2006). Isotropy is a much easier property to model (Currey 2002). Because bone is constantly stressed in the longitudinal direction throughout a lifetime, it is strongest in this direction, at least when resisting compressive forces.

A material can be described as *viscoelastic* when it responds differently depending on the speed to which the load is applied as well as the amount of load time (Wescott 2013). This feature of bone is clear when comparing low velocity and high velocity injuries. When a low velocity load impacts bone, bone has time to undergo an elastic and plastic phase prior to fracture; however, when a high velocity load impacts bone, the bone acts more like a brittle material and shatters. In the latter case, there is no plastic deformation taking place prior to fracture (Symes *et al.* 2012).

2.3 Load types

In life, the body is under constant load, by body weight and gravity, muscle forces, and various external forces. There are three primary load types that act on bone: tension, compression, and shear (Gozna 1982; Currey 2002). One may also include bending (tension + compression loads) and torsion (tension + compression + shear loads), although these are more complex to

model as they represent various combinations of the primary load types. Pure tension and compression are the forces most often studied and modeled because they are easier to model and work well with the stress-strain curve. However, the same cannot be said of shear or the various combinations of these three primary loads. Ultimately, bone is weakest in shear. When only comparing pure tension and compression, again as models often do, bone is weaker in tension.

Compression is a force that presses edges of the same bone together; in other words, under pure **compression**, strain causes a decrease in length along the axis of compression. This includes effects from muscles, weight bearing, gravity or other forces pressing down on bone. This causes the bone to shorten along the axis of compression and lengthen perpendicular to this. Bone is strongest in compression. This resistance comes mainly from the rigid mineral component of bone and is built up during our growth and development (Gozna 1982; Currey 2002; Symes *et al.* 2012). These compressive stresses, and other forces, are essentially responsible for expediting new bone deposition when the bone lining cells are stimulated to release osteoblasts (Herring 2011). Too high a compressive load can result in a compression fracture. Continuous compressive stress can result in a buildup of microfractures (Frost 1960), where the rate of healing and remodeling cannot keep up, resulting in bone failure. This is common in anatomical sites like the lower vertebral column.

Tension is a force that pulls or elongates a bone along the stressed axis; in other words, under pure **tension**, strain causes an increase in length along the axis of tension. Physiologically, this can be seen with tendons of muscles pulling on a bone's surface. In these situations, the collagen fibrils of the tendon go into the fibers of the periosteum, all aligning along the pulling axis. The histology of bone is quite altered at these attachment sites, with the "grain" of bone also aligning with the action of the tendon. If the amount of force on the bone is too great to bear, a tear

will occur at the muscle insertion site, likely resulting in an avulsion fracture. (Gozna 1982; Currey 2002; Symes *et al.* 2012).

Bending forces are combinations of both compressive and tensile forces, creating a concave (compressive) and a convex (tensile) side. Here, the elastic limit of bone is fully tested at a location along the bone shaft, commonly at the midshaft of long bones. As bone is weaker in tension than compression, the side under tension will be the first to fracture. This is located opposite the side of impact. Fractures from bending can be described as transverse, oblique, or butterfly. (Gozna 1982; Symes *et al.* 2012).

A **shear** force is one that is applied parallel to the surface of an object, a sliding movement or angular impact. For example, scissors cutting paper and sawing through bone produce shear forces. Shear can arise from compression, tension, or even a combination of the two. When a fracture results, it will be parallel to the direction of the applied force. (Gozna 1982; Symes *et al.* 2012).

Failures in torsion result from a bone being twisted or rotated, culminating from a combination of tension, compression, and shear forces. A spiral fracture is produced when torsional forces are too great to bear, and the bone fracture opens and twists upwards in a spiral. A common site for spiral fractures is the distal tibia, where the foot may be pinned while the ankle continues to twist. (Gozna 1982; Symes *et al.* 2012).

Beyond load type, two other load characteristics are important to note, **load magnitude** and **load rate** (Gozna 1982; Özkaya and Nordin 1999). The effects of magnitude are obvious. The more force impacting a bone, the more energy it will have to absorb (or fail trying). Some energy will be lost in deformation of the bone, some through fracture, and a lot of it is dissipated into surrounding tissues. More energy is required to produce complex fracture patterns. The rate of a

load is also vital to know, as aforementioned, bone is viscoelastic, so how fast a load impacts bone will affect how bone reacts as evidenced by morphological differences between blunt force and ballistic trauma.

2.3.1 The mechanics of sawing

As this dissertation focuses on the impact of bone tissue properties upon saw mark evidence in bone, sawing will be the load type of interest. The cutting motion of a saw blade is primarily shear; however, compression forces are also introduced by the weight of the sawyer pushing down upon the bone in order to cut through the material. This often results in some minor blunt force trauma at the bottom of a cut, as evidenced by a breakaway spur and notch, which will be further explained in chapter three. Monroy Vazquez, Giardini, and Ceretti (2014) describe sawing through metal as shearing forces separating a chip from the workpiece, while further explaining, “in the actual cutting of the metal, the tool deforms some of the material and then separates it through plastic deformation.” This phenomenon likely occurs in bone as well, although there is no apparent literature on this topic. As a saw progresses down a cut, it leaves horizontal striations (hardness of the saw and material cut dictating saw mark presence). In some instances, striations are not horizontal, but are instead wavy or “hopping,” reflective of the saw teeth that cut through the material. These *tooth hops* can be used to assess blade tooth size or TPI (Symes *et al.* 2005). The process of tooth hop formation is not known, but it is likely their presence is from repositioning of a saw blade, starting or stopping a cut, or similar processes related to the sawyer. These hypotheses would mean that tooth hops are more frequently the result of hand saws where more variation would be introduced by the sawyer; however, tooth hops can still result from power saws (i.e., reciprocating power saws), especially in a kerf floor when a cut is incomplete.

Unlike experimental setups studying pure tension or compression where the amount of force applied remains consistent, shear forces generated from sawing, specifically hand-powered sawing, will be inconsistent. Most saw blades cut in one-direction, either on the push or pull stroke, with cutting typically applied on the push stroke for Western saw blades. How fast a handsaw cuts through material is reliant upon the sawyer, which will vary by individual (size, strength, fatigue, experience, etc.), level of depth in the bone, hardness of the bone, and binding of the saw within the cut, either from blade twisting, soft tissue, or bone dust adhered to the blade. Another concern with shear forces generated from a saw depends upon the saw teeth because the bone tissue simultaneously affects the condition of the blade teeth. Blade teeth dull over time and the amount of energy used by the sawyer increases as the teeth dull, making it progressively harder to cut (Bariska *et al.* 2016). This is an example of the reality of skeletal trauma, and many other biomechanical phenomena; there will be a combination of extrinsic factors involved in any “one action” which will impact how the tissue responds. Combine this with intrinsic variability impacting a bone (age, sex, etc.), and the situation is highly complex to model with any simple laboratory experiment.

2.4 Bone tissue response to mechanical loads

We have so far reviewed the hierarchical structure of bone, its mechanical properties, and the various loads under which it can be stressed. But it is necessary to expand this discussion to bone on a larger scale, as segments of bone or as whole bones within anatomical contexts. This is important because most biomechanical research is performed on thin sections of bone (Symes *et al.* 2012), such as a 1 x 1 cm square of cortical bone derived from the anterior femoral midshaft.

While understanding the minutia of isolated tissue samples is fundamental to understanding bone as a material, fractures occur in complete bones incorporated within a complex anatomical context. Also, outside of the laboratory, whole bones are faced with a combination of different forces that may not all be entirely calculable (Symes *et al.* 2012).

Cortical bone is represented in all arrangements of complete bones: long, short, flat, irregular, and sesamoid bones. Long bones are of primary interest because they are the most frequently injured (Gozna 1982). A functional goal of long bones is to carry compressive loads and bending moments over a long axis for weight-bearing and locomotion, as compared to short bones which do not typically undergo bending forces (Keaveny, Morgan, and Yeh 2004). Inside of this thickened tube sits the marrow cavity, of which there can be red marrow (hematopoietic tissue) and yellow marrow (fat). Gurkan and Akkus (2008) show that bone marrow contents, mesenchymal stem and progenitor cells, are responsive to hydrostatic pressure and fluid shear, for example, which if this mechanical environment is disturbed (by changes in age, disease, etc.), these resident stem cells may be affected as well, altering the homeostasis of bone.

Trabecular bone tissue has extremely different mechanical properties from cortical bone due to its porous nature. It is hypothesized that the boney struts develop along the direction of principal stresses, with open spaces filled by marrow (Murray 1936; Roesler 1981; Hert 1994; Biewener *et al.* 1996; Cowin 1997; Currey 2002). It is hard to pinpoint all forces acting on a bone at any one time and which of these is/are principal; and yet, in some bones or bone regions it seems more obvious. The human vertebral bodies, for example, are loaded axially and located between stress-distributing intervertebral discs; therefore, the principal compressive stresses run from one vertebral body directly inferior into the vertebral disc of the adjacent vertebra (Silva *et al.* 1997; Cole and van der Meulen 2011). Trabecular bone demonstrates the greatest strength when loaded

in compression parallel to the trabecular system, like these vertical forces on the vertebral column (Downey and Siegel 2006).

Ultimately, the mechanical properties of trabecular bone tissue are less well understood than cortical bone tissue, but generally trabecular tissue is described as less stiff (Currey 2002). **Figure 2-2** provides a comparison of cortical and trabecular bone stress-strain values while placing both within the context of other common materials (ceramics, stainless steel, hard resins, and other collagenous biological structures) for comparative reference. **Figure 2-3** presents elastic modulus values for human and bovine trabecular bone as viewed from different structural levels. Trabecular regions can also typically withstand greater strains (Nordin and Frankel 1980). The density (proportion of bone in overall anatomic volume) and trabecular architecture have been found to have the most influence over mechanical behavior for an anatomical region (Hodgkinson and Currey 1990; Carter and Hayes 1976; Gibson 1985). When trabecular bone is loaded under compression, the post-yield region prior to failure is much longer than that seen in cortical bone due to buckling of trabeculae. Thus, trabecular bone works well as a shock absorber at the joint surfaces for this reason, as it is less rigid than cortical bone, so a few microfractures will not compromise the overall structural integrity of the joint.

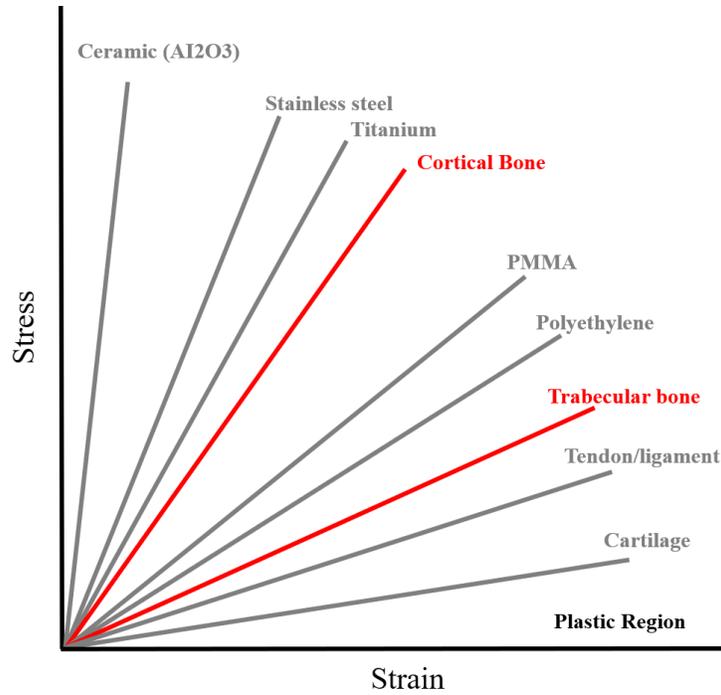


Figure 2-2 Relative Young's modulus (E) of various materials including cortical and trabecular bone (not to scale).

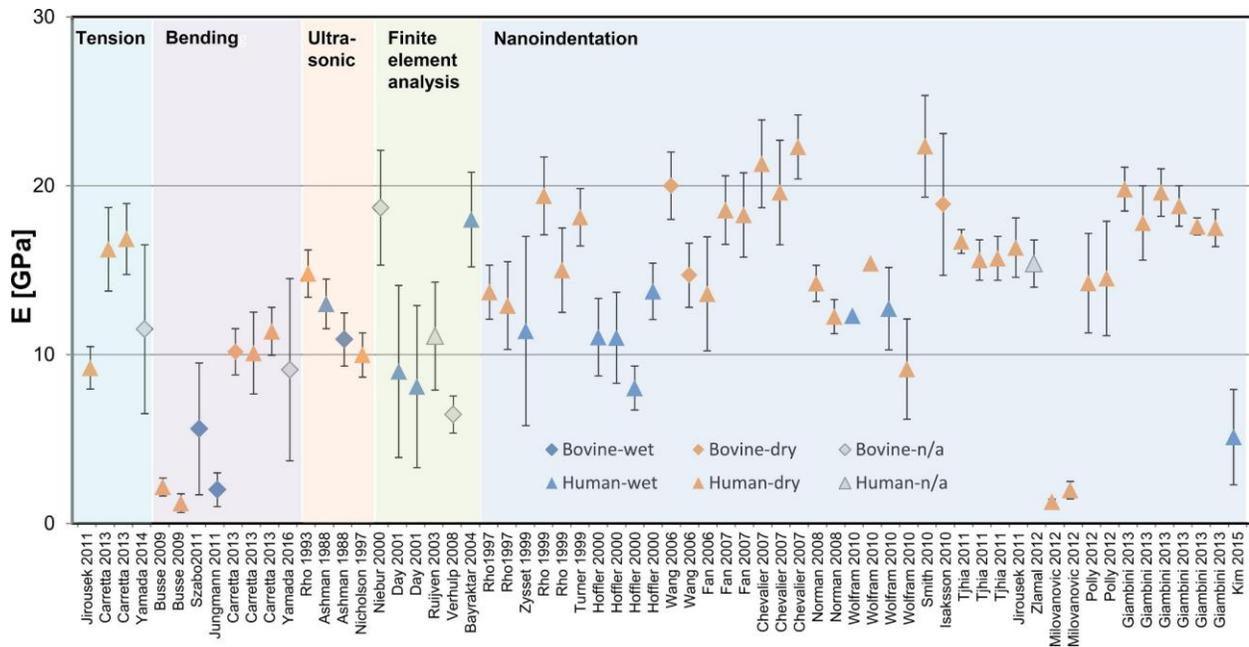


Figure 2-3 Trabecular elastic modulus of human and bovine bones as stated by numerous studies. Values from different structural levels of bone and through varied methods. (Figure from Wu *et al.* 2018; obtained with permission).

Whole bone forms are the result of a combination of cortical and trabecular tissue regions and the distribution of these macroscopic tissue types will influence the overall mechanical properties of a bone. Whole bones grow in such a way that normal, daily loads typically remain in the elastic region of the stress-strain curve (Currey 2002), avoiding catastrophic failure. Experimental studies utilizing whole bones to assess overall bone strength focus on three features: total bone mass (often measured as bone mineral content), geometric distribution (geometry, architecture), and material properties or tissue composition (Cole and van der Meulen 2011). By increasing overall mass and distributing that mass to high-stress loci, and enhancing material properties, bone strength increases. Naturally, mechanical properties will be least distorted if the shape of one part of a bone gradually blends into the next. Sudden changes in geometry would create excessive stress.

Wolff's law states that bone responds to the physical demands under which it is placed (Wolff 1892; Frost 2003). Therefore, bones and their component regions look different because they are under different loads. However, this law alone cannot explain how the process of bone formation or resorption functions, nor can the law allow predictions of bone shape under all mechanical stressors (Frost 2001). Wolff's law, when originally presented, focused on whole bones and bone cells, where a force is applied and a cellular response is generated, with cells either building up or removing bone at a site. It is a negative feedback loop but limited in discussion to just bone tissue. Overtime, increasingly more research developed around the topic of biomechanical strains and how bone can be modeled around them, including Frost's "mechanostat" (Frost 2001). Some internal "mechanostat" senses biomechanical strain and ensures that the skeleton adjusts to the appropriate mass and distribution to counter these strains (Frost 1992). Under increased mechanical usage, such as during exercise or resistance training,

bone would build. Under decreased mechanical usage or decreased skeletal loading, bone would resorb. Normal, daily mechanical usage would ultimately result in a conservation of bone mass.

As these strains change throughout our lives, so too do the bones. Cases of chronic disuse, where muscle function has decreased or is absent, are found to resemble cases of osteoporosis. But even in cases of permanent disuse or paralysis, bones and associated structures will not completely disappear (Frost 1986). Spaceflight, for example, reduces skeletal loading, tipping the scales of skeletal homeostasis towards an increased resorption of bone with a decrease in bone formation (Androjna *et al.* 2012). Androjna *et al.* (2012) also report other body alterations that occur during space-living that influence bone mass and strength, including: 1) calcium metabolism and bone hormone endocrinology; 2) cardiovascular deconditioning impacting blood supply; and 3) loss of associated skeletal muscle mass contributing to daily loading patterns on bone.

It is also likely that hormones, drugs, and other various factors can alter mechanical usage thresholds leading to increased growth or resorption of bone, depending on the factor affected (Frost 1992). The presently-developing Utah paradigm of skeletal physiology broadens the discussion of bone formation to the tissue-level (adding tendons, ligaments, joints, and fascia (Frost 1995)) and incorporates anatomical features and roles (Burr and Martin 1992; Frost 1992, 2000; Jee and Frost 1992; Schönau 1996; Takahashi 1995, 1999), suggesting that strong muscles make strong bones and weak muscles make weak bones (Frost 2001). But the question remains, how is bone able to respond to changes in biomechanical strain?

Recall that both bone collagen and hydroxyapatite have electrical charges to them that respond to force. Bassett (1965) described this piezoelectric property of bone, predicated on bone's crystalline structure (like semi-conductive, deformed rocks generating small electrical currents), with a similar negative-feedback loop as Wolff's law, providing an ionic perspective of how

Wolff's law could function. The bioelectrical components of bone are not visible or measurable when isolated. When bone is mechanically stimulated by an outside force, piezoelectric transducers generate electric potentials proportional to that force (Bassett 1965). For example, when pushing on a bone, it is likely that stretch-regulated ion channels are stimulated as well, and when opened, allow the flow of interstitial fluid and electrical potential (Hollinger *et al.* 2005). This signal is then recognized by osteocytes (tied into a network with other bone cells), which signal the release of another transducer to alter bone architecture to resist that applied force (Bassett 1965; Hollinger *et al.* 2005). Compressive forces often result in negatively charged regions of bone with the physical response being the addition of bone, while tension results in positively charged regions of bone with the physical response being the removal of bone (Bassett 1965). Negative charges attract positive ones, which for bone means attracting more Ca^{2+} and therefore depositing more mineral in regions under compressive stress. Wolff's law alone is an overly simplistic model of bone tissue response to stress and we see that there are many molecular and physiological mechanisms at play to consider, so not all boney responses can be fully explained (Pearson and Lieberman 2004; Currey 2012). A more recent theory, summarized by Hollinger *et al.* 2005, suggests that instead of only responding to excessive force, fatigue damage, or disuse, the signals for bone to remodel are always active (be it at a low level for daily strains) unless an inhibitory signal is received. Regardless of the specifics, bone tissue models itself in response to applied loads.

When examining the lower leg, for example, the femur and tibia are the two major bones contributing to weight-bearing and are strongest when loaded in compression. They are relatively large bones with dense regions of cortical bone along the shafts and larger joints filled with trabecular bone. However, the fibula, despite its close relationship with the tibia, does not aid in

weight-bearing. Instead, this bone serves mainly as a site for muscle attachments and would be loaded more with tensile forces (Bankoff 2012). Physically, the fibula is a long, slender bone that has a variable shaft morphology amongst individuals.

If we were to concentrate on an anatomical region, such as the femoral neck, further detail about bone organization and relationship to surrounding tissue comes to light. While standing or in the stance phase of walking or running, a bending force is applied to the femoral neck, which increases the amount of compression on its inferior surface and tension on its superior surface. However, when the *m. gluteus medius* contracts (origin: lateral iliac surface along iliac crest; insertion: superolateral surface of the greater trochanter), the total compressive force on the femoral neck increases, causing the amount of tension to decrease. As bone is weaker in tension, this muscular constriction reduces the risk of injury by minimizing tensile forces (Bankoff 2012). From this example, the muscle can both apply tensile force when pulling on a bone, but also redistribute or minimize the effect of certain forces. However, in cases of muscle fatigue during intense exercise, this ability to lighten the load on bone diminishes, increasing the risk for injury (Pirnay *et al.* 1987; Bankoff 2012).

2.5 Factors affecting bone mechanical properties

At the organ level, bone primarily functions as a structural support for the body and as a reservoir for calcium and phosphorus required in various physiological processes. Bone as a tissue forms and reshapes itself across its organizational levels to best meet these functions (Murray 1936; Frost 1986; Frost 1992). But bone varies amongst individuals. Structural differences alter as the body ages or is traumatized. Structural differences may also occur as a result of mechanical

stimuli, sex (sex steroids), other hormones, diet, pathological conditions, lifestyle, and other individualizing intrinsic and extrinsic features. As Hall states in the 1985 introduction added to a reprint of Murray (1936), “the critical question now is not whether skeletal growth and structure are controlled both intrinsically and extrinsically [...], but how the two levels are coordinated to effect skeletogenesis.” The skeleton is often assumed by the general public to be rigid and unchanging, with the exception of osteoporosis, but it is far more complicated than its often credited for and many of these factors (age, sex, nutrition...) cannot be divorced from each other, nor from external stimuli affecting bone formation. Major intrinsic and extrinsic factors affecting the skeleton will be discussed below, with a primary focus on age and sex, but know that they do not act in isolation. The importance of mechanical stimuli is thoroughly addressed above (section 2.4), so will not be discussed further here. Likewise, it is important to draw attention to the fact that genes and epigenetic factors, soft tissue structures (i.e., vasculature) and cartilage (as the precursor and scaffold to endochondral bone) may also be affected by intrinsic and extrinsic factors during growth and development, influencing the final boney product in countless ways; however, these factors will not be discussed.

2.5.1 Age and aging

From birth, bones undergo longitudinal and radial growth and development, until they reach adult mass and size, continuing to remodel to reflect changes in mechanical stress and physiological need throughout the remainder of our lives. This early bone development is done either directly from a mesenchymal precursor (intramembranous growth as seen in bones of the skull necessary for brain protection, feeding, and respiration) or through a cartilaginous intermediate (endochondral growth as seen in most of the postcranial skeleton). Secondary sex

characteristics develop during puberty. Once peak mass is reached (between 25-30 years of age), bone mass will typically decrease approximately 1% every year thereafter (Buckwater *et al.* 2006). Involutional bone loss is seen in both sexes; however, more bone loss occurs in women with the onset of menopause. Bone tissue loss is referred to generally as osteoporosis, although statistical categories distinguish this from osteopenia. It should be noted that it is the prevalence of osteoporosis that increases with age; osteoporosis is not an idiosyncratic disease of the elderly (Boskey and Coleman 2010). The effects of osteoporosis will be discussed with the effects of age, while menopause will be discussed with the sex steroids, but again, these effects tie heavily to one another.

Woven bone quickly arranges into mature lamellar bone, with primary differences between adult and subadult bones occurring in size, shape (at joints and muscle insertion sites), and the occurrence of cartilage at the metaphyses. Fetal bones consist of this woven bone, cartilaginous tissue, and/or Haversian bone depending on what stage of development the individual is in (Hillier and Bell 2007). Fetal skull bones will be especially different from their adult counterparts. For example, the cranial vault bones will consist of but one layer of woven bone, rather than two thin layers of cortical bone surrounding a layer of diploë (Currey 2002).

Frost (2003) states that the largest voluntary loads on load-bearing bones determine most of their strength after birth. Ruff 2003 adds that body size is the most significant element shaping the lower limb during subadult development (weight-bearing), while body size and muscle strength are both significant through growth of the upper limb elements (especially for males). However, Frost (1987;2001) reminds us, that body mass alone, is not the primary mechanical load that lower extremity bones (and vertebrae) experience; rather, body mass is a resistance that our muscles must overcome in order to locomote. Likewise, normal bone mass is adapted to withstand even greater

loads than those simply associated with locomotion (Frost 1987). Pearson and Lieberman (2004) add that overall, cortical bone is most responsive to strain before sexual maturity, when concerning new bone growth (modeling) and remodeling.

Within the regions of the body, the local components of a single bone may also grow at different rates. For example, Wang *et al.* 2010 find that fractures of the distal radius metaphyseal region in children (more often boys and potentially biased by activity levels) have a similar incidence as measured in postmenopausal women. This region had an increased rate of fracture over other sites, such as the distal tibia. It is likely that differential growth rates, with more rapid modeling occurring in the distal radius, has resulted in a greater dissociation between growth and mineral accumulation, making this region weaker and susceptible to injury. Bass *et al.* 1999 also suggest that regions growing faster may be more severely affected by a childhood illness than regions that take longer to grow or are near-adult size. Flachsmann *et al.* 2000 show that adolescent joints were particularly susceptible to shear forces at the cartilaginous growth plate, with the possibility of epiphyseal separation from the diaphysis, affecting the overall morphological fate of that bone if not corrected. These types of examples, regarding growth towards adult bone mass and differential growth rates of bones and their regions are vital to keep in mind when trying to understand mechanical properties of and between different whole bones, but especially when comparing mechanical properties of bone throughout the transition from subadult to adult bone.

In adults, remodeling is the more frequent process that takes place. Bone is restored, with old bone taken away and new bone added in order to keep its overall strength and structure while repairing microdamage. It is a synchronous activity of osteoblasts and osteoclasts. This remodeling process is fundamentally the same between cortical and trabecular bone, although more frequent in trabecular bone as this region is metabolically more active (Rho *et al.* 1998), making it quicker

to replace but also to remove. Thus, bones with proportionally more trabecular bone (i.e., vertebral bodies, the femoral neck, and the distal radius) will be at increased risk for fracture later in life when the balance between osteoblastic and osteoclastic activity is shifted towards increased removal of bone rather than growth. This process of remodeling further complicates the issue of age affecting bone material properties, especially when comparing human and nonhuman bones that do not remodel in the same way or when comparing individuals or bones with distinct remodeling rates. An individual's *actual age* does not equate to the *apparent age* of the tissue (Currey 2002), even within the same bone cross-section. Periosteal bone may be freshly laid down while endosteal bone may be older and just about to be resorbed. Even the endosteal bone of a 9-year-old child will have been around for several years (Currey 2002), long enough to be influenced by daily loads and mechanical stressors.

Similarly, as cellular sensitivity decreases with age and the body's vasculature weakens, cells are slower to respond or recognize damage and blood flow carrying vital nutrients for proper repair slows. In the shafts of long bones, the endosteum or the interior lining of bone facing the marrow, is also metabolically more active from greater cytokine exposure and greater biomechanical strain than periosteal bone, so comparably more bone at the endosteal region is typically resorbed (Hall 1978; Clarke 2008). Therefore, the marrow cavity normally expands with age and the cortical bone diameter of a shaft thins. In studies of individuals over 85-years of age, fracture incidence was 10-15 times more likely than individuals aged 60-65-years of age (Ammann and Rizzoli 2003).

Involutional osteoporosis (i.e. postmenopausal osteoporosis and age-related osteoporosis) affects both women and men in any population (Riggs, Khosla, Melton 2002; Compston 2001). Often in women, postmenopausal osteoporosis is linked to estrogen levels. But the sex steroids,

while significant, are not the only factors responsible for osteoporosis. Osteopenia or osteoporosis may also be induced by secondary hyperparathyroidism, low levels of vitamin D, low levels of dietary calcium, defunct osteoblast function, among other factors (Riggs, Khosla, Melton 2002; Weaver *et al.* 2016). Physiological pathways slow for many reasons in the aging population and do not often occur in isolation. A defect in any factor affecting bone growth, such as the cells that build or remodel bone, the vasculature that brings nutrients to bone, the actual nutrients available to us, the musculature that can strengthen bone, and so on can be responsible for the decline in bone quantity leading to the low bone density levels characteristic of osteopenia and osteoporosis.

When examining bone strength histologically, both trabecular and cortical bone lose strength and increase stiffness with age (Boskey and Coleman 2010). Whole vertebrae, vertebral trabeculae, and femoral trabeculae lose approximately 50-75% of compressive strength throughout adult and elderly life (Cole and van der Meulen 2011). Like compressive strength, vertebral trabeculae also decrease in elastic modulus and toughness by approximately 81-85% and ultimate strain or compressibility by 26% after peak bone mass has been reached (Cole and van der Meulen 2011). Thus, bones of older adults and elderly are weaker and more brittle, leaving them more susceptible to fractures. Empty lacunae within cortical bone cross-sections suggest osteocyte apoptosis, which is likely due to a breakdown of communicating canaliculi between adjacent osteocytes. This canaliculi breakdown has been observed in cases of estrogen deficiency and glucocorticoid treatment (Bourne 1972). With less osteocytes, bones cannot easily maintain themselves, so overall bone quality decreases.

The quantity of the mineral content within bone is also well-known to decrease with age. It is the mineral quality and quantity that take center stage when defining the material hardness of bone, thus affecting saw mark presence in the first place. Proportionally, bone becomes more

mineralized over time (Currey 2002; Boskey and Coleman 2010). This increased mineralization increases fracture risk as bone becomes more brittle, decreasing its ability to plastically deform or absorb forces prior to fracture. The mechanical integrity of bone's collagen network also deteriorates with age, which correlates with a decreased toughness in bone (Wang *et al.* 2002). Wang *et al.* 2000 describes the collagen network as a higher structural tier, made up of collagen fibers. Collagen fibers are made from collagen fibrils, which are composed of type 1 collagen molecules arranged into triple helixes. Bailey *et al.* 1999 found that collagen content decreased with age, resulting in an increased mineralization of bone likely due to a decrease in bone turnover with typical remodeling; there were no changes in collagen's biochemical composition. However, most studies have focused on mineral changes in bone as it is easier to assess in living patients through nondestructive methods.

2.5.2 Sex-specific hormones

Until recently, biomechanical strain on bone during growth and development was thought to be the primary process of bone formation. Sex steroids were thought to only indirectly impact the skeleton (Riggs *et al.* 1998). Albright, Smith, and Richardson (1941) were the first to relate postmenopausal osteoporosis to estrogen deficiency, finding that treatment with estrogen balanced bone density. As more methods for bone density analysis were introduced, this finding by Albright, Smith, and Richardson (1941) were validated.

The primary sex hormone found in females are estrogens (i.e., estradiol and estrone), while in males, the primary sex hormones are androgens (i.e., testosterone). However, these hormones are not unique to a sex. Surgical removal or incapacitation of the sex organs may also affect hormonal levels, as the majority of estrogens in females come from ovarian secretion, while the

majority of testosterone in males comes from testicular secretion. Prior to the study presented by Riggs *et al.* (1998), it was assumed that these sex hormones would mainly act indirectly on the skeleton, by affecting calcium homeostasis, but we now know that this is not the case. The remainder of this section will primarily focus on effects of the sex steroids. In addition to hormonal influence, females can also be affected by the strains of birth and breast-feeding, which can consume a great deal of calcium and other nutrients from the mother in order to feed the infant. This will likely be more problematic for individuals with multiple births or species with many offspring and multiple litters throughout their lifetime. Muscular differences between men and women are also present which can influence bone density, although these differences can also be tied to hormonal levels and are not just related to gender differences in physical activity.

Estrogen is a conserver of bone mass (Compston 2001; Riggs, Khosla, Melton 2002). If an individual is deficient in estrogen, bones will undergo a higher rate of bone turnover. Likewise, during remodeling, the resorptive portion of that process is extended (reducing osteoclast apoptosis). Thus, bones will undergo faster remodeling and bone removal will become the primary process, leading to a decrease in bone density (Compston 2001; Riggs, Khosla, Melton 2002). Riggs *et al.* (1998) proposed a new model for involutional osteoporosis, which found that estrogen was the primary cause of bone loss in postmenopausal women (both accelerated and slow, gradual bone loss phases, see below) as well as a slow, gradual loss of bone in older men.

Testosterone primarily acts to reduce bone resorption, thus conserving bone mass as did estrogen. This is because testosterone is aromatized into estrogen, so bone conservation here is indirect. Thus, testosterone will also impact the lifespan of osteoblasts and osteoclasts. When an individual is deficient in testosterone, overall bone mass will decrease as osteoclasts remain longer to resorb more bone (Compston 2001; Riggs, Khosla, Melton 2002). Testosterone is also converted

to 5 α -dihydrotestosterone (DHT), which is the primary source of androgenic activity on target tissues in the body.

Riggs, Khosla, and Melton (2002) do state, that unlike estrogen, testosterone increases periosteal apposition (radial growth) of bone, with this difference partly accounting for the larger skeleton males achieve during puberty. Males also have two extra years of puberty and a longer pubertal growth spurt than females, which also means males are taller on average. Thus, males are often 10% taller with an approximately 25% greater peak bone mass (Riggs, Khosla, Melton 2002). Greater mass equates to thicker, bigger bones for the average male. Likewise, it is now thought that sex steroids, estrogen and testosterone, affect osteoblasts in different regions of the skeleton, contributing further to sexual dimorphic differences between males and females, with testosterone favoring appositional growth of bone (Wakley *et al.* 1991) and estrogen opposing it (Turner, Colvard, Spelsberg 1990). More research is necessary to elucidate the formation of sexually dimorphic skeletal features in humans.

Once peak bone mass is reached, females have two primary forms of involutional bone loss, an accelerated phase that occurs immediately post-menopause (occurring for 4-8 years after menopause) and a late-phase, gradual bone loss. The accelerated, post-menopausal bone loss in women may be prevented or slowed with estrogen replacement; thus, a decrease or loss of estrogen is a primary cause of bone loss in females. Males do not undergo menopause (although they do experience a shift in sex hormone ratios) and primarily experience late-phase, gradual bone loss. A study of castrated men in Czechoslovakia found that these men experienced a similar, accelerated bone loss as postmenopausal women (Stepan *et al.* 1989). However, bone loss typically experienced by males is not from lack of circulating sex steroids; it is from their decreased bioavailability. Circulating testosterone binds to serum SHBG (sex-hormone-binding globulin)

and since SHBG increases with increasing age, testosterone bioavailability decreases with increasing age (Slemenda *et al.* 1997). Thus, men are not necessarily deficient in testosterone as females are with estrogen.

Riggs, Khosla, and Melton (2002) find that the accelerated, postmenopausal bone loss in women accounts for a 20-30% loss of trabecular bone and a 5-10% loss of cortical bone. Meanwhile, these authors find that the long-term, gradual bone loss leads to a 20-25% loss of trabecular and cortical bone. The mechanisms of bone loss differ between males and females, with females primarily experiencing trabecular loss overtime and males experiencing trabecular thinning overtime (Cole and van der Meulen 2011). These two styles of bone loss relay different effects on bone mechanical properties. In a computer simulation mimicking “10% bone loss,” bone lost from overall trabecular loss decreased bone strength by 70% whereas trabecular thinning only decreased bone strength by 20% (Silva and Gibson 1997; Cole and van der Meulen 2011).

2.5.3 Other hormones

Besides the sex steroids, the skeleton may be impacted by other important hormones, including growth hormone, thyroxine, parathyroid hormone, calcitriol, and calcitonin (Silverthorn 2013). Growth hormone leads to an increase in long bone length during development and it also increases mineralization, which increases bone density. Thyroxine also stimulates bone growth. Parathyroid hormone increases osteoclast proliferation and thus resorption of bone, while also stimulating calcium reabsorption directly through the kidneys and indirectly through the small intestines. Calcitriol promotes absorption of dietary calcium and phosphates, the primary components of the bone mineral. Finally, calcitonin blocks osteoclast activity and stimulates bone’s uptake of calcium. Pathological conditions or miscellaneous stressors affecting the

homeostasis of any one of these hormonal pathways could prove detrimental to overall bone health and strength. For this discussion, attention will be concentrated on bone loss associated with aging, rather than bone modeling during growth and development. (Silverthorn 2013).

The late-phase, gradual bone loss experienced by both men and women may not directly occur from a lack of or inactivity of respective sex steroids. For example, secondary hyperparathyroidism is characterized by an increase in serum parathyroid hormone as well as an increase in markers of increased bone turnover (Riggs, Khosla, Melton 2002). Too much parathyroid hormone without similar increases in dietary calcium means that calcium is pulled from calcium reservoirs—bones (Riggs, Khosla, Melton 2002). Thus, this type of bone loss may be slowed with an increased intake of dietary calcium. A study by Riggs *et al.* (1998) found that estrogen deficiency is an indirect player on parathyroid hormone levels, with a lack of estrogen ultimately upsetting the scales of calcium metabolism, leading to an increase in parathyroid hormone. They add to this that estrogen may play two different roles regarding bone, one where it directly affects bone cells and another where, indirectly, estrogen is mediated by fluctuating levels of parathyroid hormone. These are just two hormones among many, and their relationship is highly complex. We can only begin to scratch the surface with hormonal influences, but the effects they do have on the skeleton are significant and tie heavily to age and sex differences, further increasing bone variability amongst individuals within and between human subgroups (ex. adult males vs. geriatric females) and even different taxonomic groups that undergo similar physiological processes.

Research by Kalmijn *et al.* (1998) explored the effects of cortisol, a steroid hormone produced by the adrenal glands, on bone growth and remodeling. As cortisol secretion remains relatively constant throughout our lives or increases with age, its influence on bone may be

minimal for the general population as compared to the effects of other hormones (Kalmijn *et al.* 1998). Cushing syndrome or hypercortisolism, a disorder characterized by increased levels of cortisol in the blood, can increase bone resorption, decrease bone formation, and affect calcium metabolism by reducing uptake in the intestines and excreting more in the urine (Leong *et al.* 2009).

2.5.4 Dietary calcium and vitamin D

There are many factors related to diet that can impact skeletal properties, the most obvious being calcium and vitamin D consumption. General caloric intake and intake of nutrients beyond calcium and vitamin D are also important, including proteins, carbohydrates, fat, iron, phosphorus, fluoride, infant nutrition (breastfeeding or formula), etc. (Weaver *et al.* 2016). A body deficient in vital nutrients would not be a healthy body and an unhealthy body is unlikely to support a healthy skeleton. For example, eating disorders such as anorexia nervosa would leave an individual deficient in numerous nutrients that both directly and indirectly affect the skeleton. Similarly, muscle and body mass would also decrease, further impacting skeletal growth and maintenance. And finally, individuals suffering from anorexia nervosa may also experience premenopausal amenorrhea resulting in a lower bone mineral density (Compston 2001).

As bone is primarily made of calcium (hydroxyapatite mineral) and since we cannot make our own calcium, it must then be incorporated into our diets. Additionally, vitamin D is necessary for calcium absorption, although our skin manufactures this following UV exposure and we can supplement this with vitamin D in various food sources. These are the primary nutrients in our diet impacting skeletal properties, so any disruptions of these metabolic pathways can compromise bone mechanical properties. Other nutrients are shown to support bone health and strength,

including magnesium, fluoride, and Omega-3 fatty acids, although focus will be primarily on calcium and vitamin D.

Calcium is a vital component to vertebrate physiology. For example, calcium flow drives muscle action potentials and can stimulate graded potentials in the nervous system. Calcium is also necessary for the formation of blood clots, maintenance of a normal heart rhythm (through sinoatrial node action potentials, cardiac myocyte contractions, and contraction coupling), and normal function of various hormones and enzymes (Silverthorn 2013). It is well-known that calcium intake decreases the risk for osteoporosis and associated fractures (Chapuy *et al.* 1992; Cole and van der Meulen 2011). Likewise, vitamin D supplements have also been found to reduce the risk of (hip and nonvertebral) fractures commonly seen in the geriatric population (Chapuy *et al.* 1992; Cole and van der Meulen 2011). Like other factors impacting bone health, dietary factors do not act in isolation. For example, hypovitaminosis D and a diet deficient in calcium can lead to increased levels of parathyroid hormone, meaning calcium is pulled from storage—the bones (Chapuy *et al.* 1992).

2.5.5 Drugs

Dietary factors, outside of those related to calcium and vitamin D consumption may impact skeletal mass and mechanical properties, either directly or indirectly. These include, but are not limited to, chemotherapy, prescription drug use (i.e., antiarthritics, antidepressants, and antipsychotics), non-prescription drug use, illegal drug use (i.e., cocaine and methamphetamines), tobacco use, and alcohol consumption (Saville 1975; Appel 2015; King *et al.* 2015; Liu *et al.* 2015). Drug abuse can also lead to osteomyelitis (bone infection), osteoporosis, and resorption of bone in areas in close contact with the drug (i.e., nose and mouth for inhaled or orally ingested

drugs). In a study of the effects of long-term cocaine use on bone properties of rats, Appel (2015) found that bone density of the cocaine group was significantly lower than the experimental group. Depending on the drug and its intended purposes, bone properties may improve or not. Antiarthritics would encourage bone resorption or inhibit bone formation (Liu *et al.* 2015). Chemotherapy decreases osteoblasts and increases osteoclasts (King *et al.* 2015). Bisphosphonates, to help slow or prevent osteoporosis, inhibit resorption of hydroxyapatite by osteoclasts, ultimately reducing fracture risk; they may also have a beneficial effect on osteoblasts by preventing apoptosis, meaning that bone formation lasts longer (Feng *et al.* 2013).

2.5.6 Disease processes and previous injury

Disease processes that occur throughout an individual's lifespan may also create pathological changes to bone affecting its material and mechanical properties. Some examples have already been presented, like osteoporosis, hyperparathyroidism, and Cushing syndrome. Another example is type 2 diabetes mellitus, a common condition in modern society, which results in bones that are more susceptible to fracturing than similarly aged individuals without diabetes, despite having a higher bone mineral density (Gilbert and Pratley 2015). Many disease processes can impact bone health, by affecting bone mineral, collagen, muscle fibers, blood flow, calcium metabolism, and other related factors working jointly in the body.

Previous skeletal injury can also cause bone material properties to be atypical for an area, especially if the injury is a recent one still in the process of healing or in cases with malalignment during fracture healing. When bone is healing from a fracture, the regions between newly built woven bone (that replaced a prior "soft" cartilaginous callus) and mature lamellae bone (prior to woven bone remodeling) are disjunctions in the structure that can be weak points for another injury

(Clarke 2008). This reactivity varies with metabolic rate (Buckwater *et al.* 2006; Shapiro 2008; Herring 2011).

2.6 Nonhuman bone

Up to this point, the primary focus has been on human bone, as that is of most forensic relevance. However, animal bone proxies have often been and continue to be used in forensic research in various topics of skeletal trauma analysis. Thus, intertaxonomic variability needs to be further explored. How do these nonhuman bone models vary from their human counterparts and what intrinsic or extrinsic factors may affect their bone tissue mechanical properties? Currey (2002) states that mechanical properties of adult, quadrupedal, mammalian limb bones do not overly differ in terms of mechanical strength; however, once you stray away from these parameters, the tissue differences become apparent. For microscopic saw mark analysis, we are primarily concerned with hardness, amount of bone, and organization of that bone as this will ultimately affect the presence of toolmarks in bone cross-sections. Again, the two nonhuman bone models of interest in this dissertation are deer and pig.

Fortunately, there are many similarities between human, pig, and deer bones, as they are all examples of mammalian bone. Mature species exhibit lamellar bone, with organization of bone into osteons. The primary differences in tissue relate to proportions and measurable qualities of cortical and trabecular bone, as well as the presence of plexiform bone in addition to lamellar bone. At the microscopic level, intertaxonomic differences may be noted in size, structure, and patterns of osteons and Haversian canals (Martiniaková *et al.* 2006; Marceau 2007). These species are also subjected to many of the same or similar intrinsic and extrinsic influences as humans (i.e.,

differences attributable to age, sex, diet, hormonal, and pathological conditions) so discussion here will be limited to what specifically makes deer and pigs unlike humans.

2.6.1 White-tailed deer (*Odocoileus virginianus*)

One of the more obvious differences between human and deer (as well as pig) is the use of a quadrupedal locomotion, as opposed to the uniquely human, striding bipedality. Specifically, deer are unguligrade quadrupeds, which means that they walk on the central distal phalanges. From an evolutionary perspective, a reduction in the number of digits occurred, shifting from five to two remaining digits, the third and fourth; thus, deer fall under the genus *Artiodactyla*, which translates to “even-number of toes.” Humans on the other hand are plantigrade, with weight being distributed through tarsals, metatarsals, and phalanges. Ungulate species, like deer, show adaptations reflective of speed, having long, straight limb elements with a reduced number of digits, while running only on the distal phalanges (Kreutzer 1992; Reitz and Wing 1999). Overall, this increase in limb length increases the stride length and speed of the animal. So unlike humans, body weight is distributed through all four limbs, with the hindlimbs providing most of the propulsive power required for quadrupedal locomotion. Thus, while both human and deer femora are weight-bearing and would aid in propulsion during locomotion, the upper limb bones have very different functions as human humeri have been freed from their weight-bearing roles during locomotion.

Skedros *et al.* (2003) examined the limb bones of wild-shot Rocky Mountain mule deer, hypothesizing that the distal elements of a limb would be subjected to more fatigue damage (microcracks) and would therefore exhibit more remodeled (secondary) bone than proximal limb elements, meaning that the proximal elements will have a higher mineralization and lower porosity than the distal elements. Skedros *et al.* (2003) found that %ash decreased from proximal to distal

while simultaneously finding an inverse relationship to secondary osteon population density, which suggests their hypothesis to be correct. Thus, proximal elements exhibit different material properties from distal elements. This property is not unique to deer. Ioannidou (2003) reports further that deer pronk (jumping gait) does not significantly increase the density of deer bones compared to similar sized species that do not locomote in this manner.

Male deer are referred to as bucks and female deer as does. Bucks, after 1-year-old, develop spikes (around 2-years-old unless under optimal nutrition) or full antlers. Antlers have vastly different mechanical properties than bone. They grow rapidly, are nourished externally (through the velvet layer), and their growth is primarily linked to diet (calcium and phosphorus, primarily from bark and twigs), not age. Bucks have adapted to this intense storage and shifting of calcium/phosphorus to the antlers for growth, which may leave other areas of the skeleton (scapulae, ribs, etc.) temporarily depleted of calcium. Antlers are fully grown by August, with the surrounding velvet shed in September. It is not possible to accurately age a buck by the number of points on an antler (dental aging methods are the most accurate). Antlers are shed in the winter after the rut and begin to regrow in the spring. Increased sunlight exposure that occurs in spring is detected through a buck's eyes, which signals the pituitary gland and ultimately leads to an increase in testosterone-levels, driving antler growth. The mating season (rut) typically occurs in the fall with births occurring in the spring. With the gradual rise in male hormones, bucks shed their antler velvet, gain muscle size (particularly around the neck), and begin "shadow-boxing," where they use their antlers to mock-fight trees and their branches in preparation for mating competition. As rutting continues, however, males will lose overall body weight due to increased activity and decreased nourishment. Following impregnation, a doe will often give birth to twins, although triplets are also common; first time mothers will typically have one fawn. Pregnant does thus deal

with a dietary restriction during the winter months, give birth in the spring (around May), and then begin nursing (with weaning around September), which can cause a doe to become emaciated. (Rue 1962)

Body weight is a trait that can fluctuate heavily in deer depending on the diet, age, sex, and season. Moen and Severinhaus (1981) report that body weight can fluctuate as much as $\pm 30\%$ throughout the year, with most of this change coming from fat stores. Rue (1962) reports that the heaviest buck recorded at the time (from 1919) weighed 161 kg (354 lbs), but on average, bucks weigh approximately 68-136 kg (150-300 lbs) while does weigh approximately 40-90 kg (88-198 lbs). Unlike humans and domestic (farm-raised) pigs, deer must forage for food and are subject to severe weather conditions (i.e., drought), changing of seasons, human-induced environmental impacts, trauma or disease without veterinary care, etc., all of which will likely increase the variability amongst individuals in a wild sample. Deer will also typically stay within their home range throughout their lives, which is approximately one square mile in size (Rue 1962). White-tailed deer are not migratory animals, although they may occasionally move or expand their home range in winter if the weather is harsh (primary cause to move) or the food is inadequate for survival (Rue 1962). There are cases where deer have died of starvation rather than move from their home range (Rue 1962). Deer are herbivores, although may occasionally ingest undigestible material. Their diets consist primarily of grasses, other leafy greens, fruits (i.e., apples and acorns), twigs, and bark. Deer living near farms or in baited/enticing areas, may have diets incorporating corn or commercial feed, farm crops, garden plants, and salt licks (for added minerals).

Histologically, the cortical bone of deer long bones consists of Haversian and plexiform bone tissue, the quantities of which vary depending on the age of the deer. Immature deer have more plexiform bone, which is primarily located at the periosteal surface, with Haversian bone

located at the endosteal surface. As individuals age, long bones remodel to primarily consist of Haversian bone. This Haversian bone ages in the same manner as human Haversian bone, with an overall increase in porosity over time (Hillier and Bell 2007). Approximately 90% of all white-tailed deer will reach adult bone size by six months of age (Purdue 1987). Adult deer lamellar bone is characterized by more and densely packed primary osteons as well as lower counts of secondary osteons than average adult human bone, the latter of which is related to lower remodeling rates of cortical bone (Skedros *et al.* 2003). As remodeling decreases bone toughness and crack resistance, deer bone would be biomechanically more tough than human bone; likewise, an increased count of secondary osteons in remodeled bone means more cement sheaths between them, further decreasing the tensile strength of human bone as compared to deer (Currey 1959). Genant, Gluer, and Lotz (1994) report that density accounts for 50-80% of the variance in bone strength. Marceau (2007) adds that white-tailed deer bones had similar enough geometric and densitometric similarities to human bone that they would serve as suitable models in taphonomic experimental studies, with the most comparable examined bones of deer to their human counterparts being the femur and humerus.

2.6.2 Domestic pig (*Sus scrofa*)

Pigs are quadrupedal mammals; although, they are digitigrade rather than unguligrade (Reitz and Wing 1999). Thus, pigs walk on their elongated toes and exhibit a reduction in the number of metapodia and digits. This reduction in number and increase in length of metapodia and digits is an earlier adaptation to running, but not to the same extent as unguligrade species (deer, horse, etc.) (Reitz and Wing 1999).

Domestic and wild pigs can vastly differ in size due to breed, age, and sex (Marceau 2007). Furugouri *et al.* (1981), when controlling for age and diet, reported size differences in the limb bones amongst Landrace and Middle Yorkshire pig breeds and while their bones aged in a similar pattern, the Middle Yorkshire pigs matured at a faster rate. Regarding the femur, Furugouri *et al.* (1981) also showed that the femora significantly differed in water content, which we know from the above discussion can significantly affect how a bone responds to impacts, including perimortem (fresh) and postmortem (dry) bones. At birth, feral hogs weigh between 0.5-1.8 kg (1-4 lbs). Adult wild hogs in the United States have been seen to become particularly large, averaging 68-100 kg (150-220 lbs) depending on sex, with some hogs reportedly reaching 227 kg (500 lbs) or more (Elmore 2019), which would undoubtedly affect the material properties of the bones that must support and move that weight around during daily locomotive activities. Overall, wild **boars** (intact males) are larger than **sows** (intact adult females). **Gilts** are females less than 1-year-old; **gilts** may also appear in the literature to generally refer to female pigs that have not farrowed or given birth. In addition to size differences attributable to sex, domestic male pigs are often castrated around 2-3 weeks of age (**barrows**) (Crenshaw *et al.* 1981). Richmond and Berg 1972 report that barrows have significantly longer limb bones. Pigs are also heavily bred for food, with females having multiple and constant litters. This strain from constant lactation can affect calcium levels within the skeleton, decreasing mineralization and thus bone hardness (Marceau 2007).

As mammals, pigs require many of the same essential nutrients as deer and humans (water, carbohydrates, fats, proteins, vitamins, and minerals) to uphold normal maintenance, growth, reproduction and lactation, and other physiological activities. And as domesticated animals, their diets have been far better studied and regulated than that of white-tailed deer. The National Research Council (NRC) provides estimated dietary requirements of pigs, which shall be reviewed

in brief here. However, these requirements are for pigs under “normal” conditions, which cannot account for any genetic or environmental variation, variability in feed, and diseases among other stressors. The NRC has heavily emphasized research on caloric needs, as well as appropriate levels and ratios of calcium and phosphorus, amino acids, and several specific vitamins and minerals, in the diet that lead to the healthy growth and development of pigs. (Crenshaw *et al.* 1981; Weeden 1992; National Research Council *et al.*, 2012; Merck Manual 2016). In particular, dietary calcium and phosphorus play a key role in the growth, development, and maintenance of a healthy skeleton. Crenshaw *et al.* (1981) tested the bone strength and material properties of pig bones (gilts, barrows, and boars) fed differing ratios of calcium and phosphorus in their otherwise same diets (group 1 receiving .4,.4% C,P and group 2 receiving .8,.8% C,P). The primary component of their diet was corn (76.9,74.3%) and soybean meal (20.6,21.0%). Subgroups of these pig dietary groups were then slaughtered at different ages (18, 82, 145, and 192 days old). Crenshaw *et al.* (1981) found that early on in development, differences in pig bone mechanical properties were primarily driven by diet, with the pigs fed higher proportions of calcium and phosphorus having better bone quality. It was not until 145-days-old (approximately 4-months-old) where significant differences in bone quality were noted between males and females. Likewise, it was not until 192-days-old (approximately 6-months-old) where significant differences in bone quality were noted between boars and barrows.

Like white-tailed deer, pig bone at the tissue level is comprised of a mixture of plexiform and Haversian bone, with more tightly packed Haversian systems than exhibited in adult human bone (Owsley *et al.* 1985). Martiniaková *et al.* (2006) reports that the adult femoral diaphysis presents such a mixture, with plexiform bone found primarily along the medullary cavity, which is counter to the pattern found in deer bone as presented by Hillier and Bell (2007). The

significance of this is unclear; however, like deer, plexiform bone will ultimately be replaced by Haversian bone. Marceau (2007) likewise states that “osteonal banding,” where primary osteons appear in tightly packed and organized rows, does present along the endosteal surface of immature pigs, with osteonal banding referring to plexiform or plexiform-like bone.

Marceau (2007) concluded that domestic pig bones, like white-tailed deer, had similar enough geometric and densitometric similarities to human bone that they would serve as suitable models in taphonomic experimental studies, with the pig tibia and radius the most comparable to their human counterparts. However, pig bone is also noted to have a higher rate of bone turnover as illustrated by more resorptive lacunae, with Martiniaková *et al.* (2006) finding more resorptive lacunae in pig versus cow femora. These authors hypothesize that this higher turnover rate may not solely be linked to taxonomic differences but may also be the result of dietary differences or age. Deer bone, as indicated above, does not have as much remodeling (Skedros *et al.* 2003).

3.0 Materials and Methods

3.1 Bone sample

Three taxonomic groups were selected for this comparative analysis: white-tailed deer (*Odocoileus virginianus*), domestic pig (*Sus scrofa*), and human (*Homo sapiens*). Original intentions were to only have femora for comparison, but this changed given tissue availability at the time of sample collection. Thus, humeri (pig) and femora (deer and human) were obtained for this project. Crenshaw *et al.* (1981) show that humeri and femora are both structurally round in cross-section, with the humerus having the most similarity in terms of biomechanical strain to the femur than other skeletal elements. For quadrupedal species, they are both weight-bearing, proximal elements in the limbs, with the lower limb elements providing more propulsion during locomotion. Humans, however, have been bipedal for several millennia, leaving the upper limb elements typically free from weight-bearing roles during locomotion, and thus, no human humeri were used in this research.

The sample size for the selected hypotheses was number of tooth hops per group; however, this could not be known prior to sawing. Thus, estimates for how many bones were necessary were made based on previous experience by this investigator and by judging overall bone lengths per species to consider how many potential cuts could be made per bone. If feasible, extra bones were acquired to ensure a high enough sample of tooth hops as possible for increased statistical power.

Pig humeri were obtained from *mypetcarnivore.com* through a licensed processing facility, although the elements were marketed as pig “femurs.” The pigs were raised at Schoenborn Family Farms in Michigan, without use of hormones, antibiotics or steroids. Pigs were mixed between

castrated males and intact females, all of which were aged between 6-7-months-old. Pigs at time of slaughter weighed approximately 114 kg (250 lbs) and were raised on a typical diet of corn mixed with soybean meal (a byproduct from soybean oil extraction) (personal communication with *mypetcarnivore.com* on November 19, 2019). A total of 16 pig humeri were purchased and only 14 were used for sawing. Bones were freshly frozen, shipped frozen, and remained frozen until they were able to be cut. It is likely that sided elements were from different individuals, as bones are of unequal size and there are differing numbers of right and left elements, although this cannot be confirmed. Elements were randomly distributed between saw blades.

Deer femora were obtained from a licensed deer processor, Joseph Ott, in southwestern Pennsylvania during deer (bow) hunting season. Prior to collection, a special research permit had been obtained from the Pennsylvania Game Commission to obtain these. Bones were disarticulated and had soft tissue removed shortly after death and were immediately frozen fresh to maintain bone tissue quality. Bones then remained frozen until sawing could proceed. The deer sample consists of limbs from both sexes, with 2 female/4 male femora assigned to the rip saw and 2 female/3 male femora assigned to the crosscut saw. As limbs were near-evenly divided between right and left, elements were sorted so that each saw blade only cut one side (right or left elements). This ensured that individuals were sorted equally between the two saw blades. The exact age of death of each deer is unknown; however, epiphyseal ends of the long bones were fused or fusing. Pennsylvania also enforces antler point restrictions that protects younger bucks from hunting, with most counties following a four-point restriction, requiring at least four-points on one side of a rack. Antlers are more of a dietary product than a result of age, so the age of even the bucks in this study can still vary and a buck with four-points is at least over 1-year old. The 3.5 to 4.5-year old bucks

appear to be the most commonly active and testosterone-driven during the rut and are thus the most-obtained age group during hunting season.

The **human** femora were obtained from Mercyhurst University (Erie, PA), via permission of Dr. Dennis Dirkmaat, D-ABFA. Remains had been donated directly to the Applied Forensic Sciences Department for scientific research, with the school's personal legal services setting donation procedures. Tissue had been removed prior to freezing and defrosted in a cooler 48-hours before sawing took place. This researcher did not assist with the collection or tissue removal process when these remains had originally been donated. Remains were only processed and analyzed in approved labs, the Hirtzel Human Anatomy and Forensic Anthropology lab (Northeast, PA), and the on-campus Forensic Anthropology Processing Laboratory. The human femora were from a 73-year-old (birth year: 1943), white male and no medical or life history was reported. As these elements were from the same individual, saw blades were assigned to an element side.

3.2 Research laboratory and safety protocols

Pig and deer processing (maceration and cutting) took place in the physical anthropology laboratories of the University of Pittsburgh Anthropology Department. All animals were farmed or hunted for food and were not intentionally harmed for this research project. Gloves were always worn when handling remains until they were clean enough for post-processing and microscopic analysis. A chest freezer was purchased for the laboratory for the storage of bones prior to sawing. A stainless-steel table was placed next to the freezer for defrosting and removal of soft tissue from bones to prevent the saw from binding when cutting. For sawing, a tarp was placed on and below

a sturdy table to prevent bone dust and tissue, primarily bone marrow and blood, from dirtying the lab space. This laboratory also has a certified fume hood with associated sink, both of which were used to safely macerate tissue from the bones. A small crock-pot was used for maceration and kept under the fume hood throughout the maceration process. All bones, when placed in water and heated for at least 8-hours on low heat (longer as deemed necessary to properly degrease the bones), were placed in colored mesh bags to prevent mixing of cutmarks. Green bags were assigned to the rip saw and pink bags were assigned to the crosscut saw. All laboratory spaces and equipment were cleaned following exposure to soft tissues with household bleach.

Human remains were only processed and analyzed in the approved Mercyhurst laboratories, which operate under standards set for BSL-2 and BSL-3 measures. As it is a forensic anthropological laboratory, protocols are set to follow ASCLD-LAB guidelines. A current graduate student from the Anthropology graduate program at Mercyhurst remained present at all times and assisted with bone cleaning, photography, and preparation of data sheets. As medical history for the decedent was unknown, universal precautions were followed. When handling fresh remains prior to soft tissue removal and cooking, gloves, aprons, sleeve covers, masks, and shoe covers were utilized to ensure the safety of this researcher and assistant.

3.3 Pre-processing treatment

3.3.1 Freezing

Bones were stored in air-tight freezer bags, placed in the freezer, and were kept there for no longer than 4-weeks to prevent or minimize damage to bone quality from an extended freezing

process. Technical issues outside of this researcher's control required some adaptation regarding freezing and eventual post-processing, as explained below.

The pig bones were the first sample to be obtained and had been placed into the freezer in the manner explained above. The freezer was new, so there was no possible cross-contamination from deer tissue or pathogens. Unfortunately, power to the building was turned off for a weekend and all 16 bones had begun to defrost earlier than anticipated. It was decided that re-freezing could exacerbate any damage potentially caused by the freezing process, as moisture had already started to be released into the storage bags. All bones were sawed immediately as 48-hours of defrosting had occurred and were macerated in stages for cleaning as there was only one pot. Maceration and heat-treatment processes are explained below. The freezer was then cleaned with household bleach before the deer sample was obtained and placed in the freezer.

The deer bones were obtained in stages from the deer processor depending on when he received them for processing. He had cleaned remains of most remnant tissue and had double-bagged them prior to freezer storage. Remains were then picked up and transported to the lab for storage before further defleshing or processing took place.

3.3.2 Defleshing

Prior to sawing soft tissue had to be removed to prevent the saw from binding during the cutting process. Most of the tissue had already been removed by the meat processors (deer and pig) or graduate students (human). Remnant tissue consisted primarily of the joint capsules, ligaments, tendons, and some muscle around articular regions. This was removed via scissors, knives, and/or probes, with great care taken not to scar the surface of the bone with any trauma other than what would be afflicted by the saw blades. Regardless, none of these tools would leave

comparable marks to those left by a saw, nor were surface marks (from a saw blade or otherwise) examined during this research project.

3.3.3 Saw blade sample and measurements

Two new saw blades were purchased from a local hardware store for use throughout this research project. Each blade has a different tooth type, **rip or crosscut**, and dictates how each saw is referred to throughout this dissertation (**figure 3-1**). The crosscut saw was marketed as a 22” saw blade with 7 teeth-per-inch (**TPI**). The crosscut saw blade also has a raker tooth set, meaning that teeth alternate direction (left or right) down the blade, with every few teeth oriented parallel to or continuing with the main body of the blade. The crosscut teeth were also carbide teeth, which refers to an applied heat treatment to increase teeth hardness. The rip saw was marketed as an 18” saw blade with 7 TPI; its tooth set is an alternating pattern with no raker teeth, nor were the teeth carbide.

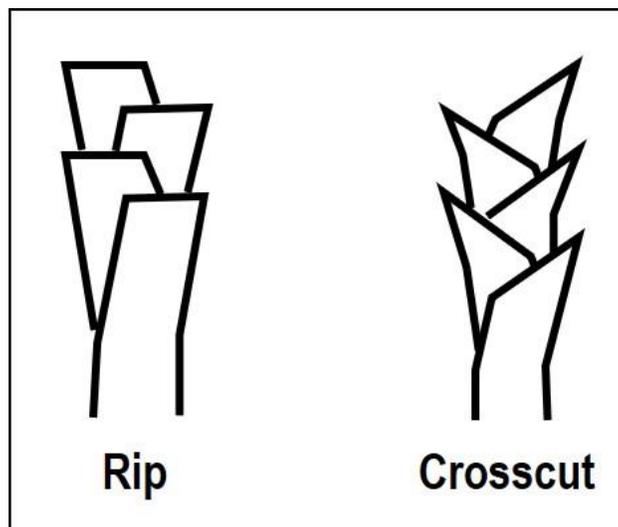


Figure 3-1 Rip versus crosscut saw teeth profiles along a saw blade.

Fifty measurements between adjacent teeth were randomly collected down each saw blade, measured with Helios dial calipers, set with pins for dental measurements. Once all bone sawing was completed, fifty more measurements were collected between adjacent teeth randomly down each saw blade. This allowed blade variability overtime to be isolated from variability in measurements introduced as a result of bone tissue differences. These measurements will be referred to as “**blade data**” throughout this dissertation, with “blade” often included as a “species.”

3.4 Sawing and number of cuts

Bones, once defrosted for 48-hours, were secured to the table in a vise and wrapped in nitrile rubber gloves and paper towels to prevent slipping during the sawing process. All bones were secured on the proximal end, with cuts proceeding proximally from the distal end (**figure 3-2**). For approximately three-quarters of the bone length, the bones were able to be cut from anterior to posterior; however, securing the humeral/femoral heads in the vise proved easiest with cuts proceeding from medial to lateral. Cutting direction is clearly identifiable when viewing striations in the bone through the microscope; however, it is not of significant interest in this project.



Figure 3-2 Crosscut saw blade in pig bone.

The deer long bones are harder and more brittle, with thinner diameters compared to the pig and human bones. During the cutting process, the vise proved to be too large, leading to one bone fracturing lengthwise. And despite great care being taken during the following cuts, several of the bones seemed to shatter early during a cut (within the first few strokes), particularly those cross-sections of the shaft. The trabecular regions were not difficult to cut through. Large blunt force regions prevented the presence of saw marks for analysis. Unintentional blunt force trauma was only an issue for the female deer femora, as the male femora were larger and thicker overall.

3.4.1 Number of cuts

Each pig humerus produced approximately 10 cuts each. Each human femur produced approximately 20 cuts, although some cuts had been lost during maceration, as is explained in the

post-processing section of this chapter. Finally, each deer bone produced approximately 10-15 cuts each. Saws were assigned to random bones for the pig sample as they were not clearly from the same individuals and had an uneven number of right and left elements. This was not the case for the deer. Left bones of the deer were assigned to the rip saw and right bones were assigned to the crosscut saw. For the two human femora, the left femur was assigned to the crosscut saw and the right femur was assigned to the rip saw. **Given the goals of this project, final number of cuts per bone is less important, as it is the number of tooth hops that each bone yields that is the final sample size (N) for the chosen hypotheses.** Finally, tooth hops on both sides of each cut were examined. While each side of a cut is related to the same cutting process, the two sides of a cut are not identical. Likewise, in a forensic case, if both sides of a cut are available, they will both be examined so that as much information can be learned about the cutting blade as possible and would thus both be included as the “sample” in the forensic case report.

3.5 Post-processing treatment

3.5.1 Pig and deer bones

After sawing, bones were placed into colored mesh bags (green for rip saw cuts and pink for crosscut saw cuts). Bags were placed into an eight-quart crock-pot filled with water and a cap full of an enzymatic laundry detergent, Tide®. Water was filled to the brim in order to ensure that the bones were properly submerged. The crock-pot was set to low and the lid cracked to prevent fully cooking the bones. The goal was as much tissue removal as possible and as much degreasing

of the bone as possible. Thus, timing varied between elements given the size and difficulty of tissue removal.

Once tissue removal was deemed successful, the bones were then given a 10-minute bleach bath (1 cup of Clorox® bleach to 4 cups of water). This was to further degrease and whiten the bones so that the saw marks could be more easily examined with oblique lighting under the microscope, without being too shiny or greasy. Once 10-minutes had passed, bones were then submerged in a plain water bath to remove any excess bleach that could further whiten or dry out the bones as they dried. After the water-only bath, bones were air-dried for at least 48-hours before they were given labels for bone number, cut number of that bone, and side of a cut. Black dots were placed on a bone section closest to the proximal end (thus, for every “cut,” the dot marked the distal side of that cut; refer to **figure 3-3**). Once fully dried and labeled, bones were placed in individual plastic bags lined with paper towels and left unsealed to prevent any potential moisture buildup in the bag that could lead to mold growth.



Figure 3-3 Pig bone post-processing. Top left shows pig bone 5 cut by the rip saw (green tag); dots on bone dictate proximal side of a cross-section and number represent cross-section number from proximal to distal. Top right shows microscope setup during data collection and bottom shows oblique lighting used to better visualize striations from saw marks.

3.5.2 Human bones

Processing the human bone was time sensitive given that the laboratory location was not easily accessible to this researcher. Thus, the chemicals and equipment used to process the human remains, freeing them of adherent soft tissue and grease, were stronger than those used to process the pig and deer bones in order to expedite the cleaning process.

To accelerate post-processing organization and labeling of cut number and sides of a cut, hollow sections of femora were strung together in sawing order on a thick cotton string. One saw was used per bone, so each bone was then cooked in a separate pot to prevent potential mixing of samples. Given the equipment available in the lab, a “small pot” and a “medium pot” were used. The small pot (left bone; n = 21 cuts) was given 1 tablespoon of OxiClean™ and 4 tablespoons of Tergazyme®, an enzyme-active powdered detergent; the medium pot (right bone; n = 25 cuts) was given 1.5 tablespoons of OxiClean™ and 6 tablespoons of Tergazyme®. Both pots were filled with water, placed on a joint-hot plate, with the smaller pot set to 150°F and the medium pot set to 250°F, and allowed to heat for 8-hours overnight. Unfortunately, despite following laboratory chemical protocols, the medium pot most likely had too much water, which led to the pot boiling over, overcooking some of the bone cross-sections—these were five epiphyseal region cuts with high amounts of trabecular bone. The bones in the small pot, after 8-hours, still had soft tissue. Therefore, the water was changed for the small pot, new chemicals were added (0.5 tablespoon of OxiClean™ and 2 tablespoons of Tergazyme®), and the pot was left to cook for another 6-hours at 150°F while the bone from the medium pot was dried, photographed, and microscopically analyzed. Overall, the left bone yielded 21 cuts and the right bone yielded 20 cuts.

3.6 Microscopic saw mark analysis and number of tooth hops (final sample size)

A Wild® camera lucida microscope was used to measure all bone data (**figure 3-3**). In the case of the human bone, the microscope was transported to the Mercyhurst University forensic anthropological facilities for analysis. An AmScope® dual light source was used to provide oblique lighting to better visualize the saw marks under the microscope, while simultaneously providing a light source for the adjacent data sheet (**figure 3-3**). Once a tooth hop was found and positioned for measurement, the mirror tube was opened so that the data recording sheet visually overlapped the specimen under the microscope. This allowed for tracing and measuring of tooth hops. Detected tooth hops were measured individually, from floor to floor, with the same Helios dial calipers, set with pins for dental measurements, that was used to measure the blade data. If two floors of a hop were incomplete, the tooth hop was alternatively measured from peak to peak. **Figure 3-4** provides an example of tooth hops on a bone cross-section. All measurements were collected in centimeters to the nearest 0.05 cm and converted to millimeters, while also accounting for the scope magnification (all measurements were divided by 9.375 prior to conversion). **Appendix A** provides an example data collection sheet from one of the cross-sections.

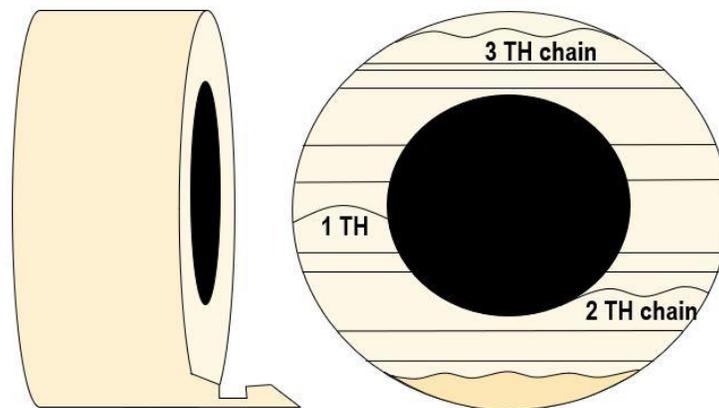


Figure 3-4 Bone cross-section with examples of isolated and chained tooth hops (TH).

3.6.1 Data organization

A separate data recording sheet was used for each cut surface (**appendix A**). Tooth hop floors (or peaks when appropriate) were traced onto the paper. No points marking valleys or peaks overlapped unless they were adjacent tooth hops in a chain, in which case, they shared a point between them. All tooth hops were measured individually. Chain status was recorded (2 or 3+ in a row) for analyses examining the accuracy of isolated tooth hop measurements versus those in chains in this and related research projects (Grosso, Begley, Toth 2020). Each bone, cut, and tooth hop received an individualizing identification number for easy recall and comparison in statistical analyses.

3.7 Statistical analyses

Question components addressed statistically are assessed with the basic null hypothesis that there was no difference amongst the relevant groups addressed by each question (questions outlined below). All statistical analyses were performed using the Statistical Package R, version 3.5.2 (R Core Team 2018) and package RCommander version 2.5-2. Descriptive data were calculated for each sample. The combined data of all tooth hop measurements were first assessed with the Shapiro-Wilk test of normality to evaluate whether the data needed to be examined in parametric or nonparametric statistical tests. Extreme outliers were noted with boxplots and replaced with “NA” where appropriate in the data set. The normality assumption was found not to be violated (results appear in the following chapter), so parametric tests were selected. There is one exception to this, noted below and reiterated in the appropriate results section of the following

chapter. Results were considered significant at the $p < 0.05$ level, using two-tailed tests. Results from assumption tests are reported in **appendix B**.

3.7.1 Questions 1 and 2: How variable are tooth hop measurements from the same instrument when using human and nonhuman bone?

Question 1, evaluating how tooth hop measurements from a single instrument vary when examining multiple usages in human bone, was addressed with a one-way analysis of variance test. Tooth hop measurements from human bone cut from the crosscut saw and human bone cut by the rip saw are compared to distance between teeth measurements of the respective blades (new blades, prior to sawing). A two-sample test of variance compared the standard deviations of bone-measured data to blade-measured data.

Question 2, evaluating how tooth hop measurements from human bone compared to nonhuman proxies cut by the same saw blades, was also addressed with a one-way analysis of variance test. Here, as no significant differences were found between the means of the two saw blades (rip and crosscut), groups were combined so that all human measurements (rip and crosscut) were compared to all deer and all pig measurements (also combining rip and crosscut data). Blade data (new blades, prior to sawing) were also included. Similarly, two-sample tests of variance were used to evaluate significant differences in standard deviations of each group.

3.7.2 Questions 3: What properties of bone affect tooth hop variability?

Qualitative differences amongst species data was recorded for each cross-section during the data collection process (i.e. breakaway spur presence and general length/shape). These

differences are not addressed statistically. However, as age of the tissue could be estimated and biological sex was known and intentionally mixed for the deer sample, potential effects of these features on tooth hop measurements could be explored. An independent sample t-test compares the tooth hop means measured from males and females; a two-sample test of variance compares the standard deviations of each group. Unfortunately, age ranges differ between taxonomic groups so cannot be isolated from differences due to taxon. However, a general trend can be examined, as pig bones are the youngest (5-7-months-old) and deer bones are skeletally mature (3½-4½-years-old). One human does not allow a full exploration of the impact aging has on geriatric tissue.

3.7.3 Question 4: What properties of the instrument affect tooth hop variability?

Question 4 primarily addresses differences in saw tooth type, with each species sample split equally between a crosscut saw blade and a rip saw blade. A one-way analysis of variance examines saw-species groups (i.e., CC_Human and R_Human). And as above, two-sample tests of variance are used to compare groups. In general, attention is paid to the relationship species and blade groups have to one another (all crosscut groups and all rip groups separate) with a final comparison between saw blades to see how the relationships between species changed, if at all.

3.7.3.1 What are the effects of tooth wear?

To be sure that the trends witnessed were not because the saw blades were changing over time with each cut, distance between teeth measurements of saw blades were compared before sawing took place to distance between teeth measurements of saw blades after all sawing had been completed. Before and after groups were tested with an independent sample t-test as well as a two-

sample test of variance. Tooth wear was also examined qualitatively, with points of obvious breakage not included in distance-between-teeth measurements of blades after sawing.

3.7.4 Question 5: Does having more than one tooth hop in a row improve the accuracy and precision of estimating blade TPI?

Finally, Symes *et al.* (2005) reports that three or more tooth hops in a row is how tooth hop should be identified and measured for analysis. Therefore, a comparison of groups including isolated instances of tooth hops were compared to their respective groups with isolated cases of tooth hops removed. The group “two or more” was used rather than “three or more” as this would have excluded all or most of the data, including the entirety of the human sample.

This analysis also combined blade before and after data, as blade data previously only looked at before measurements or was specifically comparing blade before and after data. Having this visual provides another perspective, that blades theoretically began changing from the first saw cut into bone, so this sample (combined crosscut and rip, before and after measurements) may be more representative of saws used in postmortem saw dismemberment cases that have been ill-taken care of or worn through previous use. It was this blade data that was found to not be normally distributed, so a Kruskal-Wallis rank sum test was used to compare this blade data to human, pig, and deer combined-saw samples instead of an ANOVA.

A one-way analysis of variance was used to compare species data (isolated tooth hops included versus isolated tooth hops excluded) without blade data to test whether cases of isolated tooth hops should be excluded from any future studies or forensic cases utilizing tooth hops.

4.0 Results

This study investigates tooth hops measured from bones of three species (human, deer, and pig) cut by two saw blades of differing tooth type (crosscut (**CC**) and rip (**R**)). Groups are organized by saw and species for statistical analyses (CC_Human, CC_Blade, R_Human, etc.) as well as by species only. “Blade” references measurements between adjacent teeth collected directly from the saw blades. All tooth hop measurements are in millimeters to 0.01 accuracy. One of the latter assessments of this project was re-measuring saw blades once all sawing had been completed to examine blade wear and any potential effects it had on measurements from the bones. This new group of measurements was categorized as CC or R_Blade_After and was used in two instances: 1) for comparison to tooth hop measurements prior to sawing and 2) for comparison to species-only tooth hop measurements, combining tooth distance measurements from both saw blades, before and after. Otherwise, blade data compared to bone measurements are from new blades prior to any use.

The results of all statistical tests are presented below in relation to the relevant research questions outlined in chapter 1. Almost all tooth hop (saw-species, species, and biological sex) bone groups and blade data (before and after) had outliers removed and were found to be normally distributed with the Shapiro-Wilk test of normality, so parametric tests could be used. Only one group, the combined blades (both blades and their before and after measurements) was found to not be normally distributed, which required a nonparametric alternative for analysis. The results of assumption tests can be found in **appendix B**. *All results were considered significant at the $p < 0.05$ level, using two-tailed tests.*

4.1 Descriptive statistics of the samples

A total of **1,959 tooth hops (N)** or distance between teeth measurements were analyzed in this study (**figure 4-1**). Means and standard deviations for tooth hop measurements for each group are presented in **table 4-1** with boxplots illustrating a comparison of saw-species group tooth hop measurements in **figure 4-2**. Number of cut surfaces analyzed is presented for context when comparing number of tooth hops reported between groups as the amount of bone present will influence how many tooth hops are possible per species. Combining data from both saw blades, there were **689 tooth hops measured from the deer sample, 797 tooth hops measured from the pig sample, and 280 tooth hops measured from the human sample**. On average, when combining data from each saw-species group, each surface had approximately five to eight tooth hops. This range does *not* include cases of zero tooth hops, which happened in regions dense with trabecular bone, cases where a bone fractured from blunt force trauma early in the saw cut, or in rare cases that had enough cortical bone, but no tooth hops were detected for various reasons (i.e., random chance or a polished surface). Several cut surfaces included 20+ tooth hops on a surface, but these were not the norm and typically would occur in the midshaft to distal third of the diaphysis, on the side of the cross-section closest to the breakaway spur or notch.

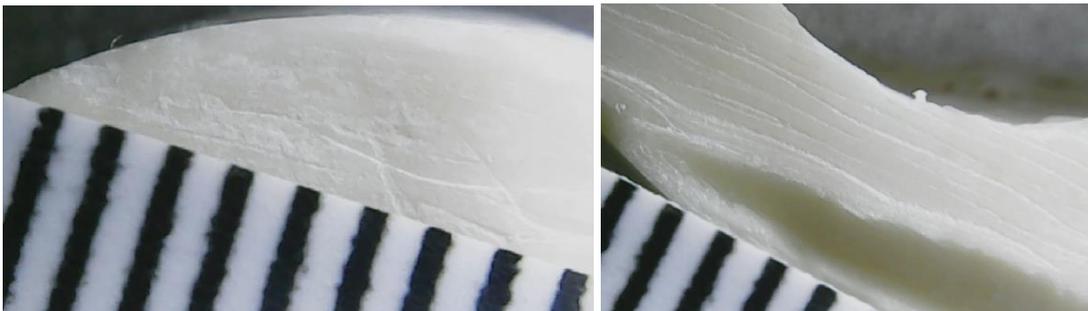


Figure 4-1 Example tooth hops (scale is mm) from deer bone.

Table 4-1 Sample distribution including mean and standard deviation for tooth hops measured from **saw-species** groups. NA counts represent the number of sides without tooth hops as well as those that had been removed as outliers.

Species	Saw	Number of cut surfaces	Number of TH (n)	Mean TH (mm)	SD (mm)	NA
Deer	Crosscut	98	328	3.60	0.48	31
	Rip	118	361	3.68	0.42	42
Pig	Crosscut	105	397	3.40	0.65	41
	Rip	114	400	3.68	0.54	33
Human	Crosscut	35	138	3.87	0.53	19
	Rip	43	142	3.45	0.59	12
Blade	Crosscut	NA	48	3.72	0.06	2
	Rip	NA	49	3.89	0.08	1
Blade (After)	Crosscut	NA	50	3.40	0.14	0
	Rip	NA	46	3.48	0.11	4

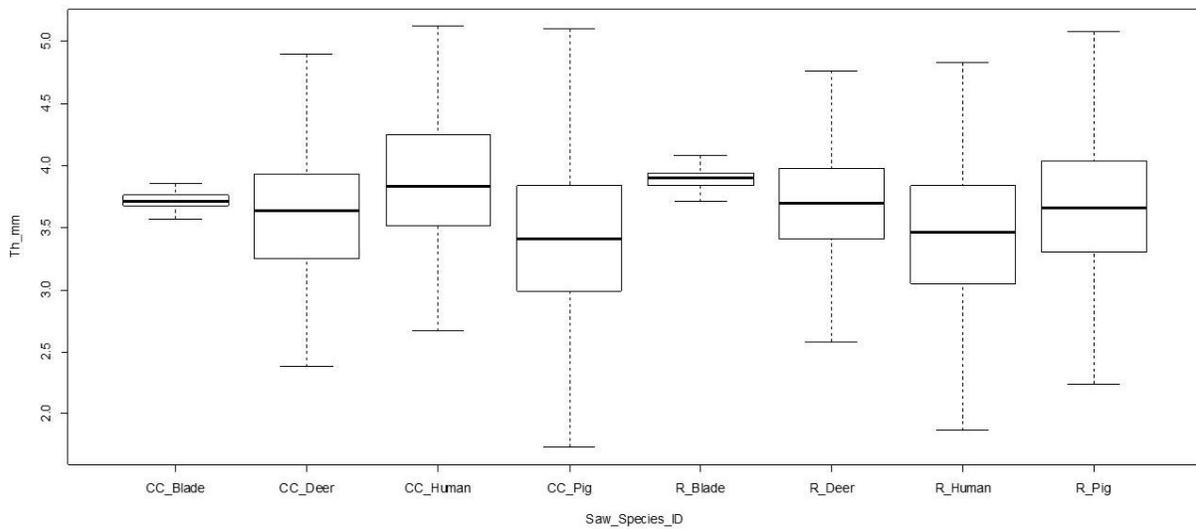


Figure 4-2 Boxplots comparing tooth hop measurements from **saw-species** groups.

4.2 Question 1: How variable will tooth hop measurements from a single instrument be when comparing multiple usages in human bone?

Results from the one-way analysis of variance comparing saw-species groups are presented in **table 4-2**. A statistically significant difference in the mean tooth hop amongst the eight saw-species groups was found ($F = 20.19$; $p\text{-value} < 0.05$). Relevant pairwise comparisons of groups are presented in **table 4-3**. Focus in this section will be only on the human and blade samples.

The mean tooth hop of human bone cut by the crosscut saw (3.87 mm) was statistically different from the mean distance between teeth of the crosscut blade (3.72 mm). Likewise, the standard deviation of each group was statistically different, with the human sample having a wider range (0.53 mm) than the blade (0.06 mm). The mean tooth hop of human bone cut by the rip saw (3.45 mm) was also statistically different from the mean distance between teeth of the rip blade (3.89). And like the crosscut saw, the standard deviations of the rip saw and tooth hops from the respective human bone were statistically different, with the human sample having a wider range (0.59 mm) than the blade (0.08 mm). Results of the two sample tests of variance between saw-species groups is presented in **table 4-4**.

Table 4-2 Summary of one-way ANOVA examining TH (mm) between groups sorted by **saw-species**.

	df	Sum Sq	Mean Sq	F-value	p-value
Saw-Species ID	7	38.7	5.523	20.19	< 0.05*
Residuals	1855	507.4	0.274		

Table 4-3 Summary of pairwise comparisons from the ANOVA using **saw-species** groups. Pairwise comparisons presented are mostly those comparing bones cut by the same blade. The first comparison illustrates the difference in means between the two saw blades (CC and R); the last comparison illustrates the difference in means between the two human samples (CC vs. R blades). ($F = 54.35$, num df = 7, den df = 520.63, p-value < 0.05*). ** = comparison of before means from both blades.

	Mean difference (mm)	SE (mm)	t-value	p-value
CC Blade – R Blade*	0.17	0.11	1.64	0.70
CC Deer – CC Blade	-0.12	0.08	-1.511	0.78
CC Pig – CC Blade	-0.32	0.08	-4.02	< 0.05*
CC Human – CC Blade	0.15	0.09	1.69	0.67
CC Human – CC Deer	0.27	0.53	5.09	< 0.05*
CC Human – CC Pig	0.47	0.52	9.07	< 0.05*
CC Deer – CC Pig	0.20	0.04	5.10	< 0.05*
R Deer – R Blade	-0.22	0.08	-2.73	0.10
R Pig – R Blade	-0.22	0.08	-2.75	0.10
R Human – R Blade	-0.45	0.09	-5.17	< 0.05*
R Human – R Deer	-0.23	0.05	-4.46	< 0.05*
R Human – R Pig	-0.23	0.05	-4.51	< 0.05*
R Deer – R Pig	0.00	0.04	0.01	1.00
CC Human – R Human**	0.42	0.06	6.74	< 0.05*

Table 4-4 Summary of two-sample test of variance (F-tests) between bone data of **saw-species** groups.

	Numerator df	Denominator df	F-value	p-Value
CC Deer – CC Human	327	137	0.80	0.12
CC Human – CC Pig	137	392	0.38	< 0.05*
CC Deer – CC Pig	327	392	0.55	< 0.05*
R Deer – R Human	360	141	0.52	< 0.05*
R Human – R Pig	141	402	1.19	0.19
R Deer – R Pig	360	402	0.61	< 0.05*

4.3 Question 2: How variable will tooth hop measurements be from the same instrument when using animal proxies (deer and pig) to model human bone?

As the initial one-way analysis of variance found no significant difference in the saw blade means prior to sawing, this subsequent ANOVA combines species data, regardless of the saw blade that cut them, as well as the combined blade data of both the rip and crosscut saws (blade before sawing measurements only). A numerical summary of the combined species data from this analysis can be found in **table 4-5**. Boxplots illustrating group tooth hop measurements can be seen in **figure 4-3**. Results from the one-way analysis of variance comparing groups sorted by species are presented in **table 4-6** and the pairwise comparisons in **table 4-7**. A statistically significant difference in the mean tooth hop amongst the four species groups was found ($F = 9.89$; $p\text{-value} < 0.05$). Of the pairwise comparisons presented, four have significant differences in tooth hop means and only one includes the human sample:

1. Deer – Blade
2. Pig – Blade
3. Pig – Deer
- 4. Pig – Human**

Results of the F-tests comparing tooth hop measurements sorted by species only is presented in **table 4-8**. Only tooth hops from bone are compared here. Of the three comparisons made, two were significantly different and only one includes the human sample:

- 1. Deer – Human**
2. Deer – Pig

Table 4-5 Sample distribution including mean and standard deviation for tooth hops measured from respective **species** group (before measurements from crosscut and rip saw data have been combined). NA counts represent number of sides without tooth hops and those removed as outliers.

Species	Number of cut surfaces	Number of TH (n)	Mean TH (mm)	SD (mm)	NA
Deer	216	687	3.64	0.45	75
Pig	219	793	3.55	0.60	32
Human	78	279	3.66	0.60	78
Blade	NA	97	3.81	0.12	3

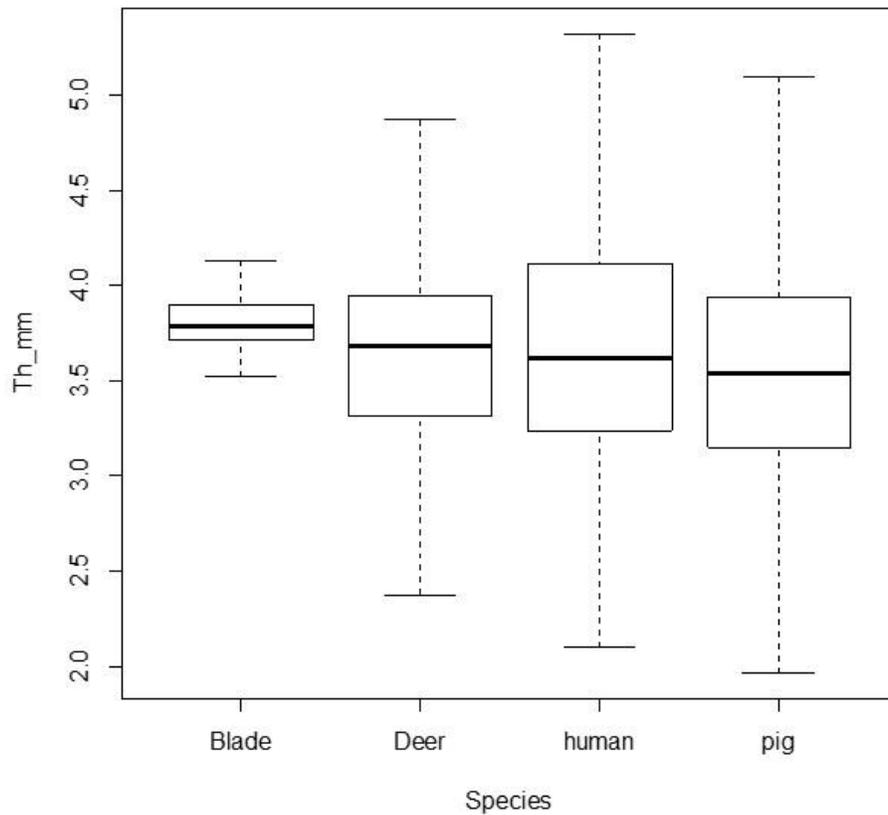


Figure 4-3 Boxplots comparing tooth hop measurements from **species** groups.

Table 4-6 Summary of one-way ANOVA examining TH (mm) between groups sorted by **species**.

	df	Sum Sq	Mean Sq	F-value	p-value
Species	3	8.3	2.769	9.889	< 0.05*
Residuals	1852	518.6	0.280		

Table 4-7 Summary of pairwise comparisons from the ANOVA combining **species** data (F = 9.89, num df = 7, den df = 3, p-value < 0.05*).

	Mean difference (mm)	SE (mm)	t-value	p-value
Deer – Blade	-0.17	0.06	-2.97	< 0.05*
Human – Blade	-0.14	0.06	-2.40	0.07
Pig – Blade	-0.26	0.06	-4.62	< 0.05*
Human – Deer	0.02	0.04	0.56	0.94
Pig – Deer	-0.09	0.03	-3.35	< 0.05*
Pig – Human	-0.11	0.04	-3.08	< 0.05*

Table 4-8 Summary of two-sample test of variance (F-tests) between bone data of **species** groups.

	Numerator df	Denominator df	F-value	p-value
Human – Pig	280	793	1.01	0.09
Deer – Human	690	280	0.57	< 0.05*
Deer – Pig	690	793	0.57	< 0.05*

4.4 Question 3: What are the properties of bone that affect tooth hop variability?

4.4.1 Qualitative results from the bones

Aside from tooth hop measurements, several features of the bones were observed by this researcher throughout the data collection process that provide greater context in understanding the differences in bone amongst the three species.

All bones were cut with the proximal end secured in the vise and cuts proceeding proximally from the distal end; therefore, cross-sections could not go above the lesser trochanter of the femur (deer, human) or above the midway point of the bicipital groove on the humerus (pig) because this end was secured in the vise. The trabecular bone from the distal ends exhibited almost no tooth hops in any of the species and if hops were found in the distal end, it was typically limited to the surrounding cortical bone. As the cortical bone around the joints is thin, tooth hop counts were biased towards those areas of thicker cortical bone, particularly around midshaft and similarly dense regions.

The human bones produced the most cross-sections per bone with dense cortical regions, as these bones were the longest. Due to an unforeseen complication during the maceration process, the most-proximal and most-distal cross-sections of the femur cut by the crosscut saw could not be examined (see section 3.5.2). These regions are primarily full of loosely packed trabecular bone (i.e., trabecular bone much less densely packed than the nonhuman proxies). Human bone had the least amount of tooth hop chains, primarily consisting of isolated tooth hops, with some cases of two in a row.

The pig bones were the shortest with thick proximal and distal ends thus producing the least number of cuts per bone. The thick epiphyseal regions made securing the proximal end of the

humerus in the vise difficult and harder to keep saw progress consistent. The deer and human bones, being femora, had more cut surfaces that could be analyzed from the distal end as the femur does not drastically twist or change shape, like the pig humerus. And despite being humeri, the pig bones were thick enough to produce a large sample of tooth hops for statistical analysis. Pig bones had the most instances of long tooth hop chains, with some chains even consisting of four tooth hops in a row. Regions of plexiform bone were also devoid of tooth hops, as this type of bone, like trabecular bone, is porous by nature.

The deer bone in several instances fractured during the sawing process, which limited how many cross-sections could be made. This primarily affected the female bones, as these were smaller and thinner overall compared to the males. In general, deer bones were more difficult to saw than pig or human, particularly with the crosscut saw blade. Breakaway spurs as well as entrance and exit chipping were larger and more frequent than any other species. Large breakaway spurs and notches are representative of blunt force trauma and therefore these regions where large chipping or fracturing took place would not exhibit much tooth hop. There were several instances where the striae on surfaces were faint, not raised far above the surface, or surfaces were so polished that oblique lighting could not make tooth hops, if present, clear enough to visualize and measure. Surface polishing was a feature in the deer bone only and primarily in the endosteal regions.

4.4.2 Biological sex

As saw blade means (crosscut and rip) before sawing were found to not be significantly different, combined saw blade information was then also used to explore potential sex differences in the deer sample while maximizing the sample size of the female sample. As the female sample

is relatively small compared to the male sample, results here should only be considered exploratory. A numerical summary of this data is presented in **table 4-9**. Boxplots illustrating group tooth hop measurements can be seen in **figure 4-4**. Tooth hops measured from males and females were found to be significantly different ($t = -3.10$, $p < 0.05$) with the results of the independent sample t-test presented in **table 4-10**. Finally, an F-test comparing male and female tooth hop measurements of the deer sample found no significant differences between variances ($F = 1.20$, num df = 105, den df = 581, $p = 0.21$).

Table 4-9 Sample distribution including mean and standard deviation for tooth hops measured from the **deer sample** sorted by **sex**. NA counts represent number of sides without tooth hops and those removed as outliers.

Sex	Number of cut surfaces	Number of TH (n)	Mean TH (mm)	SD (mm)	NA
Female	66	106	3.50	0.48	26
Male	150	582	3.66	0.44	48

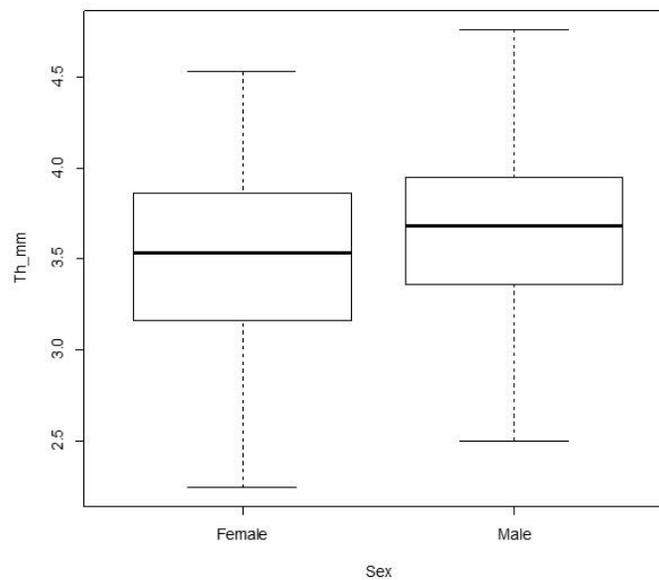


Figure 4-4 Boxplots comparing tooth hop measurements from the **deer sample** sorted by **sex**.

Table 4-10 Welch two sample t-test of tooth hops measured from the **deer sample** sorted by **sex**.

	df	t-value	p-value
Female – Male	138.76	-3.10	< 0.05*

4.5 Question 4: What are the properties of the instrument that affect tooth hop variability?

4.5.1 Tooth type

Recall that the results from the one-way analysis of variance comparing saw-species groups is presented in **table 4-2**, with statistically significant differences in mean tooth hop found in several groups (relevant pairwise comparisons presented in **table 4-3**) and results of the two sample tests of variance between saw-species groups presented in **table 4-4**. Comparisons in this section will still focus on human samples as compared to a nonhuman proxy.

The mean tooth hop of human bone cut by the crosscut saw (3.87 mm) was statistically different from the mean tooth hop of pig bone cut by the crosscut saw (3.40 mm). Likewise, the standard deviation of each group was statistically different, with the human sample having a narrower range (0.53 mm) than the pig (0.65 mm). When compared to the deer sample, the mean tooth hop of human bone cut by the crosscut saw was statistically different from mean tooth hop of deer bone but by the crosscut saw (3.60 mm). The standard deviations of human and deer bones cut by the crosscut saw were not found to be statistically different.

The mean tooth hop of human bone cut by the rip saw (3.45 mm) was statistically different from the mean tooth hop of pig bone cut by the rip saw (3.68 mm). The standard deviations of human and pig bones cut by the rip saw were not found to be statistically different. When compared

to the deer sample, the mean tooth hop of human bone cut by the rip saw was statistically different from mean tooth hop of deer bone cut by the rip saw (3.60 mm). Likewise, the standard deviation of each group was statistically different, with the human sample having a wider range (0.59 mm) than the deer (0.48 mm).

Included in **table 4-3**, are also the results of the pairwise comparisons of rip and crosscut human samples to one another. There is a significant difference between tooth hop means of the CC_Human and R_Human samples despite no significant difference occurring between the means of the two blades (blade before sawing measurements only).

4.5.2 Tooth wear

As the two blades remained constant throughout the cutting process, tooth distances were measured prior to sawing when the blades were brand new and then after sawing to see the impact of wear on the saw blade teeth over time. A numerical summary of the blade before and after data as well as the results from the independent sample t-tests assessing statistical significance of blade wear are presented below in **table 4-11**. Both blades were significantly different ($p < 0.05$) after sawing. Results of the F-tests comparing distance between teeth measurements before and after sawing occurred is presented in **table 4-12**; of these, only the variance of the crosscut saw was found to be significantly different before and after sawing.

Table 4-11 Summary of **blade data before and after sawing** as well as paired t-test of distance-between-teeth measurements comparing saw blades before and after sawing. CC and R blades were analyzed separately.

	n	Mean (mm)	SD (mm)	df	t-value	p-value
CC Before	48	3.72	0.06			
CC After	50	3.40	0.14			
CC After – Before				67.81	-14.60	< 0.05*
R Before	49	3.90	0.08			
R After	46	3.48	0.11			
R After – Before				85.99	-21.43	< 0.05*

Table 4-12 Summary of two-sample test of variance (F-tests) comparing **blade data of before and after** sawing groups.

	Numerator df	Denominator df	F-value	p-value
CC After – CC Before	49	47	5.19	< 0.05*
R After – R Before	45	48	1.58	0.12

Visual differences between the saw blades before and after the sawing process were quite evident, especially in the crosscut saw. The crosscut saw at the end of the experiment had 3 broken teeth (**figure 4-5**). Teeth in the crosscut saw flare more to the sides as the tooth set alternates down the blade, and this flaring looked more irregular after sawing. The rip saw did not have any obviously broken teeth, although some rust and mild chipping were evident on the blade. Both saws were wiped of soft tissue during the sawing process and thoroughly cleaned with soap and water after each day of sawing before being elevated to air dry.

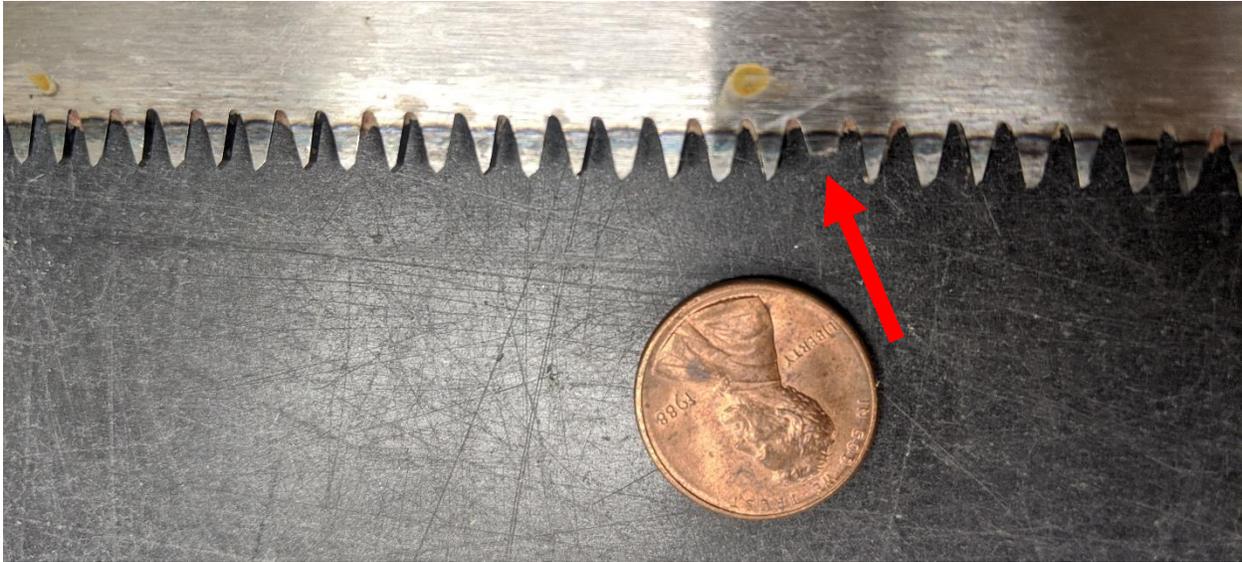


Figure 4-5 Crosscut saw once sawing completed. Arrow points broken tooth.

4.6 Question 5: Does having more than one tooth hop in a row improve the accuracy and precision of estimating blade TPI?

For this final analysis, only tooth hops that were categorized as belonging to a chain, in which case two or more tooth hops were linked, were analyzed to assess this question. **A total of 708 tooth hops amongst the three species occurred as a chain with two or more tooth hops in a row**, with 212 from the deer sample, 412 from the pig sample, and 84 from the human sample. Means and standard deviations for tooth hop measurements for each group are presented in **table 4-13** with boxplots illustrating a comparison of species group tooth hop measurements (crosscut and rip combined) in **figure 4-6**. As a comparison of bone tooth hops to new blade data (blades prior to sawing) has already been presented, it seemed pertinent to include in this research a comparison of bone data to a more realistic picture of a saw blade in a forensic setting, one where a blade may have already experienced wear and tear from prior use. Thus, the groups

CC_Blade_After and R_Blade_After were combined with crosscut and rip distance between teeth data of the freshly purchased saw blades. This blade group was not found to be normally distributed, so nonparametric testing was used. A Kruskal-Wallis rank sum test finds a significant difference between groups (combined blades before/after, human, pig, and deer) ($X^2 = 20.28$, $df = 3$, $p < 0.05$).

Finally, a one-way analysis of variance comparing groups sorted by species found no statistically significant difference in the mean tooth hop amongst the three species groups when including only instances of two or more tooth hops in a row.

Table 4-13 Sample distribution including mean and standard deviation for tooth hops measured from respective species group when only including instances of **two or more tooth hops in a row** (saw data has been combined to include both before and after measurements of the rip and crosscut blades).

Species	Number of TH (n)	Mean TH (mm)	SD (mm)	NA
Deer	212	3.51	0.47	9
Pig	412	3.44	0.59	12
Human	84	3.46	0.59	4
Blade	200	3.62	0.23	0

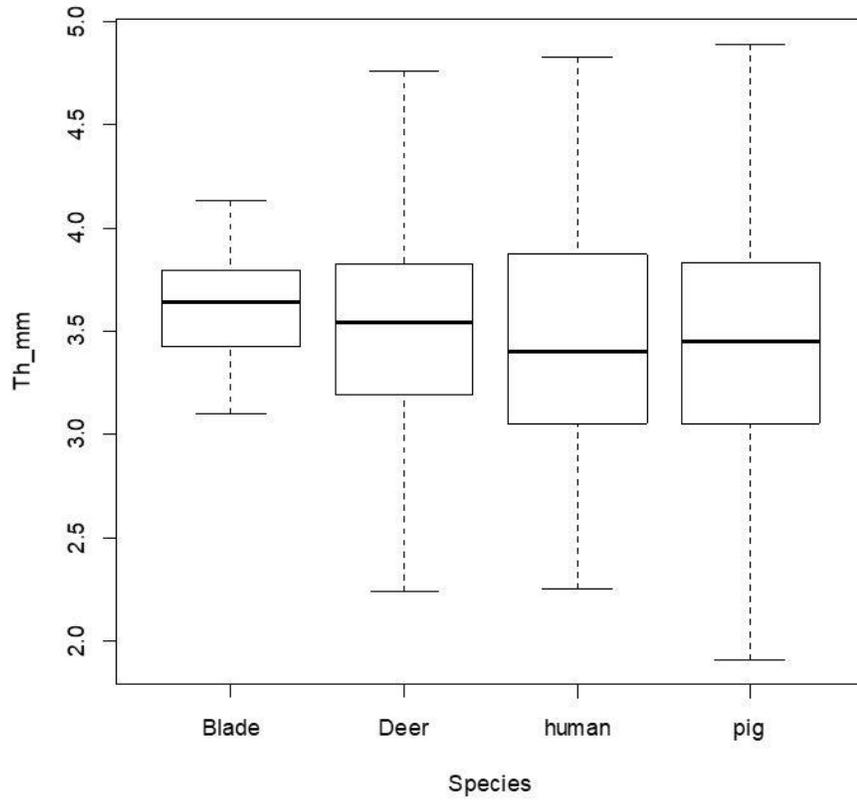


Figure 4-6 Boxplots comparing tooth hop measurements from **species** groups when only including instances of **two or more tooth hops in a row** for the bone data. Note the that blade data are combined (both before and after measurements for the crosscut and rip saw).

5.0 Discussion

5.1 Goals and research questions

The purpose of this study was to investigate the effects of tissue variability on the accuracy and precision of tooth hop measurements in bone to estimate tooth size of a saw blade. Five research questions were asked; a discussion of their results ensues in this chapter.

1. How variable will tooth hop measurements from a single instrument be when comparing multiple usages in human bone?
2. How variable will tooth hop measurements be from the same instrument when using animal proxies (deer and pig) to model human bone?
3. What are the properties of bone that affect tooth hop variability?
4. What are the properties of the instrument that affect tooth hop variability?
5. Does having more than one tooth hop in a row improve the accuracy and precision in estimating blade TPI?

Question components addressed statistically were assessed with the basic null hypothesis that there was no difference amongst the relevant groups addressed by each question. Results were considered significant at the $p < 0.05$ level, using two-tailed tests.

5.2 Sample composition

Tissue variability was created using different taxonomic groups with bones of varying hardness (deer, pig, and human) as well as of varying age and sex. The pig sample was comprised of 5-7-month-old pigs of unknown sex, all fed a diet of corn and soy meal and raised in a similar environment. All pig bones were humeri with unfused epiphyses. The deer sample was comprised of male and female deer from southwestern Pennsylvania, collected during the appropriate hunting season. With one exception, deer were estimated to be 3.5-4.5-years of age at the time of death with fused or near-fused epiphyses. One male had open epiphyses and was estimated to be 1.5-years of age at death. There were four femora from females and seven from males. The human sample consisted of the femora from one male who was 73-years of age at the time of death. No pathological conditions were included in the medical history provided to this researcher. **It is important to note here that variance for the human sample is variation presented in one individual and cannot truly represent variation in the overall human population.** At best, reference may be made to a geriatric population, but no overtly large conclusions can be drawn from one human. Realistically, humans range far more in age, hormonal level(s), diet, and are influenced by an even broader range of other environmental factors, which would ultimately make human bone the most variable group.

5.3 Question 1: How variable will tooth hop measurements from a single instrument be when comparing multiple usages in human bone?

The mean tooth hop measured from human bone cut by the crosscut saw was significantly different from the distance-between-teeth measurements collected directly from the crosscut blade. The same occurred for the rip saw groups (human bone and blade). Here, the human bone tooth hops from the crosscut saw had a higher mean than the blade, whereas the reverse was observed in the rip saw comparison. The pattern exhibited by the crosscut saw was the expected pattern. Realistically, the average tooth hop that occurs in a bone should at minimum be the same as or higher than the mean of the blade as the bone variability can only build on the variation of the blade.

There are multiple hypotheses to consider here as to how tooth hop means from bone can be lower than the respective mean from the blade teeth; I have outlined five. The *first* is that observer error can cause a measurement to be smaller; if a bone is not perfectly level in the view of the microscope, a slight angle could cause the peaks and valleys of tooth hops to appear closer together. This is likely to occur as it is near impossible to cut cross-sections perfectly flat and then get cross-section walls to lie parallel under the microscope. This mechanism of error would affect all species groups; in fact, all means from bone were lower than the (new, unused) blade mean with the exception of the human crosscut group. We will return to the issue of surface contouring in the following section as it likely plays a significant role, but here, we are primarily concerned with how one 7 TPI saw cutting human bone could be different from the other. A *second* hypothesis ties to the first in that from the initial cuts to bone tissue (pig sample cut first), both saw blade means and variances changed. Blade “after” means are all lower or near-equivalent to bone means. We will return to this issue for a discussion of tooth wear, but like the previous hypothesis, it does

not explain how one 7 TPI saw performed like this in otherwise “identical” human tissue. A *third* hypothesis is that the rip saw teeth do not create sharp force trauma in the same way as the beveled teeth of the crosscut saw. Rip saw teeth mechanically chisel out material, in which case, we could be observing an elastic response of bone tissue to sharp-blunt force trauma. With this proposal, it seems more likely that the chiseling teeth would increase tooth hop mean, beyond the crosscut (knife-like teeth) saw groups. As we will see in other species, the latter proposal seems to hold true for the means; we will return to this issue for a discussion of tooth type. A *fourth* hypothesis is that when sawing, the entire length of the blade is not always used to complete a cut. Thus, cut strokes might only be represented by a third of the blade teeth. Direct measurements from each blade had been collected from heel to toe of the blade. This fourth hypothesis is unlikely as while the distance-between-teeth as measured from the blade do vary, they vary consistently from heel to toe and not just within one section of the blade.

A *fifth*, and most likely, hypothesis as to why the mean of one human bone group fell below the average mean tooth distance from the blade and not the other is that because the human bone had the least amount of tooth hop chains (i.e., with only a few cases of two in a row and no instances of three or more), proportionally more isolated tooth hops were measured, which can mean inclusion of false tooth hops. False tooth hops would be areas in the striae that are peaked or hop like a tooth hop but are not actually tooth hops. This final hypothesis is likely the most influential here, as will be addressed by research question five. **However, while the tooth hop means are found to be statistically different as divided between the two saw blades, in reality, the amount of variation between the blades overlaps so much, that these two means would be included in the same TPI confidence interval (see below).** Also, variability introduced from cutting bone versus direct measurements from the blade is much beyond that of what observer

error and/or isolated tooth hops would introduce. **Table 5-1** presents a comparison of distance-between-teeth measurements collected from saw blades as compared to the blade TPI.

Table 5-1 Distance-between-teeth measurements (mm) as compared to the associated blade TPI. Table adapted from Symes *et al.* 2005. * = presented as same values in Symes *et al.* 2005 and could be from manufacturing variability in the measured blades or a typing error.

Distance between teeth (mm)	Blade TPI
0.30	100.00
0.50	50.00
1.30	20.00
1.80	14.30
2.50	10.00
2.80	9.10
3.30*	8.30
3.30*	7.70
3.60	7.10
3.80	6.67
4.10	6.25
4.30	5.88
4.60	5.55
4.80	5.26
5.10	5.00

The differences in standard deviation were also examined for each of the two human samples and found to be significantly different from the standard deviation of the respective blade that cut them. This is of most importance when a forensic anthropologist projects the likelihood that a specific saw is consistent with evidence from a saw cut bone in a forensic case. As mentioned with the means, the standard deviations from bone measurements are much wider than measurements from the actual blades. Human bone cut by the crosscut saw had an average 3.87 ± 0.53 mm tooth hop (3.34 – 4.40 mm), whereas human bone cut by the rip saw had an average 3.45

± 0.59 mm tooth hop (2.86 – 4.04 mm). **These ranges translate to approximately 8 – 5.5 (5) TPI for the human bone cut by the crosscut saw and approximately 9 - 6 TPI for the human bone cut by the rip saw.** Thus, each resultant TPI range includes approximately 3 TPI and **therefore, when presenting a potential TPI range in a forensic anthropological report, the analyst must at minimum include a ± 1 TPI range.**

5.4 Question 2: How variable will tooth hop measurements be from the same instrument when using animal proxies (deer and pig) to model human bone?

As the saw blade means were not significantly different, saw blade information was combined for all groups in the one-way analysis of variance (blade, pig, deer, and human). Variance between groups is also examined. Significant differences in means were found between four comparisons (deer – blade, pig – blade, pig – deer, and pig – human). All tooth hop groups measured from bone had means smaller than the blades combined average tooth hop of the blades.

5.4.1 A comparison of tooth hops measured from bone only

When comparing means of species groups to each other and not to the blades, the pig group mean was most unlike that of the other bone groups, with mean measurements from the human bones not significantly different from deer. This relationship was most like the one visualized by the crosscut saw groups, whereas human was most like pig in the rip saw groups. As the analysis here combines blade (as not all cases of postmortem saw dismemberment present the same tooth style) and bone data, which increases sample sizes and provides a broader view of tooth hops

measured from human bone, the relationships from this approach are likely the more realistic ones. It can be posited that the human and deer bone having statistically different means from the pig bone because the human bone is from a 73-years-old individual. Geriatric bone is more brittle, which would imply that the human bone used in this study is closer to deer in how it reacts to sawing, whereas the pig sample consists of 6-month-old pigs, which have bone that is more elastic. When comparing the standard deviations of each sample, the human bone expressed the same standard deviation as the pig sample, with the deer having the narrowest variation. Thus, bone elasticity may be reflected by variance while the mean more closely reflects bone hardness.

Potentially, what is exhibited by the mean and variances of the bone samples above reflects the notion that hardness and elasticity are not one and the same. Hardness tends to increase with an increase in elastic modulus (with stiffer materials having a higher elastic modulus). How hard or soft a material is dictates whether “scratch marks” will be left behind on the cut surface; thus, hardness dictates the presence or absence of striations from the sawing process. Saville, Hainsworth, and Ruddy (2007) found that the average hardness for a deer tibia was 54.8 kg mm^{-1} (external surface hardness) and 66.8 kg mm^{-1} (hardness across cortex), while the hardness for a 74-year-old human male femur was much less (39.5 and 39.4 kg mm^{-1}). The pig femur from their study showed the most similarity in hardness to the human bone (26.0 and 37.1 kg mm^{-1}) compared to other species included in the sample (deer, sheep, and cow). Ages of nonhuman proxies were not mentioned. Regarding tissue hardness, the human sample in this study may be closer to deer (as the pig bones are from known subadults), at least in terms of dense cortical sites along the diaphysis where the majority of tooth hops occurred. And in contrast, elasticity (of lamellar bone tissue) could be affecting how wide or narrow the variance would be; here, for the human sample which is all of the cross-sections from distal to proximal in the femur, the standard deviation is

identical to that exhibited by the pig bone. We could be witnessing a change in tissue hardness along the femoral shaft for the human sample, the general elastic response of human tissue for this individual, or both. Further studies and a larger human sample would be able to clarify this issue. Another point to consider here is that both bone mineral and collagen change in quantity and quality with age (among other factors), but neither are required to change to the same degree in every person. Typically, bone proportionally becomes more mineralized with age (Currey 1969; Boskey and Coleman 2010), but many factors go into this. More mineral generally means harder bone; more collagen generally means more elastic bone. Human age changes are based on degeneration of tissue and thus far more variable than dental formation and eruption charts in children, for example. Aging of tissue is a complex issue and this sample only includes one 73-year-old male.

5.4.2 A comparison of tooth hops measured from bone to blade

Only in two groups was the difference in tooth hop means statistically significant (deer and pig) when compared to blade mean distance-between-teeth measurements. As we are now seeing multiple species groups (and not just CC_Human vs. R_Human) exhibit this phenomenon, the question remains as to how means from bone measurements *can be lower than tooth distance means of the blades that cut them*. As above, I suggest that this is due to angled surfaces of the bone under the microscope. This issue cannot easily be fixed, as leveling a bone surface can be difficult. Modeling clay could be used to fix large angles; however, bone surfaces can also physically wave with the tooth hops, meaning that the cut surface is not truly flat. Symes *et al.* 2005 refers to this phenomenon as *harmonics*, where three-dimensional peaks and valleys are observed (with power saws typically exhibiting greater harmonics than hand saws). As all bone

groups experience this to some degree, with means lower (some significantly so) from the blade that cut them, this type of error is the most likely scenario.

It was also posited in the previous section that as the bone samples include cases of single (isolated tooth hops) as well as two or more chained tooth hops, it is likely that cases of isolated tooth hops are affecting the relationships of tooth hop means. **Table 5-2** presents a comparison of means and standard deviations from all measured tooth hops within a bone sample to only tooth hops present in a chain of two or more from that same sample. Note that the means dropped even lower in cases of two or more tooth hops in a row; however, the variances are near identical. From this perspective, isolated tooth hops or cases of potential “false tooth hops” either increased the means, while maintaining the overall variance of groups, or it had no significant effect on combined species group data. Alternatively, harmonics is likely more of a factor in cases of longer tooth hop chains, which can cause peaks and valleys of tooth hops to be closer together when viewed under the microscope. Pig bone presented the longest tooth hop chains, making the effect of surface harmonics potentially the greatest in this group. **Regardless, the means of tooth distances are measured in millimeters and are of less significance when having to be compared to TPI of suspect blades.** **Table 5-3** provides a comparison of tooth hop ranges from all groups mentioned in this discussion to potential TPI ranges their measurements would correlate.

Table 5-2 Comparison of combined (crosscut and rip) blade means and standard deviations for groups where isolated tooth hops are included vs. groups where only two+ tooth hop chains are included.

	Isolated tooth hops included	2+ tooth hop chains only
	Mean ± SD (mm)	Mean ± SD (mm)
Deer	3.64 ± 0.45	3.51 ± 0.47
Pig	3.55 ± 0.60	3.44 ± 0.59
Human	3.66 ± 0.60	3.46 ± 0.59

Table 5-3 Combined (crosscut and rip) blade means and standard deviations for each group associated with their approximate TPI ranges. TPI ranges are also included for 2+ tooth hop chains-only groups. Blades were both marketed as 7 TPI. TPI decimals are rounded at the 0.5 so as not to include partial teeth.

	Number of tooth hops (n)	Tooth hop Mean ± SD (mm)	Blade TPI range
Blades (Before)	97	3.69 – 3.93	7 – 6
Blades (Before & After)	200	3.39 – 3.85	8 – 6
Deer (all TH)	687	3.20 – 4.10	8 – 6
Deer (2+ TH chains only)	212	3.04 – 3.98	9 – 6
Pig (all TH)	793	2.95 – 4.15	9 – 6
Pig (2+ TH chains only)	412	2.85 – 4.03	9 – 6
Human (all TH)	279	3.06 – 4.26	9 – 6
Human (2+ TH chains only)	84	2.87 – 4.05	9 – 6

5.5 Question 3: What are the properties of bone that affect tooth hop variability?

Of the physical properties of bone that may affect tooth hop variability, three are discussed below, including species, age, and biological sex. Following is a brief discussion of what to do with this knowledge of bone variability and how it should impact our presentation of TPI ranges for suspect blades from saw cut bones in forensic cases of postmortem saw dismemberment.

5.5.1 Species

Conclusions of intertaxonomic differences can only properly be addressed by the pig and deer samples that included bones from multiple individuals. Even then, populations were biased towards certain age groups and sexes. The pig sample originated from farm-raised pigs, aged 5-7-months, and raised on a diet of corn and soy meal. Sex could not be determined, but pig bones sold

were from either barrows (castrated males) or gilts (intact, young female pigs that had never farrowed a litter). Sex could be attributed to specific deer and ages could be approximated from epiphyseal fusion and as reported by hunters following listed Pennsylvania hunting laws (<https://www.pgc.pa.gov/HuntTrap/Law/Pages/SeasonsandBagLimits.aspx> accessed March 1, 2019); however, diet from a wild sample is not controlled. Birth and weaning status for females could also not be determined. And as stated previously, the human sample consisted of bones from one 73-year-old male, so does not represent a full spectrum of human variation. Variation in the human sample is the variation within one bone type from one individual, as opposed to a comparison of bone type amongst multiple individuals within a species.

Qualitative results from the bones confirm deer bone brittleness as compared to the pig and human samples in this study. Both deer and pig bones received the same maceration treatment and the resultant deer femora felt like smooth, hard porcelain, whereas the pig bone was less smooth and continued to emit grease (fat) post-processing. Deer bone presented with the largest breakaway spurs as well as the largest instances of exit chipping and entrance shaving in the direction of cut progress. Breakaway spurs in the pig sample were typically shorter and in many cases, breakaway spurs and notches appeared on both sides of the cross-sections. Breakaway spurs and opposing breakaway notches are the result of blunt force trauma, where force from the sawyer exceeds the load needed to fracture the bone at the bottom-most point in a cut. To continue the discussion above, elasticity and hardness are different properties, with elasticity being an intrinsic property of a material, thought of as the molecular bonds between components of a material. Hardness is measured extrinsically, referring in general to the indentation of a material and its ability to resist “flow” away from the indentation site. Larger breakaway spurs, large and frequent exit/entrance

chipping, as well as clear spurs and notches on opposing sides of a saw cut are the result of less-elastic (i.e., more brittle, stiffer) material and not just the result of a longer bone.

As with trabecular bone, regions of plexiform bone in the pig samples proved just as problematic in retaining or being able to visualize striations from saw cuts. While plexiform bone is more like cortical bone overall, it has a dense vascular plexus which increases its porosity, giving plexiform bone a “brick wall” appearance. Martiniaková *et al.* (2006) reported plexiform bone in the endosteal zone surrounding the medullary cavity in adult pig femora, whereas Hillier and Bell (2007) described mature pig femora presenting Haversian bone in the posterior region of the shaft, with the remaining bone being plexiform bone. Hillier and Bell (2007) reported plexiform bone in the periosteal zone in immature deer femora, with Haversian bone beginning to form from the endosteal surface and then further developing on the posterior side of the bone. Skeletally mature deer will have had plexiform bone replaced by Haversian bone. Thus, all skeletally mature deer in this study had most of the plexiform bone already remodeled as Haversian bone. So, in terms of microscopic structure, human and deer samples were comprised of Haversian bone, whereas the pig sample retained more plexiform bone. Plexiform bone is present in fast-growing, large mammals, such as the young pigs in this study, which could impact the overall appearance of tooth hops on a cross-section surface. Certainly, the presence/absence of tooth hops along the endosteal regions of pig bones was affected. How plexiform bone directly impacts the mean and standard deviation of tooth hops cannot be discerned from this study, but it likely lies between cortical and trabecular bone values. Cortical bone has a higher elastic modulus, which correlates with hardness, than trabecular bone (Zysset *et al.* 1999), taking the species into consideration. Zysset *et al.* (1999) report a range for the elastic modulus of human femoral cortical bone as 20.1 ± 5.4 GPa (measured longitudinally) versus trabecular bone treated similarly as 11.4 ± 5.6 GPa; the authors concluded

that the elastic modulus of human bone tissue depended strongly on tissue type, anatomical location, and individual (hardness did as well, but was less reliant).

Deer bone hardness and density provided an additional problem to the presentation of saw striae and tooth hops in several specimens. Some cross-sections from the deer bone appeared so polished and smooth that no striations were overtly visible or clear enough for microscopic analysis, despite the presence of oblique lighting. When striations are absent or not clear in specimens, no tooth hops can be measured. This phenomenon was not frequent, but when present occurred around the femoral midshaft, where cortical bone was extremely thick. Some cross-sections of pig bone emitted grease post-processing which meant the surface was too shiny to see striations and thus, tooth hops.

5.5.2 Age

Regarding age, the pig bones were the youngest, at 5-7-months-old. Deer were mostly skeletally mature adults and the human individual was an elderly adult. If tissue or individual age was the primary factor affecting tooth hop measurements and not intertaxonomic variation, human (assuming is representative of geriatric males) would have been the most accurate with a small standard deviation, followed by the deer, with the pig bones being the most elastic, least brittle, and therefore, would have the widest standard deviation. The relationship amongst groups was not this clear, so age was not the sole or primary factor affecting tooth hop measurements.

Because the humans sample consisted of measurements from one individual (and the smallest sample size), there is a greater uncertainty as to how this would be representative of the entire human population or even a human male geriatric population. Potentially, a geriatric male population would present just as wide or wider variation. As geriatric bone is typically more brittle

than subadult or adult bone, this type of bone may be best represented by the adult deer sample rather than the subadult pig sample. This trend was noted in the tooth hop means when species measurements were compared (combining data from both saw blades). However, the opposite relationship was observed in standard deviations, with the range of variation presented in the human femur being identical to that measured from the pig femora. Previous hypotheses presented (isolated vs. two or more tooth hop chains; influence of surface harmonics) are more likely to explain any significant differences in means. Regarding variance, deer bone can provide an extremist look at what hard, brittle bone could look like, whereas subadult pig bone can provide a glimpse of soft, or more elastic bone. A true human population would vary along this spectrum, with subadult and young adult bones being the most elastic tissue and geriatric bone being the most brittle or stiff tissue.

5.5.3 Biological sex

Only the deer sample had femora of known sex, with more bones coming from males, although male and female bones being evenly split between the two saw blades. It is hypothesized that because males exhibit more testosterone, bones from males within the deer population would be harder and/or denser than females since testosterone is a primary influencer of appositional bone growth. Significant differences were found between mean tooth hops measured from male and female deer, with no significant differences in tooth hop standard deviations. Tooth hop mean measured from the male bones was closer to true mean of the saw blades, although this could be biased due to a larger sample of tooth hops coming from male bones. Regarding standard deviations of groups, the trend does continue that tooth hop variance is affected more by species

rather than age or sex of the tissue. However, given the small sample size, little can be confirmed about the influence of biological sex on tissue mechanical properties.

Crenshaw *et al.* (1981) had examined the influence of age, sex, and dietary calcium/phosphorus levels on bone mechanical properties in pigs. Early in development, bone strength was driven by dietary differences with pigs fed higher amounts of calcium/phosphorus having increased bone quality. At 145-days-old (approximately 4-months-old), significant differences in bone quality were detected between males and females. At 192-days-old (approximately 6-months-old), significant differences in bone quality were detected between barrows and boars. Thus, at different life stages, bone quality was affected by different properties, with diet being most important early in life and sex hormones increasing in importance once secondary sex characteristics began to develop. A similar trend could be argued for humans (sufficient energy intake and particular nutrients being vital for subadult growth), although the timeline would differ as our lifespans are much longer; additionally, one could add a revival of sex hormone influence post-puberty (especially in humans with longer life expectancies) because hormones change drastically again in both postmenopausal women and elderly men with sex hormones decreasing in presence or bioavailability.

5.5.4 What to make of this variation in human bone?

Ultimately, many factors from an individual's life (beyond even that of age, sex, and diet) impact bone quality and quantity (mineral and collagen) that it is better to err on the side of caution when providing tooth hop ranges as measured from human bone. From the single human in this study, a mean TPI \pm 1 TPI is the minimum a forensic anthropologist should present in court (when saw data is not combined). **But given the variety of ways that humans can vary, I suggest**

presenting a mean TPI \pm 2 TPI (ex. 7 \pm 2 TPI or 5 – 9 TPI) when establishing consistency between saw cut bones from forensic cases to suspect saws. Even though both pig and deer samples present similar TPI ranges, each is biased to a particular age group, sex, and/or diet that would be minimizing true variation of the population. Another option would be to present both ranges, with a \pm 1 TPI range as a narrow confidence interval and a \pm 2 TPI range as a wide confidence interval. Recall that TPI is just one general class characteristic of a saw blade and other features would be included in the analysis (such as tooth type, power type, and tooth set), all of which would be used to say whether or not a suspect saw is consistent with saw marks found in bone or if no suspect saw is found, to suggest qualities it would have encompassed.

5.6 Question 4: What are the properties of the instrument that affect tooth hop variability?

Of the physical properties of blades that may affect tooth hop variability, three are discussed below, including tooth size (TPI), tooth type, and tooth wear.

5.6.1 Tooth size

The two saws used in this research were marketed as 7 TPI; however, when comparing an standard English ruler to the teeth of each saw blade, the crosscut saw measured as 7 TPI whereas the rip saw measured as 6 TPI. Statistically, there was no significant difference noted between the distance-between-teeth (mm) measurements of either blade. Therefore, a 1 TPI difference between two blades is insignificant. But knowing this, a significant difference in means (mm) from the bone

measurements was not overtly a surprise and in the end, had little effect on resultant TPI range presented.

5.6.2 Tooth type

As mentioned above, it was hypothesized by this researcher that crosscut teeth would act more like sharp force trauma, given the beveled nature of the saw teeth. Each tooth would be “cutting” across the grain of bone. Meanwhile, the rip saw teeth, not being beveled, would act more like sharp-blunt or micro-blunt force trauma, with teeth chiseling bone from the kerf. Crosscut teeth are designed to cut across the grain of a material, where rip teeth are designed to cut along the grain of a material while pushing out pulp from a kerf. But neither of these saw blades were meant to cut bone; both were designed to cut wood, a much softer material. Similarly, the crosscut teeth are carbide, meaning that they had been heat treated to increase tooth hardness.

Both blades were much easier to use when sawing bone at the distal end, where cortical bone was thin and trabecular bone the dominant tissue pattern. Progressing proximally, cortical bone thickened, and it became more difficult to saw through the tissue, primarily with the crosscut saw blade. The crosscut saw would often bind in the cut, which meant having to remove the blade and trying to re-initiate the sawing process in order to complete the cross-section.

Means were not significantly different for tooth distances measured from the two saw blades, so attention will be paid primarily to differences in standard deviation for the bone samples; possible reasons for differences in means were also thoroughly addressed in the first two sections of this chapter. When comparing bones cut by the crosscut saw, human and deer had insignificant differences in standard deviations (significant differences in standard deviations were found between human-pig and deer-pig). When comparing bones cut by the rip saw, human and pig had

insignificant differences in standard deviations (significant differences in standard deviations were found between human-deer and deer-pig). As pig and deer bones are more representative of the populations they come from, more can be concluded from them than can be firmly said of the human samples. Also, both pig and deer have significantly different standard deviations from each other in both crosscut and rip groups. Measurements from pig bones were more variable than those from the deer, no matter the tooth type. When looking within a species, it was the crosscut saw that created more variation than the rip saw and not the reverse, as had been anticipated. This likely represents the difficulty of sawing with crosscut teeth in bone rather than how each tooth type generally cuts through the material for which it was designed (wood). This is similarly reflected in tooth wear below, as the crosscut saw changed the most as more bone was progressively cut throughout the study.

5.6.3 Tooth wear

There is no doubt that tooth wear occurred throughout this study. Mean distance-between-teeth measurements (measured before sawing and when sawing concluded) were significantly different for both saw blades. Regarding a test of variance, only the crosscut saw was significantly different when comparing standard deviation before and after sawing. Visually, the crosscut saw changed the most as exhibited by broken teeth (and broken teeth had been avoided when measuring the “after-group”). Tooth wear likely affected the crosscut saw more because the teeth were set wider from the body of the blade and had been heat-treated, which increases tooth hardness and is intended to keep saw teeth sharper for longer. However, teeth treated in this manner are more brittle and break when cutting material like bone that is harder than what it was intended to cut.

If tooth wear was the primary influencer of tooth hop variation in the bones, the bone cut first would more accurately reflect the new, unused saw blade, with the bone cut last being the most variable. Pig bones were cut first, then human bone, and lastly, deer bone. Deer bone had the smallest standard deviation for both the crosscut and rip saw. Pig and human expressed larger standard deviations.

5.7 Question 5: Does having more than one tooth hop in a row improve the accuracy and precision of estimating blade TPI?

Table 5-3 provides a comparison of tooth hop ranges from all groups mentioned in this discussion (including a comparison of group data using all tooth hops versus group data with only two or more tooth hops) to potential TPI ranges their measurements would correlate. Differences in tooth hop means of the three bone samples (human, pig, and deer) were no longer significant when only analyzing cases of two or more chained tooth hops. **However, resultant TPI ranges of groups, both including isolated instances of tooth hop measurements or not, were the same.** Symes *et al.* 2005 suggests looking at tooth hop in instances where you have three or more tooth hops in a row, but I argue here, that it does not make a difference when these measurements are converted to potential TPI ranges. And when considering sample size of tooth hops, if an analyst were only to look at instances of 3+ or even 2+ tooth hop chains, over half of the data in this study would be eliminated. But knowing the resultant TPI range is the same, there is no need to disregard this much valuable information.

6.0 Conclusions

6.1 Bone tissue effects on saw mark evidence in bone

Bone tissue variability is the primary influencer of measured tooth hop means and standard deviations from saw cut bone. Variation from the saw blade teeth is existent, but minimal in comparison to what variation is introduced to these measurements from sawing through bone. Mean tooth hops as measured from bone can be smaller than mean tooth distances directly measured from blades, but when measurements are converted to TPI ranges, this difference is inconsequential. Most likely, surface harmonics (especially prominent in long tooth hop chains) and difficulty leveling cross-sections under the microscope creates smaller ranges, as can instances of isolated “tooth hops” if these are not real or incomplete. But again, when converted to TPI ranges, these differences are inconsequential.

6.2 Limitations of this study

The results of this study are limited due to the samples included. Age, for example, cannot be isolated as age varied with taxonomic group (young pigs, adult or skeletally mature deer, and a geriatric human). Of primary concern is that conclusions from human tissue can only reflect the variation of human tissue within one individual, a 73-year-old male. Variation is variation expressed along the length of the left and right femur, and not variation from a human population. At best, this individual may represent a geriatric male population; however, this cannot be known

without an increase in sample size or for a way to compare the tissue to other geriatric males in the human population.

The pig sample is of mixed sex, but sex cannot be attributed to any of the specimens. However, it is known that they are intact females or castrated males, which would mean that intact male pigs are not represented, and this sample likely aligns more closely with young, female pigs. Therefore, the effects of testosterone on bone tissue cannot be fully explored in the pig sample. Similarly, the deer sample, while of known sex, does not have a controlled diet and there are proportionally more males than females included in the current study. More female cross-sections are under preparation and will be included for comparison in a future study.

6.3 Significance and implications for forensic anthropological case reports

This research has at least six implications to consider for future forensic anthropological research and presentation of saw mark evidence in forensic case reports.

1. Even if significant differences are found between tooth hop measurements (mm) within one forensic case, this does not necessarily translate to a significant difference in converted TPI estimates. Other class characteristics of saw blades should be evaluated in instances where the analyst believes more than one saw blade was used (i.e., power, tooth type, tooth set, etc.).
2. Tooth hop measurements from human bone groups (rip and crosscut saws) in this study produced ranges of a mean TPI \pm 1 TPI. This should be the narrowest confidence interval presented in a forensic anthropological report.

3. Given the variation that can be exhibited by human tissue, a wider confidence interval should also be presented in a forensic anthropological report, that is mean $TPI \pm 2 TPI$. Tooth size is just one of many general class characteristics that can be used to evaluate consistency of marks found in bone to suspect saws.
4. Blades do wear over time, so this knowledge can be taken into consideration when estimating the potential blade TPI range of the saw blade that cut bone. However, even after making hundreds of cuts in bone, the TPI ranges estimated from tooth hops in each group were still the same, no matter if the bone was cut at the beginning of the study or at the end. Thus, blade wear minimally affected blade TPI estimation and variation exhibited by the bone was primarily caused by bone tissue variability. Feature such as missing blade teeth, and/or teeth breaking off in bone from wear or misuse, would be more valuable here, potentially serving as an individual tool characteristic if recovered and able to be matched to a suspect blade.
5. Isolated instances of tooth hop can be just as effective in producing an estimated TPI range for a blade as cases of two or more tooth hops in a row. Analysts do not require three or more in a row, although for less experienced researchers, two or more can help with tooth hop identification. Number of tooth hops should be reported as the sample size (N) used to estimate blade TPI in forensic reports.
6. Because measurements are converted to TPI ranges (as this is how blades are marketed and referred to by the general public), fresh pig and deer bone femora can be used as proxies for human bone. However, I would caution the use of deer, particularly the metapodials as the increased hardness and density as compared to human bone could cause us to narrow our confidence intervals. A mean $TPI \pm 2$

TPI should account for this, as a broader confidence interval, and would also work for pig femora proxies.

Validation studies such as that presented in this dissertation play a vital role in the forensic science community. The FBI does not report the frequency of dismemberment cases in the United States, so it is difficult to calculate the number of forensic cases utilizing microscopic saw mark analysis. Similarly, not every dismemberment case from the forensic anthropology side makes it to court, especially since dismemberment is often used to hide the identity of the decedent. During my master's program at Mercyhurst University, working with Dr. Steven Symes, D-ABFA, renowned saw mark expert, my experience encountering microscopic saw mark analysis would likely be biased as compared to another forensic anthropological laboratory. In a 3-year period (2011-2013), I assisted Dr. Symes with five forensic cases that had saw marks. However, a validation study examining tooth hops could also be related to "chop marks" made with serrated blades and similar blade or sharp force properties that rely on bone hardness in order to leave a mark.

Our role as forensic scientists is to be proactive in developing and testing the accuracy and reliability of our methods. Forensic methods do not just result in inconsequential conclusions that ends up on a dusty shelf; our methods can lead to conclusions that drive legal decisions and in criminal cases, may result in a death sentence.

6.4 Recommendations for future research

Ideally, mechanical tests could be implemented to offer more knowledge on mechanical and material properties of the bone prior to sawing. Hardness testing was to be incorporated in this

research; however, a hardness tester could not be obtained, and early defrosting of the pig bones pressed this researcher to saw earlier than anticipated to ensure that sawing occurred as soon as the bone was defrosted. Hardness and elastic modulus can be estimated from other studies; however, the more information that can be directly gleaned from the sample at-hand would be preferred.

The bones analyzed in this study were humeri (pig) and femora (human and deer). Humeri and femora are proximal elements in the limbs. Often, researchers utilize deer metapodials for forensic research as these bones do not greatly vary in shape down the shaft and have very thick cortical bone (ideal for showing saw marks). However, Skedros *et al.* 2003 found that percent-ash and secondary osteon population density progressively change from proximal to distal elements, which likely reflect changes in mechanical and functional loading along the limb. A future comparison of deer femora to metapodials would reflect the usefulness and effectiveness of deer metapodials in saw mark (and other sharp force trauma) research.

Appendix A Example Data Collection Sheet



Figure A-1 Example data sheet from specimen DP4.2N.(TH#1-23). This is deer bone #4 (D4) cut by the crosscut saw (P) and the second cross-section from the proximal end, examining the unmarked, distal (N) surface. Points indicate tooth hop valleys with a single hop having two same-number points, one per valley. Joining tooth hops share a valley. The bottom right shows calculations for tooth hops, considering scope magnification and conversion. The bottom left is an outline of the bone surface with approximate locations of each tooth hop indicated.

Appendix B Assumption Tests for Statistical Analyses

Table B-1 Shapiro-Wilk test of normality for tooth hop measurements (mm) for **saw-species** groups.

Species	Saw	df	W-value	p-value
Deer	Crosscut	327	1.00	0.50
	Rip	360	1.00	0.36
Pig	Crosscut	396	1.00	0.31
	Rip	399	1.00	0.34
Human	Crosscut	137	0.99	0.22
	Rip	141	0.99	0.81
Blade	Crosscut	47	0.99	0.83
	Rip	48	0.99	0.86
Blade (After)	Crosscut	49	0.97	0.19
	Rip	45	0.96	0.09

Table B-2 Shapiro-Wilk test of normality for tooth hop measurements (mm) for **species** groups. The blade data here are only before measurements, but combined saw blades (CC and R).

Species	df	W-value	p-value
Deer	690	1.00	0.05
Pig	793	1.00	0.15
Human	280	1.00	0.64
Blade	99	0.99	0.50

Table B-3 Shapiro-Wilk test of normality for tooth hop measurements (mm) for the **deer sample** sorted by **sex** (saw data combined to maximize sample size from females).

Sex	df	W-value	p-value
Female	105	0.98	0.19
Male	581	1.00	0.12

Table B-4 Shapiro-Wilk test of normality for tooth hop measurements (mm) for **species groups, excluding isolated tooth hops** (except for blade data). The blade data here are combined before and after measurements from both saw blades (CC and R).

Species	df	W-value	p-value
Deer	211	1.00	0.85
Pig	411	0.99	0.14
Human	83	0.99	0.68
Blade	199	0.98	0.01*

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