Temporal Rate of Post-mortem DNA Degradation in Archived Tissue Samples: Evidence from Liver and Muscle

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Guidelines identifying best practices for harvesting tissues that lead to optimal DNA preservation are few but are important curatorial concerns for genetic resource collections. We conducted a temporal study to establish rate of DNA degradation of tissue samples extracted from field-caught museum specimens. Five individuals of *Sigmodon hispidus* were collected and their liver and muscle tissues were harvested. Each tissue type was sectioned into 15 subsamples, and each was preserved in liquid nitrogen at different time intervals (2, 4, 8, 16 and 32 minutes; 1, 2, 4, 8 and 16 hours; and 1, 2, 4, 8 and 16 days) following death. DNA was extracted using an automated robotic instrument and molecular mass profiles were determined fluorometrically. Post-mortem DNA degradation was continuous and dependent on time, but also was significantly affected by differences among individual cotton rats. DNA fragments of ≥10,000 base pair in length were present in muscle samples across all time intervals, whereas DNA fragments of this size in liver samples were no longer present after 8 to 16 hours post-mortem. DNA molecular mass profiles showed that muscle samples retained 80% of their longest fragments (≥10,000 bp) until 1 day post-mortem, whereas liver samples retained the same percentage only until 8 minutes after death. Although rates of decay were measured from samples in a laboratory (not field) setting, rates of decay presented here can guide field and museum workers in best practices. Results suggest that opportunistic samples, such as those from roadkill specimens, are more likely to be of use for a variety of molecular methods when muscle is preserved. Considerations of differences in rates of degradation may also guide selection of tissue types housed in genetic resource collections, especially under space-limited circumstances.

Key words: Archived tissues, genetic resource collection, high quality DNA, tissue harvesting guidelines.

The advent of comparative genetic methods in the late 1960s, based on karyotypes and the molecular structure of proteins or nucleic acids (Hubby and Lewtonin 1966; Murphy et al. 1996) marked the beginning of procuring and biobanking tissues for future genetic studies (Sheldon and Dittmann 1997; Bradley and Dowler 2019). Soon after, harvesting of tissues became a standard scientific practice leveraged to address questions in systematics, evolution, and molecular biology (Moritz and Hillis 1996; Sheldon 2001; Bradley and Dowler 2019). Subsequent development of the polymerase chain reaction (PCR, Saiki et al. 1985) in the early 1980's allowed researchers to obtain DNA sequences for comparative analyses from very small samples of biological material (Sheldon 2001; Lonsinger et al. 2019). Continuing advances in genetic methods further stimulated growth of individual researcher and museum-based collections of tissues (Sheldon 2001; Corthals and Desalle 2005; Phillips et al. 2019). Consequently, curator-affiliated biological research collections housed in museums progressively assumed the task of maintaining tissue samples for long-term storage in what are known today as genetic resource collections or biobanks (Corthals and Desalle 2005; Dunnum et al. 2018; Cook and Light 2019). The infrastructure supporting data collection, organization, and cross-referencing to voucher specimens positioned museums as the ideal institutions for long-term stewardship of tissue specimens (Winker 2004; Corthals and Desalle 2005; Schindel and Cook 2018; Cook and Light 2019; Thompson et al. 2021). Indeed, genetic resource collections are an important form of scientific infrastructure that facilitates future research in an efficient and cost-effective manner (Sheldon 2001; Winker 2004; Watanabe 2019).

Despite increased organization and centralization of biological specimens, few best practices for long-term curation of these materials have been outlined and even fewer adopted (Zimkus and Ford 2014). One such example of guidelines and standards was produced by the

Systematic Collections Committee of the American Society of Mammalogist that advised that tissues should be harvested and cold-stored as soon as possible after the death of the organism to provide the best quality material for later genetic analysis Phillips et al. 2019). As a result, collections with the necessary infrastructure and financial abilities, now typically store and maintain their holdings in ultra-cold mechanical freezers (-80˚C) or in high-volume stable liquid nitrogen tanks (-196˚C) that offer premium long-term storage conditions (Hanner et al. 2004). However, in the absence of expensive cryogenic or ultra-cold infrastructure, scientific collections may opt to store tissue samples in ethanol at various concentrations. Even though this practice is effective in preserving DNA in tissues, it poses the disadvantages of conserving small quantities of high molecular weight DNA even after just 2 years of storage (Kilpatrick 2002). Consequently, when archiving animal tissues, the best curatorial practice for storage and preservation of these biological samples for later genetic studies is their long-term storage at cryogenic temperatures such as those obtained with liquid nitrogen (Sheldon 2001, Corthals and Desalle 2005; Soniat et al. 2021). Preserving tissue samples in such a way allows them to freeze immediately when in contact with liquid nitrogen. This avoids intracellular ice formation that occurs when biological materials are cooled gradually (Taylor 1957) as happens when preserving tissues in dry ice or even in mechanical ultra-cold (-80°C) freezers.

DNA is a stable macromolecule that easily can be extracted from degraded biological materials (Lindahl 1993; Fordyce et al. 2013; Lopez et al. 2020). However, the more degraded the source of DNA is, the more limited are the laboratory techniques, analyses, and molecular applications (Lonsinger et al. 2019) that can be conducted on them. Often field conditions preclude immediate post-mortem tissue collection, or some tissues are opportunistically obtained, such as from roadkill and thus collected many hours or days after death (Cheviron et al.

2011). Characterizing post-mortem degradation behavior, especially long after death will contribute to the decision-making process of harvesting a particular tissue sample for certain types of genetic analyses. Moreover, establishing best field practices for post-mortem tissue harvesting is a curatorial concern that needs to be addressed from a collection management perspective. Established expectations about DNA degradation rates paired with recorded information about post-mortem collection time can guide borrowers and collectors of material for genetic studies. These perspectives are relevant in view of the cost of long-term storage and management of genetic resource collections (Sheldon 2001; Corthals and Desalle 2005; Bradley et al. 2012; Baker et al. 2014).

Different biorepositories have different tissues collecting protocols, for example the Robert J. Baker Genetic Resource Collection (Texas Tech University, Lubbock, TX) curates heart, kidney, liver, muscle, spleen, lung, colon, feces, gonads, and blood as part of standard procedures (Bradley et al. 2020). However, liver and muscle are the most loaned tissues for genetic sequencing research, thus the focus of the present manuscript centers on these two tissue types. Similarly, most studies of DNA degradation focus on liver and muscle tissues because both types are easily harvested in the field and regularly are collected for long-term museum storage across different genetic resources collections (Corthals et al. 2015). The challenge of sampling liver is that autolysis rapidly increases after death due to high enzymatic activity of this organ (Bär et al. 1988; Hanner et al. 2004). This often renders liver to be of only limited value for DNA studies when harvesting of tissue occurred long after death (Bär et al. 1988;). In contrast, muscle tissue has a much lower level of vascularization and degrades at a lower rate than other tissues (Bär et al. 1988; Hanner et al. 2004). However, a precise time frame for optimal post-mortem liver or muscle harvesting for subsequent high-quality DNA analysis is not

112 available in the current literature. This lack of information makes the decision of when to
113 harvest tissues in the field difficult, especially when the time of death of an organism is not
114 known. Also, considering that space is a limited resource in many cryogenic collections, a better
115 understanding of degradation rates would help prioritize field harvesting of tissues and inform
116 best practices when field conditions do not allow rapid cryopreservation (Dessauer et al. 1996;
117 Cheviron et al. 2011).

118 Consequently, the objectives of this study were to determine the rate at which tissue
119 samples of liver and muscle degrade post-mortem, and also to provide parameters for tissue
120 sampling for optimal long-term archival. Obtaining DNA degradation rate will provide an
121 optimal time window for best sampling of post-mortem tissues for later DNA genetic analysis.
122 We hypothesized that quality of DNA will quantifiably decrease with time post-mortem in both
123 tissues and that liver will degrade at a faster rate than muscle.

124

125 **MATERIALS AND METHODS**

126 *Focal species*.*Sigmodon hispidus*.— (hispid cotton rat) was selected as the focal species
127 given that accessibility and relatively large body size, 80-150 grams (Schmidly and Bradley
128 2016), allowed for large quantities of tissue to be obtained and archived for multiple time-point
129 comparisons. Individuals of *S. hispidus* were collected using Sherman live-traps following the
130 American Society of Mammalogists guidelines (Sikes et al. 2016) and a TTU animal care and
131 use protocol (Protocol #17023-02). Traps were set before dusk at the Texas Tech University
132 Department of Natural Resources Management Range Barn, in Lubbock Texas (Erskine Road
133 and Texas Tech Parkway) and collected after dawn. To minimize variation among individuals,

only adult males were targeted. Five male individuals were collected and liver and muscle tissues were harvested as described below.

Sample preparation and laboratory procedures.—Each collected specimen was assigned a unique identifier number (TK 195782, TK 195786, TK 195788, TK 195792, and TK 195793) referencing it to the Robert J. Baker Genetic Resources Collection of the Natural Sciences Research Laboratory, Museum of Texas Tech University. The skull of each individual was prepared and catalogued as a voucher specimen. Immediately after removal of liver and muscle, organs were sectioned into 15 pieces of approximately the same size with a mean weight of 0.124 g for liver (s.e.= 0.005) and 0.113 g for muscle (s.e. = 0.004). Pieces of tissue were individually placed in pre-labeled cryogenic vials and capped. Between the time of dissection to immersion in liquid nitrogen, tissues were stored at room temperature (25° C). Vials were then placed in a dewar flask containing liquid nitrogen at different time intervals after death (2, 4, 8, 16, 32 minutes post-mortem; 1, 2, 4, 8, 16 hours post-mortem; and 1, 2, 4, 8, 16 days post-mortem). So as to linearize time intervals for statistical analyses, they were assigned a rank from first (1) to the fifteenth (15) time period. These were used as levels of the independent variable in linear models described below.

Samples were sent to RTLGenomics (Lubbock, Texas) where DNA was extracted using a Kingfisher 96-well extraction robot with the Kingfisher Cell and Tissue DNA kit (ThermoFisher Scientific, Waltham, Massachusetts). After extraction, samples were diluted to a target concentration of 1ng/µl and transferred to an Advanced Analytical Technologies fragment analyzer (Agilent, Santa Clara, California) to quantify DNA molecular mass distributions using the DNF-464-0500 High Sensitivity Large Fragment 50kb kit. This method quantifies DNA

sizes and relative abundances from 0 to 50,000 base pairs (Advanced Analytical Technologies 2016) expressed as relative fluorescent units (RFUs).

Data analysis.—Distribution summary statistics of median and variance, as well as the cumulative molecular mass profile were used to characterize DNA size distributions for each sample. The molecular mass profile is presented in Soniat et al. (2021); it is defined as the slope of the regression line describing the relationship between DNA quantity and molecular mass (i.e., size in bp).

Three statistical models were used to determine the relationship between DNA quality and time: a fixed effect model, a mixed-effects random intercept model, and a mixed-effects random intercept plus random slope model following the protocol of Zuur et al. (2009). Mixed effects linear models (i.e., random intercept or random intercept plus random slope) take into consideration differences in DNA fragment sizes attributable to individual differences (i.e., the five different cotton rats) as well as measurements taken through time on the same individuals (repeated measurements, 15 time measurements each for 2 tissue types). The random intercept model tested for differences among individuals in terms of the intercept, in other words accounting for differences among individuals in overall size of DNA fragments. A random intercept plus random slope model tested for not only differences among individuals in terms of the intercept (overall size of DNA fragments) but also differences among individuals in how DNA fragment distributions changed across time. A likelihood ratio test (Zuur et al. 2009) was performed to compare goodness of fit of the fixed effect, random intercept, and random intercept plus random slope models. When no significant differences were detected between models, the simpler model was chosen as the one that best fit the data. When one model showed significant difference over another in its goodness-to-fit, that model was selected. All mixed models were

conducted using the NLME (Non-Linear Mixed Effects Models) function (Pinheiro et al. 2019) in R (R Core Team 2019).

RESULTS

Liver and muscle samples showed a progressive shortening of DNA fragments with increased time post-mortem prior to preservation (Fig. 1). As predicted, liver tissue degraded at a more rapid rate than muscle. A strong individual component to rate of DNA fragmentation in both tissue types is illustrated by substantive differences among individuals regarding intercepts. Likelihood ratio tests contrasting fixed effect models and random intercept models were significant for median (P <0.001), variance (P <0.01), and molecular mass profile (P <0.01) for liver, as well as for molecular mass profile for muscle (P <0.01) indicating that accounting for differences among individuals significantly improved fit of models (Table 1). For muscle samples, differences in model fit between the fixed effect model and random intercept model were not significant (Table 1) for median (P = 0.054) or variance (P = 0.056). Nonetheless, mixed models were used throughout to account for non-independence of times measured within individuals so as to properly partition degrees of freedom by considering the repeated measures.

Likelihood ratio tests comparing random intercept and random intercept plus random slope models revealed no significant differences between the two models for median (P = 0.183), variance (P = 0.863), or molecular mass profile (P = 0.253) of DNA fragments sizes for liver. For muscle tissue there was no significant difference in fit of median DNA fragment sizes (P = 0.062) among models (Table 1). However, for muscle tissue the random intercept plus random slope models fit the data better for variance (P = 0.012) and molecular mass profile (P <0.001) of DNA fragments sizes. These results indicate that not only were there differences among

individuals in overall amount of DNA, but the manner that fragment size changed with time was also different among individuals.

Median DNA fragment sizes in liver tissue were relatively smaller than in muscle samples (Figs. 1A, 1B). Median fragments for liver and muscle decreased in size through time for most individuals, however, this decrease was more consistent in liver than in muscle (Figs. 1A, 1B). Large DNA fragment sizes in muscle tissue were maintained for longer time post-mortem (Figs. 1A, 1B) than in liver. Linear mixed models indicated that the slope of the relationship between time post mortem and median DNA fragment size was greater in magnitude for liver (-1460.57) than for muscle (-1225.68) (Table 2). Nonetheless, 95% confidence intervals of the slope for liver (-1150.35 to -1770.79) and muscle (-569.591 to -1881.77) overlapped, likely due to the greater variability of slopes for muscle, and suggest no significant difference between liver and muscle regarding the rate in change in fragment size with time post-mortem.

Variances of liver DNA fragment sizes were more variable not only across time but also among individuals when compared to muscle tissue (Figs. 1C and 1D). Similarly, the slope of the relationship between variance in the fragment length and time (b_1 = -9060688) of liver tissue were more pronounced than in muscle samples (b_1 =-2811150). Linear mixed models indicated that the variance of fragment lengths decreased significantly with time (represented by a negative slope) in liver (P <0.001). In contrast, the decrease in variance of fragment sizes for muscle (a negative slope) through time was non-significant (P = 0.281) (Table 2). Calculations and comparison of 95% confidence intervals of the slope of the relationship between variance and time post-mortem for liver (-6,508,864 to -11,612,512) and muscle (2,256,169.3 to -7,878,469) indicated that they overlapped, suggesting no significant difference in the rate of change in the variance of fragment length between liver and muscle tissues.

Liver and muscle tissues showed a change in molecular mass profiles with a decrease in the proportion of large DNA fragments at later times. The slopes of the relationship describing the molecular mass profiles decreased as time post-mortem increased (Figs. 2 and 3). This decrease in molecular mass profile with time was more prominent in liver than in muscle (Fig. 2 and 3). Linear mixed models indicated a significant interaction between time and molecular mass profile and corroborated that the slope decreased with increased time post-mortem prior to preservation (Table 3).

DISCUSSION

Collections of preserved tissues are the primary source of genetic material for comparative studies in several biological fields (Sheldon 2001; Corthals and Desalle 2005; McLean et al. 2016). Genetic resource collections and their ability to link genetic material with voucher specimens are a valuable source for basic and comparative genetic research (Corthals and Desalle 2005; Bradley et al. 2014; McLean et al. 2016; Thompson et al. 2021) especially now that in-situ biodiversity (i.e., natural habitat) is declining, specimen collection is decreasing (Malaney and Cook 1998, Rohwer et al. 2022) and the demands for ex-situ genetic repositories are increasing (Sheldon 2001; Lopez et al. 2020). Genetic resource collections advocate for preservation of biological samples in liquid nitrogen to secure long-term integrity of collections (Sheldon 2001; Soniat et al. 2021). Sample procurement and preservation methods greatly affect quality of the collection and subsequently affect quality of biological material available for science (Sheldon 2001; Corthals and Desalle, 2005; Corthals et al. 2015). In the present study, temporal differences in post-mortem immersion in liquid nitrogen (from 2 minutes to 16 days) were evaluated for liver and muscle samples to create baselines of degradation to establish best practices in terms of cryo-preparation of samples.

Median fragment sizes of DNA size distributions quantified from 2 minutes to 16 days post-mortem decreased continually with time prior to preservation in liquid nitrogen for both liver and muscle tissue. This decrease in DNA fragment size was more pronounced in liver than in muscle samples. For example, in liver, a reduction of 50% in median fragment sizes occurs between 16 and 32 minutes post-mortem, but in muscle the same reduction of 50% of median fragment sizes occurs later, between 1 and 2 days after death (Fig. 4). As expected, shortening of DNA fragment size was dependent on time post-mortem prior to cryopreservation and this degradation occurs more rapidly in liver than in muscle samples, likely due to high post-mortem autolysis (non-microbial autodigestion) activity of this organ in comparison to muscle (Bär et al. 1988; Corthals et al. 2015). Even though nuclear mass in muscle cells is relatively lower than other tissues (Bär et al. 1988) it retains longer fragments of DNA for longer periods of time when compared to liver. Muscle continued to contain DNA fragments of relatively large size across all time periods. Variability of median DNA fragment sizes (i.e., differences in regression lines among individuals) in both liver and muscle samples also appear to be different, and greater in liver than in muscle (Figs. 1C and 1D). This suggests that muscle degradation is more uniform than liver, and that muscle is more stable than liver. This result is consistent with Camacho et al. (2013) that described muscle tissue as being less prone to DNA degradation than other tissues.

When characterizing DNA degradation as the proportion of DNA fragments at a particular base pair threshold, the molecular mass profile shown in Figs. 2 and 3 also showed a continual decrease in the amount of DNA across time. The decrease in liver and muscle molecular mass is represented by a negative slope across all 15-time measurements (Figs. 2 and 3). These slopes become progressively less steep as time intervals increased from 2 minutes toward 16-day post-mortem. Liver degradation was more pronounced than muscle as shown by

the more rapid change in the incline of slopes in Fig. 5. For example, muscle samples retained 80% of the longest fragments (≥ 10,000 bp) until 1 day post-mortem, but liver retained the same percentage only until 8 minutes post-mortem (Fig. 5).

Ten-thousand base pair and larger segments of DNA were present across most time intervals in both liver (until 8 to 16 hours post-mortem) and muscle (including 16 days post-mortem). Similar results were found by Camacho et al. (2013) where large DNA molecules of 10,000 base pairs in length were present until 7 days post-mortem in *Rattus rattus* muscle tissue. Tissue samples used in the Camacho et al. (2013) study were not cryopreserved and were extracted with different protocols, and this could explain why we found 10,000 base pairs fragments of DNA in muscle until 16 days post-mortem. In another study, Graham et al. (2015) found that DNA degradation of dorsal muscle in white fish (*Coregonus clupeaformis*) after 4 days post-mortem was so great that DNA was completely unquantifiable, and after 2 days DNA fragments were shorter than 2,000 base pairs. This discrepancy in degradation rate between the present study and Graham et al. (2015) could be attributable to the fact that fish skeletal muscle indeed degrades at a faster rate than mammalian muscle (Listrat et al. 2016). Another study, conducted by Bär et al. (1988) found that complete degradation of liver DNA occurred between 24- and 36-hours post-mortem in a diseased human body. The current study shows that intermediate quality liver DNA (DNA fragments shorter than 10,000 base pairs) was still present in samples taken between 1- and 16-days post-mortem. Discrepancies in results with Bär et al. (1988) could be attributed to their use of different laboratory techniques such as storing samples at -20°C at various durations prior to DNA extraction. According to Soniat et al. (2021), degradation of DNA still occurs in cold storage even at -80°C.

The purpose of this study was to determine a critical time frame in which to collect high quality tissue samples for long term storage. Various authors have defined thresholds for quality in different ways. Low quality DNA has been considered those less than 250 base pairs (Lindahl 1993), or less than 3,500 base pairs according to other authors (Bär et al. 1988). High-quality DNA has been defined as fragments above 10,000 base pairs (Graham et al. 2015), 15,000 base pairs in length (Bär et al. 1988), or 20,000 base pairs (Blethrow et al. 2018). These seemingly arbitrary thresholds can be viewed in light of what molecular approaches can be reliably employed with different qualities of DNA. For example, the length of mitochondrial Cytochrome *b* and Cytochrome Oxidase I gene sequences, two commonly used genetic markers in mammalian genetic research are 1,140 and 1,541 base pairs in length (Zehner et al. 1998; Tobe et al. 2010), and only fragments of DNA large enough to include a gene's entire locus would result in successful amplification, that is without the need to employ internal primers to amplify either locus in multiple reactions. For the purpose of discussion here poor-quality DNA is considered to be fragments smaller than 3,500 base pair in length, and high-quality DNA being longer than 10,000 base pairs. The quantity of DNA fragments larger than 10,000 base pairs declines after 8 hours post-mortem in liver but are still present in muscle at 16 days post-mortem (Fig. 4). Although these observations can be used to guide collection, it should be noted that tissues were stored in-doors at ambient temperature prior to freezing in this study. Hotter or colder field conditions would certainly shift timeframes of expected DNA degradation. There are a variety of emerging library preparation and DNA sequencing workflows that can make use of relatively degraded DNA (e.g., methods that involve DNA fragmentation during library preparation such as probe-based capture enrichment; McCormack et al. 2012), whereas others benefit from high quality DNA (e.g., long-read sequencing; Bai et al. 2021), restriction enzyme

site associated reduced representation genome fingerprinting; Peterson et al. 2012). For the latter, specific time-points for each tissue beyond which they are no longer useful is difficult to definitively state because of the different technical aspects of these methods. For example, genome assembly is aided by long-reads and large library insert sizes, but a range of relative high molecular mass fragments would still improve assembly beyond expectations for highly degraded DNA. For restriction-site associated methods, degraded DNA will lead to low recovery of loci compared to high quality DNA, but in some instances, such as those involving rare material, the recovery of a subset of loci for such specimens is preferred to no data (Lah et al. 2016). However, given that higher quality DNA has numerous applications (Graham et al. 2015; McLean et al. 2016), archiving high quality DNA should be the goal of genetic resource collections, and it is hoped the guidelines provided in this study will be of practical utility to field biologists and curators.

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480 **FIGURES**

481 **Figure 1.** Median and variance of DNA fragment sizes for liver (A and C) and muscle (B and D)
482 across time post-mortem prior to preservation in liquid nitrogen. Individual 1 is represented by
483 circles, individual 2 represented by squares, individual 3 by triangles, individual 4 by stars, and
484 individual 5 represented by diamonds.

485 **Figure 2.** DNA molecular mass profile for liver samples represented by the proportion of DNA
486 density (RFU) across different base pair size thresholds. Y axis values indicate proportion.
487 Individual 1 is represented by circles, individual 2 represented by squares, individual 3 by
488 triangles, individual 4 by stars, and individual 5 by diamonds.

489 **Figure 3.** DNA molecular mass profile for muscle samples represented by the proportion of
490 DNA density (RFU) across different base pair size thresholds. Y axis values indicate proportion.
491 Individual 1 is represented by circles, individual 2 represented by squares, individual 3 by
492 triangles, individual 4 by stars, and individual 5 by diamonds.

493 **Figure 4.** Median DNA fragment size for all five individuals. Liver samples represented by
494 bottom regression line and solid dots. Muscle samples represented by upper regression line and
495 clear dots. Dark gray shaded area denotes the upper half of DNA fragments in the samples and
496 its intersection with liver and muscle regression lines indicates the critical time when 50% of
497 DNA fragments are lost to degradation. Horizontal black line indicates 10,000 base pair
498 fragments, one criterion of high quality DNA.

499 **Figure 5.** Mean molecular mass profile of DNA fragments sizes for all 5 individuals. Y axis
500 values indicate proportion. Liver samples represented by bottom regression line, solid dots.
501 Muscle samples represented by top regression line, unshaded dots. Horizontal line denoted 80%

of total DNA (arbitrary proportion to illustrate large quantities of DNA mass) in samples and its intersection with liver and muscle slopes across time.

TABLES

Table 1. —Results from Likelihood Ratio Tests examining differences among fixed effect, random intercept, and random intercept plus random slope models in their goodness of fit to DNA fragment size distribution characteristics (median, variance and molecular mass profile) for liver and muscle tissue. df = degrees of freedom, AIC = Akaike Information Criterion, and p-value = probability.

Dependent variable	Model	df	AIC	Likelihood ratio	p-value
Liver median	Fixed	3	1516.70		
Liver median	Intercept	4	1503.28	15.426	<0.001
Liver median	Intercept+Slope	6	1503.89	3.386	0.183
Liver variance	Fixed	3	2838.56		
Liver variance	Intercept	4	2820.38	20.182	<0.001
Liver variance	Intercept+Slope	6	2824.08	0.296	0.863
Muscle median	Fixed	3	1523.34		
Muscle median	Intercept	4	1521.63	3.711	0.054
Muscle median	Intercept+Slope	6	1520.05	5.578	0.062
Muscle variance	Fixed	3	2825.63		
Muscle variance	Intercept	4	2823.98	3.650	0.056
Muscle variance	Intercept+Slope	6	2819.10	8.886	0.012
Liver mol. mass	Fixed	5	-104.37		
Liver mol. mass	Intercept	6	-177.31	74.938	<0.001
Liver mol. mass	Intercept+Slope	8	-176.06	2.751	0.253
Muscle mol. mass	Fixed	5	-656.20		
Muscle mol. mass	Intercept	6	-710.97	56.595	<0.001
Muscle mol. mass	Intercept+Slope	8	-782.18	75.214	<0.001

Table 2. —Results from Linear Mixed Models examining the relationship between DNA fragments size distributional characteristics (median or variance) and time post-mortem based on tissue type. t-value = Student test value**,** df = degrees of freedom, and p-value = probability value.

Dependent variable	Distributional moment	Slope	t-value	df	p-value
Liver	Median	-1460.57	-9.23	69	<0.001
	Variance	-9060688	-6.96	69	<0.001
Muscle	Median	-1225.68	-3.66	69	<0.001
	Variance	-2811150	-1.09	69	0.281

Table 3. —Results from Linear Mixed Models examining the relationship between DNA fragments size distributional characteristics based on molecular mass profile and time post-mortem based on tissue type. t-value = Student test value**,** df = degrees of freedom, and p-value = probability value.

Dependent Variable		Slope	t-value	df	p-value
Liver	Time	-0.047	-11.90	817	<0.001
	Mol. mass profile	-0.112	-22.86	817	<0.001
	Time x mol. mass	0.003	6.66	817	<0.001
Muscle	Time	-0.027	-4.41	817	<0.001
	Mol. mass profile	-0.111	-33.24	817	<0.001
	Time x mol. mass	0.002	4.38	817	<0.001