A Multivariate Approach to Postmortem Interval Estimation Using DNA Degradation Patterns in Necrophagous Insects

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1. Introduction

Accurate estimation of the postmortem interval (PMI) remains one of the most persistent challenges in medicolegal death investigation, particularly in cases involving advanced decomposition where traditional early postmortem indicators have lost interpretive value. As soft tissue degrades and internal organs are no longer reliable, investigators increasingly rely on forensic entomology, using insect colonization patterns and developmental timelines to infer time since death.

In practice, however, entomological PMI estimates are influenced by numerous biological and environmental factors, including species composition, developmental stage, temperature regime, microclimate, and carcass context. These variables are rarely controlled outside of experimental settings. As a result, PMI estimation operates within a complex and variable system, raising important questions about how to model, quantify, and communicate the reliability of insect-based estimates in forensic casework.

Parallel advances in forensic genetics have highlighted the potential value of molecular degradation metrics as indirect indicators of time since death and sample integrity. Studies of DNA persistence and short tandem repeat (STR) amplification success in skeletal remains, degraded tissues, and environmentally exposed samples demonstrate consistent patterns of declining DNA quantity and profile completeness over time and under adverse conditions. In principle, necrophagous insects feeding on decomposing remains may provide both classical entomological information and molecular data in the form of DNA degradation patterns. The central question is whether these molecular measures can meaningfully supplement traditional indicators and improve PMI estimation in a statistically defensible way.

The present study adopts a forensic statistics focused approach to this problem using a simulated but empirically grounded dataset that reflects realistic ranges of DNA degradation and PMI estimation error in necrophagous insects. The dataset incorporates multiple insect species, developmental stages, and environmental conditions, along with molecular measures such as DNA concentration and STR profile completeness. Because the data are simulated, the goal is not to validate an operational method, but to evaluate the statistical behavior, interpretability, and potential added value of molecular degradation metrics within a multivariate modeling framework.

This study is guided by three research questions:

(1) How do DNA degradation patterns differ across insect species and developmental stages?
(2) How do environmental conditions influence DNA concentration, STR profile completeness, and PMI estimation error?
(3) Do molecular degradation metrics improve PMI estimation beyond traditional biological and environmental predictors when analyzed using multivariate models?

These questions are framed in terms of statistical feasibility and model behavior rather than empirical validation. By foregrounding the structure and limitations of the simulated dataset, this paper positions necrophagous insect DNA degradation as a case study for evaluating the role of multivariate modeling of complex forensic systems.

2.1 Postmortem Interval Estimation and Forensic Entomology

Estimating the postmortem interval (PMI) is a central objective in medicolegal death investigation, yet it becomes increasingly difficult as decomposition progresses (Amendt et al., 2007; Byrd & Castner, 2019). Early postmortem indicators such as algor, rigor, and livor mortis are only informative within limited time frames and lose reliability as tissues degrade (Amendt et al., 2007; Haskell & Williams, 2017). In later stages, investigators must rely on alternative indicators that remain informative over longer intervals, most notably forensic entomology (Byrd & Castner, 2019).

Forensic entomology leverages the predictable relationships between colonization, species-specific life histories, and developmental timing. Necrophagous insects such as blowflies and beetles colonize remains in relatively consistent patterns, and their development is strongly influenced by environmental conditions (Amendt et al., 2007; Campobasso et al., 2001). By identifying species, determining developmental stage (e.g., larva, pupa, adult), and accounting for temperature, investigators can estimate PMI using established developmental timelines (Byrd & Castner, 2019).

Despite its utility, entomology-based PMI estimation operates within a highly variable system. Species composition varies by geography, season, habitat, and colonization patterns may differ across otherwise similar scenes (Byrd & Castner, 2019). Environmental factors such as temperature, sunlight exposure, carcass size, and access by scavengers can significantly alter both insect activity and decomposition rates (Campobasso et al., 2001; Owings et al., 2024), Field studies consistently demonstrate that small environmental differences can produce substantial variation in decomposition trajectories and insect succession, underscoring the inherent uncertainty in PMI estimation (Campobasso et al., 2001).

Recent efforts have focused on developing more structured and statistically informed PMI models that account for this variability (Owings et al., 2024). These include temperature-adjusted developmental models, succession-based frameworks, and approaches that incorporate multiple predictors (Haskell & Williams, 2017). However, even these models can lose accuracy when applied outside controlled conditions, highlighting the need for additional data sources and more robust analytical frameworks to improve interpretability and reliability in forensic casework.

2.2 DNA Degradation and STR Profiling in Forensic Contexts

DNA degradation is a central concern in forensic genetics because it directly affects the ability to obtain interpretable short tandem repeat (STR) profiles from compromised biological

samples. As tissues are exposed to environmental stressors such as heat, moisture, microbial activity, and ultraviolet radiation, DNA undergoes fragmentation and chemical modification, reducing both the quantity and quality of amplifiable material. Studies of skeletal remains and degraded tissues consistently demonstrate that increased degradation is associated with reduced polymerase chain reaction (PCR) success, locus dropout, and incomplete STR profiles (DiZinno et al., 2002; Linacre & Tobe, 2013). These patterns highlight the importance of considering both DNA concentration and degradation state when interpreting genetic evidence in forensic contexts.

STR profiling remains the cornerstone of human identification in forensic laboratories, and its performance under degraded conditions has been extensively characterized. As DNA breaks down, longer STR amplicons are preferentially lost, producing characteristic patterns of allele dropout and partial profiles. This degradation effect often results in “ski-slope” electropherogram patterns, where smaller loci are successfully amplified while large loci fail (DiZinno et al., 2002). In practice, analysts evaluate both quantitative measures, such as DNA concentration (e.g., nanograms per microliter), and qualitative indicators such as the proportion of loci successfully amplified. STR profile completeness, expressed as the percentage of loci yielding interpretable results, therefore serves as a practical indicator of overall DNA integrity that integrates multiple aspects of degradation.

Environmental conditions play a critical role in shaping DNA degradation trajectories and, by extension, STR profiling success. Elevated temperatures, prolonged exposure to sunlight, and fluctuating moisture conditions accelerate DNA breakdown, while cooler, shaded, or protected environments tend to preserve DNA for longer periods (Campobasso et al., 2001; Linacre & Tobe, 2013). Importantly, these same environmental factors also influence decomposition processes and insect activity. As a result, DNA degradation patterns and entomological indicators are not independent but are instead responding to overlapping environmental drivers. This relationship suggests that molecular degradation metrics may provide complementary information to traditional entomological measures in PMI estimation.

DNA-based methods have also been incorporated into forensic entomology, providing a foundation for integrating molecular approaches into insect-mediated PMI research. Insects collected from decomposing remains have been used for species identification, detection of human DNA within gut contents, and association of biological material with specific individuals or scenes (Wells & Stevens, 2008). These applications demonstrate that necrophagous insects can retain recoverable DNA and that molecular techniques are already part of forensic entomological practice. However, existing research has largely focused on qualitative identification and presence, or absence, questions rather than systematic quantification of DNA degradation within insect samples.

This gap reflects a key limitation in current literature. While both entomological and molecular approaches provide valuable information independently, there has been limited effort

to model DNA degradation metrics as continuous variables within a structure analytical framework. Measures such as DNA concentration and STR profile completeness have the potential to quantify molecule degradation in a way that can be directly incorporated into statistical models. The present study builds on this premise by treating these measures as candidate variables that can be evaluated alongside species, developmental stage, and environmental conditions within a multivariate framework.

3. Environmental and Biological Variability in PMI Estimation

This section establishes the theoretical basis for the multivariate modeling approach by demonstrating how biological and environmental variables interact to influence both entomological and molecular indicators.

Postmortem interval estimation based on entomological and molecular indicators occurs within a system shaped by interacting biological and environmental factors. Variables such as insect species, developmental stage, temperature, and exposure conditions influence both decomposition processes and the reliability of forensic measurements. These factors do not operate in isolation; rather, they interact to produce variability in both insect activity and molecular degradation, complicating efforts to generate consistent and generalized PMI estimates.

Insect species play a central role in this variability due to differences in colonization behavior, ecological preferences, and developmental rates. Blowfly species, such as *Lucilia sericata* and *Calliphora vicina*, are typically among the earliest colonizers of decomposing remains, while beetle species, such as *Dermestes maculatus* and *Necrobia rufipes*, are more commonly associated with later stages of decomposition (Byrd & Castner, 2019). These species differ not only in arrival times, but also in feeding behavior, habitat preference, and physiological response to environmental conditions. As a result, the same postmortem interval may produce different entomological and molecular patterns depending on which taxa are present.

Developmental stage further contributes to variability in both entomological interpretation and molecular integrity. Larval stages are characterized by active feeding and rapid growth, often occurring in environments with high microbial activity and tissue breakdown. In contrast, pupal and adult stages may be associated with different environmental exposures and physiological states that influence both development rates and DNA preservation. These differences introduce additional complexity when attempting to interpret PMI using insect evidence, as both biological state and environmental context must be considered simultaneously. (Amendt et al., 2007; Haskell & Williams, 2017).

Environmental conditions, particularly temperature and exposure, exert a strong influence on both decomposition and insect-mediated processes. Temperature is one of the primary drivers of insect development, with higher temperatures generally accelerating growth rates and shortening developmental timelines, while cooler conditions slow these processes (Campobasso

et al., 2001). Similarly, exposure conditions such as direct sunlight versus shaded environments can alter microclimate conditions, affecting both insect activity and tissue decomposition. Sun-exposed environments often produce higher temperatures and more rapid desiccation, whereas shaded environments may retain moisture and support prolonged biological activity (Owings et al., 2024).

The same environmental factors influence DNA degradation, linking molecular and entomological indicators through shared external drivers (Linacre & Tobe, 2013). Because both entomological indicators and molecular degradation metrics respond to the same environmental driver, their effects are inherently linked. This overlap suggests that variation in PMI estimates cannot be attributed to a single factor but instead reflects the combined influence of biological and environmental conditions acting simultaneously.

The combined influence of species, developmental stage, and environment creates a highly variable system in which PMI estimation must be interpreted cautiously. Even when similar conditions are present, small differences in microclimate or insect access can produce divergent decomposition trajectories and associated forensic measurements (Watson & Carlton, 2003). This variability highlights limitation of approaches that rely on single indicators or simplified models, as they may fail to capture the full complexity of the system.

Overall, the pattern suggests these findings demonstrate that PMI estimation in forensic contexts is inherently multivariate, shaped by interacting biological and environmental factors that influence both entomological and molecular evidence. This complexity provides a strong rationale for the use of statistical approaches capable of evaluating multiple variables simultaneously. Rather than attempting to isolate individual predictors, multivariate frameworks allow for the assessment of combined effects, improving interpretability and providing a more realistic representation of forensic conditions.

4. Limitations of Current PMI Methods and the Need for Multivariate Modeling

Despite advances in forensic entomology and molecular analysis, current approaches to postmortem interval (PMI) estimation remain limited by their reliance on simplified or single-variable interpretations. Traditional methods often emphasize individual indicators, such as insect developmental timelines or environmental temperature, while treating other contributing factors as secondary or uncontrolled sources of variability. Although these approaches can provide useful approximations, they may fail to capture the complexity of real-world decomposition processes, where multiple biological and environmental variables interact simultaneously.

In forensic entomology, developmental models are frequently constructed using controlled laboratory conditions in which temperature, species, and environmental exposure are carefully regulated. While these models provide valuable baseline data, their applicability in casework is constrained by the inherent variability of outdoor and uncontrolled environments.

Factors such as microclimate differences, carcass accessibility, and ecological variation can significantly alter both insect colonization patterns and developmental rates, leading to discrepancies between predicted and observed PMI estimates (Campobasso et al., 2001; Owings et al., 2024). As a result, estimates derived from single developmental timelines may not fully reflect the complexity of decomposition in applied forensic contexts.

Similar limitations are observed in molecular approaches to forensic analysis. While DNA degradation patterns provide useful information about sample integrity and environmental exposure, they are often interpreted in isolation from other biological and ecological factors. This separation can limit their utility in PMI estimation, as molecular degradation is influenced by many of the same environmental variables that affect insect activity and decomposition processes. Without integrating these overlapping influences, molecular indicators may provide only partial insight into the timing and progression of decomposition.

More broadly, forensic interpretation has been critiqued for relying on implicit or simplified predictive reasoning rather than explicitly structured analytical frameworks. As noted in prior research, the use of single indicators or informal inference can obscure underlying uncertainty and lead to overconfidence in forensic conclusions (Thompson, 2015). This limitation is particularly relevant in PMI estimation, where multiple interacting variables contribute to observed outcomes and where variability is an inherent feature of the system rather than an anomaly.

These challenges highlight the need for analytical approaches that explicitly account for the multivariate nature of forensic data. Biological and ecological systems, including decomposition and insect colonization, are inherently multivariate, requiring methods capable of evaluating multiple variables simultaneously and identifying meaningful patterns within complex datasets (Levine, 1993). Multivariate statistical techniques provide a framework for examining the combined effects of species, developmental stage, environmental conditions, and molecular degradation metrics, rather than treating each factor independently.

The application of methods such as multivariate analysis of variance and hierarchical regression allows for the simultaneous assessment of multiple predictors and their contributions to PMI estimation. These approaches are particularly well suited to forensic contexts, where variables are interdependent and where the goal is not only to describe individual effects but to understand how combinations of factors influence outcomes. By incorporating both biological and molecular variables into a unified analytical framework, multivariate modeling offers the potential to improve interpretability and provide a more realistic representation of forensic conditions (Frey, 2015).

Taken together, the limitations of current PMI estimation methods and the complexity of decomposition processes provide a strong rationale for the use of multivariate statistical approaches. Rather than attempting to reduce variability through simplification, these approaches acknowledge and model the inherent complexity of forensic systems. This perspective forms the

foundation for the present study, which evaluates whether molecular degradation metrics can meaningfully contribute to PMI estimation when analyzed within a multivariate framework.

5. Application to Simulated Dataset and Analytical Framework

Building on the limitations identified in current PMI estimation approaches, the present study applies a multivariate framework to a simulated but empirically grounded dataset designed to reflect the complexity of forensic entomology and molecular degradation processes. The use of simulation allows for controlled exploration of variable interactions within a system that is inherently difficult to standardize in real-world medicolegal contexts. Rather than attempting to eliminate variability, this approach incorporates it directly into the analytical design, allowing for systematic evaluation of how biological, environmental, and molecular factors contribute to PMI estimation outcomes.

The simulated dataset was generated using parameter ranges and variance structures informed by existing forensic entomology and DNA degradation literature. Mean values for DNA concentration, STR profile completeness, and PMI estimation error were assigned based on empirically reported trends, while random variation was introduced within each experimental condition to reflect realistic biological and environmental variability. The simulation assumed normally distributed outcomes with stable variance across groups and did not explicitly parameterize higher-order interaction effects. As a result, the purpose of the simulation is not to validate empirical relationships, but to evaluate whether a multivariate analytical framework can coherently model and integrate these variables under controlled conditions. The findings should therefore be interpreted as demonstrating statistical feasibility and model behavior rather than confirming real-world predictive performance.

The dataset is structured as a fully balanced factorial design incorporating four forensically relevant necrophagous insect species, three developmental stages (larva, pupa, and adult), and two environmental conditions (shaded/cool and sun-exposed/warm). These factors were selected based on their established relevance in forensic entomology and their demonstrated influence on both decomposition processes and DNA degradation. By including both Diptera and Coleoptera taxa, as well as early- and late-stage decomposers, the dataset reflects realistic variation in species composition encountered in forensic investigations. Similarly, the inclusion of developmental stage and environmental exposure captures key sources of variability that have been consistently identified in the literature as influencing PMI estimation.

In addition to biological and environmental variables, the dataset incorporates molecular degradation metrics in the form of DNA concentration and STR profile completeness. These variables are treated as continuous measures reflecting the quantity and quality of recoverable DNA, providing a quantitative representation of molecular degradation. As discussed in prior sections, these metrics are influenced by environmental conditions and decomposition processes, making them suitable candidates for integration into a multivariate analytical framework. By

modeling these variables alongside traditional entomological indicators, the study evaluates whether molecular measures provide additional explanatory power in PMI estimation.

The analytical approach is designed to assess both the individual and combined effects of these variables. A multivariate analysis of variance (MANOVA) is used to evaluate the joint influence of species, developmental stage, and environmental condition on DNA concentration, STR profile completeness, and PMI estimation error. This approach allows for simultaneous examination of multiple dependent variables, capturing relationships that may not be evident when variables are considered independently.

To further evaluate the contribution of molecular degradation metrics, hierarchical multiple regression modeling is applied. Initial models include biological and environmental predictors, followed by the addition of molecular variables to assess whether they significantly improve model performance. This stepwise approach allows for direct comparison of model explanatory power and provides insight into the extent to which DNA degradation metrics contribute meaningfully to PMI estimation beyond traditional indicators.

Because the dataset is simulated, the emphasis of the analysis is not on empirical validation but on statistical feasibility, interpretability, and model behavior. Simulation enables the exploration of realistic parameter ranges and variance structures informed by forensic entomology and forensic DNA literature while maintaining control over the analytical environment. Using this approach, it becomes possible to evaluate model assumptions, effect sizes, and interaction structures, providing insight into how multivariate models perform in complex forensic contexts.

By integrating biological, environmental, and molecular variables within a unified analytical framework, this study addresses a key gap identified in the literature: the lack of structured approaches for incorporating multiple sources of variability into PMI estimation. The simulated dataset serves as a test case for evaluating whether such integration is both analytically feasible and practically meaningful. In doing so, it provides a foundation for future empirical research and supports the development of more robust and transparent approaches to PMI estimation in forensic practice

6. Results

The simulated dataset (N = 240) was analyzed using multivariate analysis of variance (MANOVA) and hierarchical multiple regression to evaluate the effects of species, developmental stage, and environmental condition on DNA concentration, STR profile completeness, and PMI estimation error. The dataset followed a fully balanced 4 x 3 x 2 factorial design, with ten observations per experimental condition.

6.1 Descriptive Statistics

Across all observations, the dependent variables demonstrated biologically consistent distributions. Mean DNA concentration was 106.38 ng/µL (SD = 31.94), mean STR profile completeness was 54.38% (SD = 18.26), and mean PMI estimation error was 25.11 hours (SD = 5.46).

Group-level patterns aligned with expected forensic trends. Beetle taxa (*Dermestes maculatus* and *Necrobia rufipes*) exhibited higher DNA concentration and STR completeness compared to blowfly species (*Lucilia sericata* and *Calliphora vicina*). Molecular integrity increased across developmental stages (larva < pupa < adult), and shaded or cooler environments were associated with higher DNA concentration, higher STR completeness, and lower PMI estimation error relative to sun-exposed conditions.

No extreme values or unstable variance patterns were observed across experimental groups, supporting the stability and interpretability of the simulated dataset.

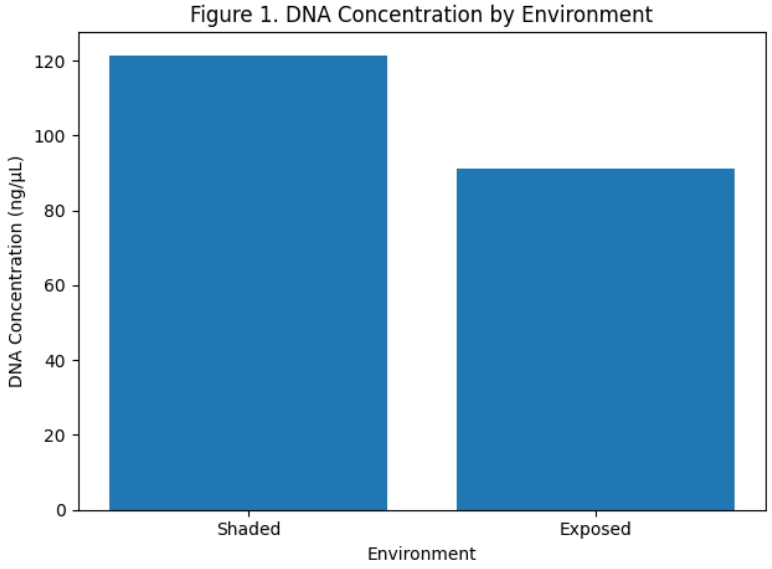

Figure 1

Mean DNA concentration (ng/µL) across shaded and sun-exposed environments. Higher concentrations in shaded conditions indicate reduced DNA degradation.

6.2 MANOVA Results

A three-way MANOVA was conducted to examine the combined effects of species, developmental stage, and environmental condition on DNA concentration, STR completeness, and PMI estimation error.

Multivariate tests using Pillai's Trace indicated that all three main effects were statistically significant (all $p < .001$), demonstrating that species, stage, and environment each contributed meaningfully to variation across the dependent variables.

Specifically:

- Species differences reflected distinct molecular degradation profiles across taxa
- Developmental stage produced systematic variation in DNA integrity
- Environmental conditions significantly influenced both molecular degradation and PMI estimation accuracy

No significant interaction effects were observed (all $p > .05$), including species × stage, species × environment, stage × environment, and the three-way interaction. These results point to the effects of the independent variables were primarily additive rather than interactive.

Assumption testing supported the validity of the analysis. Box's M was non-significant ($p = .777$), indicating equality of covariance matrices, and visual inspection of residual distributions supported multivariate normality.

6.3 Univariate Analysis and Post Hoc Comparisons

Follow-up univariate ANOVAs revealed significant main effects of species, developmental stage, and environment on both DNA concentration and STR profile completeness (all $p < .001$).

Post hoc comparisons confirmed clear and biologically meaningful patterns:

- Developmental stage followed a degradation gradient: Adult > Pupa > Larva.
- Beetle taxa demonstrated significantly higher DNA preservation than blowflies.
- Shaded environments consistently produce higher DNA concentration and STR completeness than sun-exposed conditions.

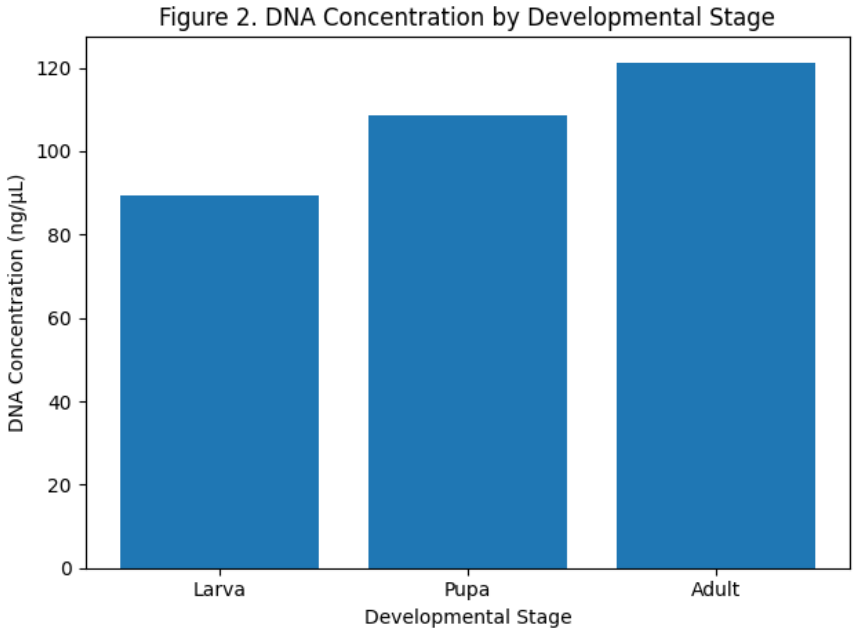

Figure 2

Mean DNA concentration (ng/μL) across developmental stages. DNA integrity increases from larval to adult stages.

PMI estimation error was most strongly influenced by environmental conditions, with sun-exposed environments producing significantly higher error ($p < .001$). Species and stage effects on PMI error were comparatively weaker, indicating that environmental exposure is the dominant factor influencing estimation accuracy.

6.4 Hierarchical Multiple Regression

Hierarchical multiple regression was conducted to evaluate whether molecular degradation metrics improved PMI estimation beyond biological and environmental predictors.

In Model 1, species, developmental stage, and environment explained 33.1% of the variance in PMI error ($R^2 = .331$, $p < .001$). Environment emerged as the strongest predictor in this model.

In Model 2, DNA concentration and STR profile completeness were added as predictors. The model demonstrated a significant improvement in explanatory power ($R^2 = .472$), with an increase of 14.1% in explained variance ($\Delta R^2 = .141$, $p < .001$).

Both molecular variables were significant negative predictors of PMI error:

- DNA concentration ($\beta = –.369$, $p < .001$)
- STR completeness ($\beta = –.318$, $p < .001$)

Model diagnostics indicated homoscedasticity and no evidence of unstable variance across predicted values.

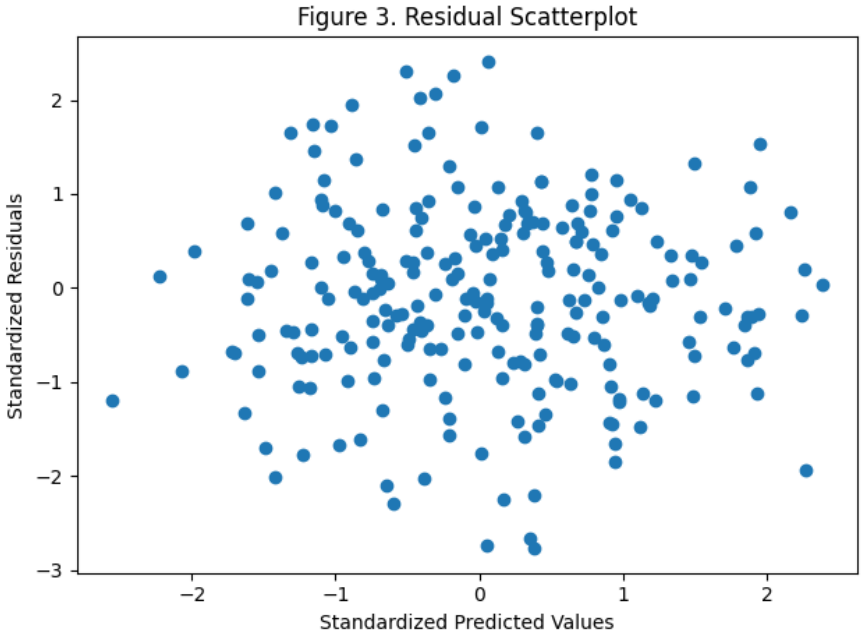

Figure 3

Scatterplot of standardized residuals versus predicted values for PMI error, demonstrating homoscedasticity and supporting regression model assumptions.

Across conditions, higher DNA integrity was associated with reduced PMI error and that molecular degradation metrics provide meaningful predictive value beyond traditional variables.

6.5 Summary of Findings

Overall, the results demonstrate that species, developmental stage, and environmental conditions significantly influence DNA degradation patterns and PMI estimation outcomes. Importantly, the inclusion of molecular degradation metrics substantially improves predictive accuracy, supporting the integration of DNA-based variables into multivariate PMI estimation models. Full statistical output, including assumption testing and model diagnostics, is provided in Appendix A.

7. Discussion

7.1 Forensic and Operational Implications

The findings of this study have several important implications for forensic practice, particularly in the interpretation of postmortem interval (PMI) estimates derived from insect evidence. Traditional entomological approaches often rely on developmental timelines and environmental approximations, which, while valuable, may not fully account for the variability observed in real-world conditions. The present results suggest that incorporating molecular degradation metrics can provide an additional layer of interpretive context, improving both the accuracy and transparency of PMI estimates.

One of the most significant implications is the potential use of DNA integrity as a supplemental indicator in forensic casework. Measures such as DNA concentration and STR profile completeness are commonly generated in forensic laboratories, suggesting potential for integration pending validation, particularly in cases involving degraded biological samples. The ability to leverage these existing outputs for PMI estimation represents a practical advantage, as it does not require the development of entirely new analytical infrastructure. Instead, molecular data that are already being collected could be integrated into entomological assessments to refine time-since-death estimates.

This approach may be particularly valuable in cases involving ambiguous or compromised entomological evidence. For example, when insect specimens are collected at transitional developmental stages or when environmental conditions have altered typical colonization patterns, molecular degradation metrics may provide additional insight into the timing and progression of decomposition. Similarly, in cases where insect evidence is limited or partially degraded, DNA-based indicators may offer a complementary line of evidence that supports or refines traditional interpretations.

The strong influence of environmental conditions observed in this study also has important implications for forensic decision-making. The finding that sun-exposed environments produce greater DNA degradation and increased PMI estimation error reinforces the need for careful consideration of scene conditions when interpreting both entomological and molecular evidence. In practice, this suggests that forensic analysts should explicitly account for environmental exposure when evaluating the reliability of PMI estimates and avoid overreliance on single indicators that do not reflect these conditions.

From a broader perspective, the integration of multivariate modeling into forensic workflows represents a shift toward more structured and transparent analytical practices. Rather than relying on implicit or experience-based reasoning, multivariate approaches allow for the explicit evaluation of multiple variables and their combined effects on forensic outcomes. This has the potential to improve not only the accuracy of PMI estimation but also the clarity with which findings are communicated in legal contexts.

In courtroom settings, the ability to present PMI estimates within a statistically supported framework may have the potential to strengthen the credibility of forensic testimony pending empirical validation. Multivariate models provide a basis for quantifying uncertainty, demonstrating how different variables contribute to an estimate, and explaining why certain conclusions are reached. This level of transparency is particularly important in adversarial legal systems, where the reliability and interpretability of forensic evidence are subject to scrutiny.

However, it is important to recognize that the application of molecular degradation metrics in forensic entomology is still an emerging area of research. While the present study demonstrates statistical feasibility, further empirical validation is required before such approaches can be fully integrated into routine casework. Future research should focus on validating these findings using real insect samples, expanding the range of environmental conditions and taxa, and developing standardized protocols for incorporating molecular data into PMI estimation models.

Overall, the integration of DNA degradation metrics into entomological PMI estimation represents a promising direction for advancing forensic science. By combining biological, environmental, and molecular information within a unified analytical framework, forensic practitioners may be able to generate more accurate, reliable, and transparent estimates of time since death, ultimately improving the quality of medicolegal investigations.

7.2 Limitations

Several limitations should be considered when interpreting the findings of this study. Most notably, the use of a simulated dataset limits the extent to which results can be generalized to real-world forensic contexts. Although the dataset was designed to reflect biologically and environmentally realistic patterns based on existing literature, it does not capture the full variability and unpredictability inherent in actual decomposition environments. As a result, the

findings should be interpreted as demonstrating statistical feasibility and theoretical potential rather than empirical validation.

A related limitation is the controlled structure of the dataset, which assumes balanced group sizes and relatively stable variance across experimental conditions. While this design is appropriate for evaluating statistical relationships, real forensic data are often unbalanced and subject to irregular sampling, missing data, and uncontrolled environmental variation. Factors such as uneven insect collection, partial specimen recovery, and scene-specific constraints may introduce additional complexity that is not fully represented in the present model.

The study is also limited by the scope of biological variables included. Although the dataset incorporates multiple species and developmental stages, it represents only a subset of forensically relevant taxa. In practice, insect communities may include a broader range of species with differing ecological roles, colonization patterns, and physiological responses to environmental conditions. Additionally, intra-species variation, such as genetic differences or local adaptations, may influence both development rates and DNA degradation patterns in ways that are not captured in a simplified model.

Environmental conditions in the present study were represented as a binary distinction between shaded/cool and sun-exposed/warm environments. While this categorization captures broad differences in exposure, it does not reflect the full spectrum of environmental variability encountered in forensic casework. Factors such as humidity, rainfall, soil composition, scavenger activity, and microhabitat differences can significantly influence both decomposition processes and DNA degradation. Future studies should incorporate more granular environmental variables to better approximate real-world conditions.

Another limitation involves the use of DNA concentration and STR profile completeness as the sole indicators of molecular degradation. While these measures are widely used and forensically relevant, they do not capture all aspects of DNA degradation, such as fragmentation patterns, chemical modifications, or sequence-specific degradation effects. Additional molecular markers, including single nucleotide polymorphisms (SNPs) or advanced degradation indices, may provide a more comprehensive representation of DNA integrity and should be explored in future research.

The absence of significant interaction effects in the present analysis should also be interpreted with caution. While the findings suggest that species, developmental stage, and environmental influences operate in an additive manner within the simulated dataset, real-world systems may exhibit more complex interactions. Environmental conditions can differentially affect species behavior and development, and these interactions may become more pronounced under extreme or fluctuating conditions. Additional research using empirical datasets is needed to evaluate whether interaction effects emerge in applied forensic contexts.

Finally, the study does not address operational constraints associated with implementing multivariate modeling in forensic practice. While the statistical framework demonstrates feasibility, practical considerations such as data availability, analyst training, laboratory resources, and standardization of methods may influence the extent to which these approaches can be adopted in routine casework. The integration of molecular degradation metrics into forensic workflows will require not only empirical validation but also the development of clear guidelines and best practices for interpretation and reporting.

Despite these limitations, the study provides a valuable foundation for future research by demonstrating that molecular degradation metrics can be incorporated into multivariate models of PMI estimation. The findings highlight key variables, analytical strategies, and areas for further investigation, supporting the continued development of more robust and integrative approaches to forensic time-since-death estimation.

The absence of statistically significant interaction effects should also be interpreted with caution. In real-world decomposition systems, interactions between species, developmental stages, and environmental conditions are expected to occur. The additive structure observed in the present analysis likely reflects the design of the simulated dataset, which was not explicitly parameterized to include interaction variance. As a result, the findings may underestimate the complexity of variable interactions present in applied forensic contexts. Future research using empirical data should evaluate whether interaction effects emerge under real environmental conditions and how they influence multivariate PMI estimation models.

8. Conclusions and Future Directions

Accurate estimation of the postmortem interval (PMI) remains a persistent challenge in forensic science, particularly in cases involving advanced decomposition where traditional physiological indicators are no longer reliable. While forensic entomology has provided a valuable framework for late-stage PMI estimation, its application is inherently influenced by biological and environmental variability that cannot be fully controlled in real-world contexts. Similarly, molecular approaches such as DNA degradation analysis offer important insights into sample integrity and environmental exposure but are often considered independently from entomological indicators.

The literature reviewed in this study demonstrates that both entomological and molecular measures are shaped by overlapping environmental drivers and are subject to substantial variability. As a result, approaches that rely on single indicators or simplified interpretations may fail to capture the complexity of decomposition processes. This limitation highlights the need for analytical frameworks that can account for multiple interacting variables and provide more transparent and interpretable PMI estimates.

In response to these challenges, the present study applied a multivariate statistical framework to a simulated dataset designed to reflect realistic variation in insect species,

developmental stage, environmental conditions, and molecular degradation. By integrating DNA concentration and STR profile completeness with traditional entomological variables, the analysis demonstrated that molecular degradation metrics contribute meaningful explanatory value to PMI estimation. The use of simulation allowed for controlled evaluation of statistical feasibility, model behavior, and interpretability within a system that is typically difficult to standardize in practice.

The findings support the broader argument that PMI estimation should be approached as a multivariate problem rather than a series of isolated indicators. Multivariate techniques such as MANOVA and hierarchical regression provide a structured means of evaluating the combined effects of biological, environmental, and molecular variables, offering improved interpretability and a more realistic representation of forensic conditions. While the study does not propose a finalized operational method, it establishes a statistical and methodological foundation for integrating molecular data into PMI estimation frameworks.

Future research should focus on validating these findings using empirical datasets derived from controlled decomposition studies and real forensic casework. Expanding the range of environmental variables beyond binary exposure conditions—such as incorporating humidity, soil composition, and seasonal variation—would provide a more comprehensive understanding of how environmental factors influence both insect activity and DNA degradation.

Additional work is also needed to broaden the taxonomic scope of analysis, including a wider range of necrophagous and predatory insect species, as well as to examine intra-species variation that may influence molecular degradation patterns. From a molecular perspective, future studies should explore alternative or complementary markers of DNA degradation, including single nucleotide polymorphisms (SNPs), fragment length analysis, and advanced degradation indices, to enhance the resolution and applicability of molecular PMI indicators.

Finally, the development of standardized analytical frameworks and practical guidelines will be essential for translating multivariate modeling approaches into forensic practice. This includes evaluating how molecular degradation metrics can be integrated into existing laboratory workflows, as well as how results can be effectively communicated in legal settings. Continued interdisciplinary collaboration between forensic entomologists, molecular biologists, and forensic statisticians will be critical in advancing these efforts.

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Appendix A

SPSS Output and Supplemental Statistical Tables

The following appendix includes selected SPSS output supporting the statistical analyses presented in Section 6. These materials are provided for transparency and reference but are not required for interpretation of the primary findings.

Table A1

Box's Test of Equality of Covariance Matrices

Box's M	143.951
F	.906
df1	138
df2	24358.020
Sig.	.777

Table A2

Levene's Test of Equality of Variances

Levene's Test of Equality of Error Variances[a]

		Levene Statistic	df1	df2
DNA_Concentration_ngul	Based on Mean	1.171	23	216
	Based on Median	.936	23	216
	Based on Median and with adjusted df	.936	23	159.929
	Based on trimmed mean	1.171	23	216
STR_Completeness_pct	Based on Mean	.735	23	216
	Based on Median	.588	23	216
	Based on Median and with adjusted df	.588	23	168.865
	Based on trimmed mean	.720	23	216
PMI_Error_hr	Based on Mean	1.403	23	216
	Based on Median	.966	23	216
	Based on Median and with adjusted df	.966	23	168.213
	Based on trimmed mean	1.360	23	216

Levene's Test of Equality of Error Variances[a]

		Sig.
DNA_Concentration_ngul	Based on Mean	.273
	Based on Median	.550
	Based on Median and with adjusted df	.551
	Based on trimmed mean	.274
STR_Completeness_pct	Based on Mean	.807
	Based on Median	.934
	Based on Median and with adjusted df	.932
	Based on trimmed mean	.823
PMI_Error_hr	Based on Mean	.111
	Based on Median	.511
	Based on Median and with adjusted df	.512
	Based on trimmed mean	.133

Tests the null hypothesis that the error variance of the dependent variable is equal across groups.

a. Design: Intercept + Species + Stage + Environment + Species * Stage + Species * Environment + Stage * Environment + Species * Stage * Environment

Table A3

Multivariate Tests

Multivariate Tests

	Value	F	Hypothesis df	Error df	Sig.	Partial Eta Squared
Pillai's trace	.716	22.559	9.000	648.000	<.001	.239
Wilks' lambda	.297	37.396	9.000	520.970	<.001	.333
Hotelling's trace	2.319	54.806	9.000	638.000	<.001	.436
Roy's largest root	2.300	165.623[a]	3.000	216.000	<.001	.697

Table A4

Results of Between-Subject Effects

Tests of Between-Subjects Effects

Source	Dependent Variable	F	Sig.	Partial Eta Squared
Corrected Model	DNA_Concentration_ngul	39.706	<.001	.809
	STR_Completeness_pct	15.157	<.001	.617
	PMI_Error_hr	5.901	<.001	.386
Intercept	DNA_Concentration_ngul	12582.366	<.001	.983
	STR_Completeness_pct	5028.453	<.001	.959
	PMI_Error_hr	7466.039	<.001	.972
Species	DNA_Concentration_ngul	134.928	<.001	.652
	STR_Completeness_pct	56.957	<.001	.442
	PMI_Error_hr	1.519	.211	.021
Stage	DNA_Concentration_ngul	148.540	<.001	.579
	STR_Completeness_pct	41.511	<.001	.278
	PMI_Error_hr	.073	.930	.001
Environment	DNA_Concentration_ngul	188.975	<.001	.467
	STR_Completeness_pct	78.936	<.001	.268
	PMI_Error_hr	116.318	<.001	.350
Species * Stage	DNA_Concentration_ngul	1.845	.092	.049
	STR_Completeness_pct	.473	.828	.013
	PMI_Error_hr	1.349	.237	.036
Species * Environment	DNA_Concentration_ngul	1.065	.365	.015
	STR_Completeness_pct	.953	.416	.013
	PMI_Error_hr	.547	.651	.008
Stage * Environment	DNA_Concentration_ngul	1.261	.285	.012
	STR_Completeness_pct	2.592	.077	.023
	PMI_Error_hr	.199	.820	.002
Species * Stage * Environment	DNA_Concentration_ngul	.934	.472	.025
	STR_Completeness_pct	.819	.557	.022
	PMI_Error_hr	.763	.600	.021