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Evaluating the fidelity of microwear replication: molding, casting, and conservation implications in Eocene–Oligocene perissodactyls

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Abstract

This research evaluates the reliability of dental microwear analysis through repeated molding and casting procedures on perissodactyl fossils. Statistically significant differences in scratch measurements were found across *Brontotheriidae* (UWBM #75645) casts ($F(9, 203.79) = 2.01$, $p = 0.039$), particularly between Generation 2 and Generation 8 (mean difference = 0.0334, 95% CI [0.0014, 0.0654], $\text{p\_adj} = 0.0330$). Non-parametric analysis further revealed significant distributional differences between Generation 1 and Generations 2 ($p = 0.0018$), 5 ($p = 0.0045$), 9 ($p = 0.0129$), and 10 ($p = 0.0129$). In contrast, no significant differences were found among *Subhyracodon* (UWBM #35783) casts for either scratch ($F(9, 88.40) = 1.17$, $p = 0.327$) or pit measurements ($F(11, 54.10) = 1.740$, $p = 0.089$). These findings suggest that the molding and casting process can introduce both mean-level changes and distributional shifts in microwear features after multiple replication cycles, potentially affecting dietary interpretations. The observed differences between Generation 2 and 8 indicate that surface fidelity remains relatively stable for several replications before detectable changes emerge. Given the importance of microwear in reconstructing short-term feeding behavior and ecological change, even subtle distortions may compromise analytical accuracy. This research emphasizes the need for standardized molding and casting protocols and recommends best practices to reduce error and improve consistency in paleodietary studies.

Keywords Dental Microwear Analysis; Paleoecology; Molding Techniques; Casting Replication; Fossil Preservation

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Chapter 1. Introduction

Understanding the diet and feeding behaviors of extinct species is crucial for reconstructing ancient ecosystems and evolutionary patterns. Among the tools that are available to paleontologists, microwear analysis (i.e., the study of microscopic patterns on tooth surfaces) has been useful in providing direct evidence for reconstructing diets and ancient habitats (DeSantis, 2016; Xia et al., 2015; Rivals & Semprebon, 2011; Semprebon et al., 2004). By examining the pits and scratches left on fossilized teeth, researchers can infer dietary preferences and ecological interactions of species.

Unlike other methods such as stable isotope analysis, dental microwear analysis provides a short-term dietary signal, capturing information from the final weeks or days before an animal's death (Grine, 1986). This unique snapshot of diet is particularly valuable for studying taxa that lived during periods of major ecological transition, such as the Eocene–Oligocene Extinction Event (Grande Coupure). During this time, dense forests gave way to more open and arid landscapes, causing significant environmental and ecological changes.

Perissodactyls (odd-toed ungulates) represent an ideal case study for examining adaptive response to these environmental transformations (Eronen et al., 2009; Janis, 1989, 1993; Zhang et al., 2020). Extinct members of this order, such as the Brontotheriidae and early rhinocerotoids like Subhyracodon, underwent substantial ecological shifts during this period. Dental microwear analysis can show how their feeding behaviors evolved in response to changing habitat conditions.

However, the accuracy and reliability of microwear data depend on the quality of the tooth surface being analyzed, and often, on the quality of its replica. Because microwear features are microscopic and can be difficult to photograph due to lighting and curvature of the enamel surface, researchers frequently rely on mold-making and casting techniques to produce detailed replicas that better capture these features (Galbany et al., 2006). This approach has become standard practice in both research and museum contexts. For example, visiting researchers can use this method to collect data from specimens housed in other institutions, allowing them to take high-resolution replicas without requiring loans or risking damage during transport.

Despite its widespread use in paleontology, concerns remain about how repeated molding might affect the fidelity of microwear features (Galbany et al., 2006). These procedures (e.g., using different materials, tools, or cleaning techniques), especially when performed multiple times on the same specimen, could potentially introduce morphological damage (O'Hara & Le Cabec, 2019). Other researchers may use a range of molding materials (e.g., silicones, resins) and cleaning methods (e.g., non USP cotton, aggressive solvents), which could accumulate wear on irreplaceable specimens over time or cause replication inaccuracies.

This research addresses these concerns by quantifying the impact of repeated molding and casting procedures on the accuracy and consistency of microwear data. By analyzing casts from multiple molds of the same perissodactyl fossils, this research aims to:

1. Determine whether repeat molding and casting procedures yield consistent microwear data or introduce systematic biases.
2. Quantify the degree of variation in microwear features on the fossil by evaluating casts from multiple mold iterations of the same specimen.
3. Develop evidence-based best practices for molding and casting techniques that minimize damage to original specimens while ensuring data reliability.
4. Provide a practical guide on molding and casting to balance research access with long-term preservation of paleontological museum collections

Chapter 2. Methods

2.1 Molding

Specimens were selected based on specific criteria: only complete teeth without adhesives or consolidants were chosen, with primary consideration given to specimens exhibiting well-preserved enamel surfaces with visible microwear features. All specimens were examined under low magnification before selection to verify the presence of identifiable microwear features.

Molds were created by sampling multiple cusps, with ten molds produced for each cusp. To ensure consistency, polyvinyl siloxane (PVS) was used throughout, as it is the standard molding material in dental microwear studies due to its high fidelity and dimensional stability (Semprebon & Solounias, 2002; Ungar et al., 2003). The pre-mixed PVS was applied directly to the cusp (Figure 1) using a pointed dispensing tip and required only two minutes to set. Mold-making procedures followed the guidelines outlined by Fox (2003).

Figure 1

Sample Area of Polyvinyl Siloxane (PVS).

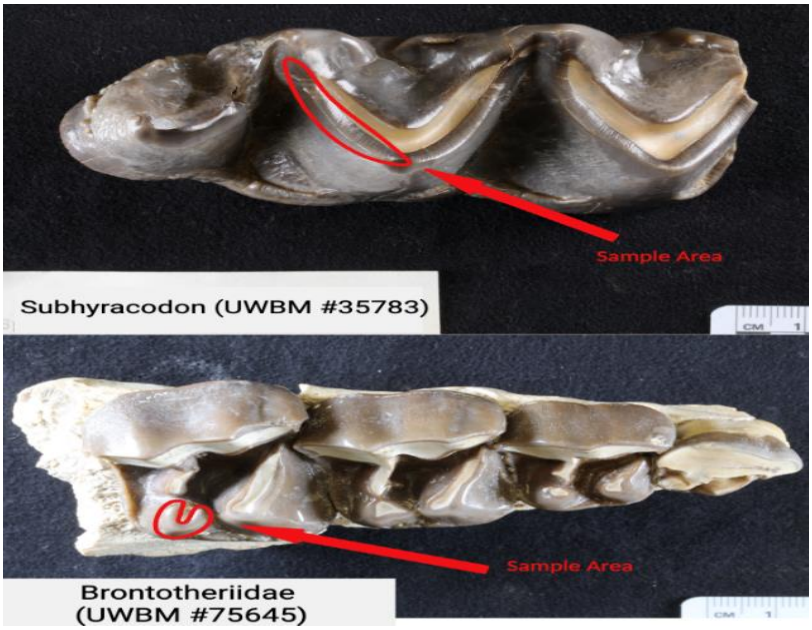

Figure 1. Representative tooth specimens showing polyvinyl siloxane (PVS) sampling areas for microwear analysis. Top: *Brontotheriidae* (UWBM #75645) specimen with sample area indicated on the occlusal surface cusp. Bottom: *Subhyracodon* specimen (UWBM #35783) with sample area marked on the tooth cusp. Red arrows and circles indicate the specific locations where PVS molds were created for microwear feature analysis. Multiple molds (n = 10) were produced from each cusp.

2.2 Casting

Defend Super Hydrophilic Vinyl Polysiloxane (VPS) Putty was used to create a containment lock around the specimen to hold the resin. All casts were produced using standard EPO-TEK® 301-2 clear epoxy resin, mixed at a 100:35 (Part A: Part B) ratio by weight. The resin was poured into the molds. Toothpicks were used to remove air bubbles without touching the mold surface. The casts were cured for 8 hours under a fume hood set to Minimum Flow (70+ FPM).

2.3 Imaging and Analysis

Once cured, the casts were removed and analyzed for surface detail retention and/or modification. Solounias and Semprebon (2002) and Semprebon et al. (2004) described a method of microwear low magnification analysis. This method was adapted with the use of a stacked imaging software. Imaging was conducted with a Canon EOS R5 Mark III camera fitted with a Canon 65 mm f/2.8 MP-E 1-5X Macro Manual Focus EF-Mount Lens, on 5x magnification. Photos were taken using a one-second shutter speed and an aperture of f/10, with Capture One software for processing. For improved clarity, images were stacked automatically through the BK PLUS Lab imaging system attached to the camera.

Dynalite MH2015 lights with barn door fixtures were used: one placed at the side, and one directed downward through a glass platform positioned 3 inches above a white surface. This setup created a high-contrast digital image that creates the illusion of a non-transparent surface by allowing the light to radiate off the white background (Mihlbachler et al., 2012).

Because dental wear surfaces are often irregularly angled and not perfectly flat, Protolina clay was used to hold the casts in position, orienting them so that the wear surface of interest was horizontal to avoid image distortion and ensure the whole image was in focus.

Pits and scratches were identified using one of two methods, selected based on the visibility of microwear features in the grayscale image. For specimens where features were visible, the paintbrush tool in ImageJ was used to mark them directly on the image. For specimens with less distinct features, the 1×1 mm cropped images were processed in Adobe Photoshop. A high-pass filter was applied to enhance high-frequency details such as edges and textures, after which features were marked using the brush tool.

After feature identification, quantitative analysis was performed in ImageJ. The freehand tracing tool was used to outline pits, and the line tracing tool to mark scratches. To track consistency across casts, the measure command recorded the surface area of pits in mm^2 and the length of scratches in mm.

Chapter 3. Results

All data visualizations were created using R statistical software. Violin plots with embedded box plots were used to display pit surface area distributions on a logarithmic scale, while standard box plots showed scratch length distributions for comparative analysis between specimens.

Figure 2

Distribution of Microwear Features (Scratches).

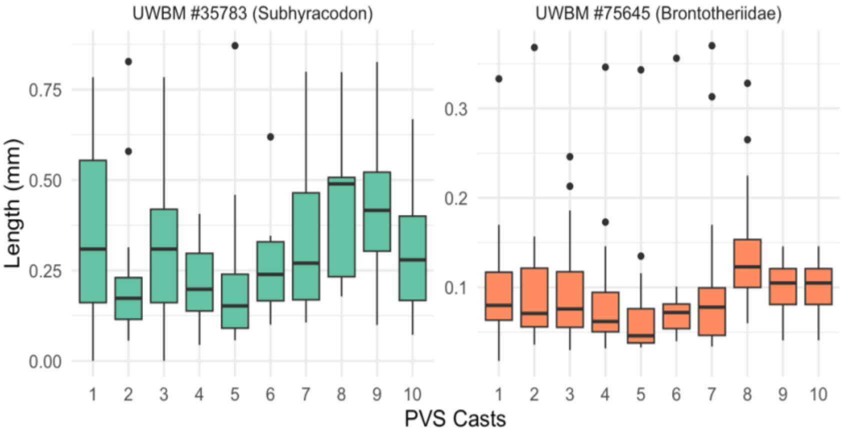

Figure 2. Distribution of microwear feature lengths (scratches) comparing Subhyracodon (UWBM #35783, left panel) and Brontotheriidae (UWBM #75645, right panel). Box plots display scratch length measurements (mm) across PVS casts, with median values, interquartile ranges, and outliers shown. The Subhyracodon specimen shows greater variation in scratch lengths compared to the Brontotheriidae specimen across casts.

Figure 3

Surface Area Distribution of Microwear Features (Pits) for UWBM #35783 (Subhyracodon).

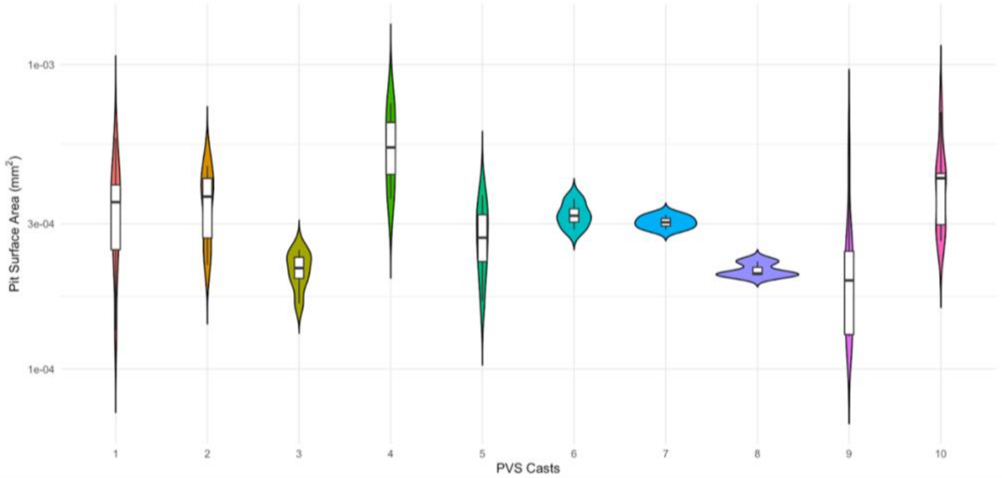

Figure 3. Surface area distribution of microwear features (pits) across PVS casts for the Subhyracodon (UWBM #35783). Violin plots show the distribution of pit surface areas (mm²) on a logarithmic scale, with box plots indicating median, quartiles, and outliers.

3.1 Parametric Analysis

To assess whether microwear measurements varied significantly across casts from the ten generations of molds, linear mixed-effects models were employed. Measurement point was included as a random effect to account for repeated measures on the same locations, while mold generation was treated as a fixed effect. Models were fitted using the lmer function from the lme4 package in R. Significance was evaluated through Type III ANOVA with Satterthwaite's method for degrees of freedom approximation via the lmerTest package. This controlled for the inherent variability between measurement locations across the tooth surface.

A Type III ANOVA was conducted from measurements of the scratch marks across 10 generations of casts from the Brontotheriidae (UWBM #75645) specimen. It revealed a significant effect of mold generation on scratch measurements (Sum of Squares = 0.0541, Mean Square = 0.0060, df = 9, F (9, 203.79) = 2.01, p = 0.039), indicating measurable variability in scratch dimensions across mold generations. No pits were observed on any casts in the 1 mm × 1 mm landmark area for this specimen, precluding pit measurement analysis.

For the Subhyracodon (UWBM #35783) specimens scratch measurements, Type III ANOVA showed no significant effect of mold generation (F (9, 88.40) = 1.17, p = 0.327), suggesting consistent scratch measurements across repeated molds.

Type III ANOVA analysis of pit surface area measurements for the Subhyracodon (UWBM #35783) showed no statistically significant differences among casts (F (11, 54.10) = 1.740, p = 0.089), indicating that pit measurements remained stable across repeated mold generations.

3.1 Post Hoc Analysis

Given the significance of the Brontotheriidae (UWBM #75645) specimens' scratch analysis, a Tukey's HSD post hoc test was conducted to compare scratch length measurements across all 10 generations of casts. Most pairwise comparisons between generations were not statistically significant (all adjusted p-values > 0.05), indicating no substantial change in scratch length through repeated molding for most generation pairs. However, a significant difference was observed between Generation 2 and Generation 8 (mean difference = 0.0334, 95% CI [0.0014, 0.0654], p_adj = 0.0330), suggesting an increase in scratch length at this point. No other pairwise comparisons reached statistical significance (p_adj > 0.05), including comparisons between Generation 8 and subsequent generations (Generations 9 and 10), indicating no continued deviation beyond Generation 8.

3.2 Non-parametric Analysis

To assess potential differences in data distribution beyond mean values, a series of Mann–Whitney U tests were conducted between the original cast of Brontotheriidae (UWBM #75645) (Generation 1) and each subsequent generation (Generations 2–10). These revealed statistically significant distributional differences between Generation 1 and several later generations: Generation 2 (p = 0.0018), Generation 5 (p = 0.0045), Generation 9 (p = 0.0129), and Generation 10 (p = 0.0129).

Chapter 4. Discussion

4.2 Interpretation of Statistical Results

The results of this research show that the fidelity of microwear replication via repeated molding and casting can vary depending on the specimen and feature type. For the Brontotheriidae (UWBM #75645) specimen, a statistically significant effect of mold generation on scratch measurements was observed (F (9, 203.79) = 2.01, p = 0.039), indicating measurable variability across repeated molds. Additionally, Tukey's HSD post hoc analysis showed a significant difference specifically between Generation 2 and Generation 8 (mean difference = 0.0334, 95% CI [0.0014, 0.0654], p_adj = 0.0330), suggesting an increase in scratch length measurements at this point in the replication sequence. In contrast, no significant differences were found in either scratch (F (9, 88.40) = 1.17, p = 0.327) or pit measurements (F (11, 54.10) = 1.740, p = 0.089) for the Subhyracodon (UWBM #35783) specimen, suggesting consistent microwear feature replication in that case.

Further non-parametric analysis revealed additional information in the data distribution. Mann-Whitney U tests comparing the original cast (Generation 1) with subsequent generations identified statistically significant distributional differences between Generation 1 and Generations 2 (p = 0.0018), 5 (p = 0.0045), 9 (p = 0.0129), and 10 (p = 0.0129). These findings suggest that certain generations introduced small but detectable shifts in scratch length distributions not captured by mean comparisons alone. Interestingly, comparisons between Generation 1 and Generations 3, 4, 6, 7, and 8 did not yield significant results (p > 0.05), indicating a non-linear pattern of variation throughout the repeated molding.

The significant difference observed between Generation 2 and Generation 8 in the parametric analysis suggests that subtle surface degradation or alteration may accumulate after multiple cycles of mold-making and casting, potentially reaching a detectable threshold by Generation 8. The absence of significant differences among earlier generations (2 through 7) in the Tukey's HSD analysis indicates that surface fidelity remains relatively stable through several replications before notable changes emerge in mean values. Furthermore, the lack of significant differences between Generations 8, 9, and 10 suggests that any degradation in mean scratch length may plateau after reaching this threshold, potentially showing cumulative wear or the loss of fine morphological details.

Generation 2 exhibited differences under both analyses, suggesting it may represent a point of mechanical or procedural deviation in the casting process. The significant differences observed in distributions for Generations 5, 9, and 10 when compared to the original cast further indicate that subtle alterations can occur throughout the replication sequence, even when mean values remain statistically similar. This suggests that the replication process may introduce variability in complex ways that are not necessarily linear with each generation.

4.2 Implications for Microwear Analysis

These findings suggest that while repeated molding and casting can yield reliable data in some cases, the process may also introduce subtle changes that affect the accuracy of microwear analysis, especially for scratches, which appear more sensitive to variation. Given that dental microwear provides dietary information from the last days or weeks of an animal's life (Grine, 1986), even small alterations in surface features could lead to misinterpretations of feeding behavior or ecological adaptation. This is particularly important when analyzing specimens from times of rapid ecological change, where accurate reconstructions of diet inform our understanding of evolutionary responses.

4.3 Potential Sources of Variation

Several potential sources could explain the observed variation in scratch measurements across Brontotheriidae (UWBM #75645) casts. One primary factor may be the physical properties of the polyvinyl siloxane (PVS) itself. Despite its reputation for detailed reproduction, PVS could potentially interact with the fossil surface in subtle yet cumulative ways across multiple applications. The chemical composition of PVS includes volatile organic compounds that might react with the fossil. Additionally, the physical process of removing the cured silicone could create microscopic displacement or distortion of the tooth surface. This mechanical stress, while imperceptible during a single molding event, may accumulate over time with repeated molding generations.

The imaging process itself is another significant source of potential variation. Despite efforts to standardize photography, subtle differences in specimen orientation, lighting angle, and camera positioning can affect the appearance of microwear features. The highly reflective nature of clear epoxy casts adds to the challenge of achieving consistent imaging. Even minor adjustments in light placement can either enhance or obscure specific details. Setting up the specimens to maintain consistent imaging conditions required frequent adjustments, including repositioning lights, reorienting specimens, and modifying camera settings. Although these conditions were kept consistent within each casting generation, some variation can still occur. Additionally, the image processing workflow in Adobe Photoshop, while guided by standardized protocols (Mihlbachler et al., 2012), involves subjective decisions about contrast and brightness that may influence feature identification and measurement.

In addition to methodological variation, the significant differences observed across casts must be considered alongside concerns about observer error in microwear analysis. Previous studies (Grine et al., 2002; DeSantis et al., 2013) have documented variability in observer scoring even when standardized protocols are followed.

The use of ImageJ for feature identification and measurement represents an attempt to standardize the quantification process. However, the identification of features as either pits or scratches is still a form of subjective decision-making. The observer's categorization of features likely impacts the data variability. New automated tools are being developed to minimize this error, for example, the R package MicroweaR, which is an open access tool to (a) sample the marks, (b) classify features into categories as pits or scratches and then into their respective subcategories (large pits, coarse scratches, etc.), (c) generate an output table with summary information, and (d) obtain a visual surface-map where marks are highlighted (Strani, 2018). Although MicroweaR offers useful and more rapid analysis, stacked images are not compatible with this software. A newly improved MicroweaR R package that can read stacked images would significantly enhance microwear analysis.

Several factors may contribute to the observed inconsistencies in microwear preservation. While this research employed polyvinyl siloxane (PVS), the fidelity of microscopic detail capture may vary among liquid-based silicones due to differences in viscosity, setting time, polymerization shrinkage, and molecular structure. Furthermore, despite adherence to standardized protocols, minor variations in specimen preparation procedures and molding compound mixing ratios may have influenced mold and cast quality.

Representative images from mold generations 1, 2, and 8 (**Appendix A**), which exhibited statistically significant differences in comparative analyses within the respective 1×1 mm sampling areas, demonstrate a temporal pattern in casting quality. Generation 1 contains isolated air bubbles, generation 2 exhibits numerous and more extensively distributed bubbles, whereas generation 8 is free of bubbles. Additionally, these images show a gradual decrease in surface detail quality: microwear features become increasingly attenuated and scratches appear shallower with each molding generation.

4.4 Limitations

It is important to emphasize that these results are based on a limited number of specimens without extensive replication. This limits the generalizability of the findings. Variability in fossil preservation, molding materials, or casting techniques could influence the observed pattern, and measurement noise cannot be fully ruled out. Therefore, these findings should be considered preliminary, serving as an initial indication that repeated molding and casting can impact microwear surface fidelity after multiple generations. Future research involving additional specimens and more replication cycles is needed to confirm whether this threshold effect is consistent across taxa and to better understand what causes surface damage during repeated molding and casting.

4.5 Conservation and Museum Practice Implications

For museum collections, these results suggest that permitting repeated molding of fossil specimens, particularly using different materials and techniques, may compromise the integrity of microscopic features over time by removing existing features, modifying existing features, or adding new ones. Museums should maintain detailed records of molding events for each specimen, including materials used and specimen cleaning procedures. Standardized molding and casting protocols should be developed and shared across institutions to ensure consistency in research methods. These practices would help minimize methodological error in microwear studies and ensure more reliable paleodietary reconstructions across researchers and institutions.

Chapter 5. Molding and Casting Guide

This guide will walk you through the molding and casting process used for microwear analyses that require casts for data collection. This guide can also be helpful when making molds of an object with only one viewable side (such as a bone trapped in its matrix).

5.1 Choosing a Specimen

Before beginning the molding process, carefully examine the fossil specimen to ensure its stable enough to withstand silicone application. Identify any fragile areas that may require reinforcement to prevent damage during molding.

Inspect the specimen for existing adhesives or consolidants, especially on tooth surfaces intended for microwear analysis. Any residues in these areas can impact your data quality. Specimens previously treated with shellac or other older consolidants (such as glyptal or PVA) should be avoided, if possible, as these substances are difficult to remove without damaging the fossil, and their glossy finish can obscure critical surface details. If you are molding a specimen that belongs to another institution, make sure that you have written permission to mold the specimen.

5.2 Gap Filling or Reinforcing Holes and Cracks

Once you have found the appropriate specimen, avoid silicone seeping into the cracks and holes and hardening, as this may cause damage to the fossil. You want to fill these with materials like Carbowax (polyethylene glycol 3350), which proves useful in temporary filling as well. All options are soluble in water and provide a temporary stabilization method.

5.3 Cleaning

Additionally, when cleaning specimens prior to molding, it is important to avoid aggressive solvents such as shellac cleaner, which can cause damage or modification to delicate microwear features. Standard protocols emphasize gentle, non-invasive cleaning methods to preserve microwear integrity (Solounias & Semprebon, 2002). If cleaning is necessary, use USP lab-grade cotton swabs, as non-USP or non-sterile swabs may contain silica particles or other additives that could damage valuable microwear surfaces or introduce new features. Lightly moisten the swab with ethanol or deionized water, selecting the appropriate solution based on the fossil's stability and the type of residue being removed. Avoid applying abrasive pressure to protect the integrity of the tooth surface.

5.4 Molding

Silicone molds are made using a two-part mixture, typically labeled Part A and Part B. Always follow the manufacturer's instructions, which specify the proper mixing ratio. You can measure the components using a digital scale. Be sure to tare the weight of the mixing cup or use a disposable measuring cup if ratios are given by volume. Select a mixing cup that allows the mixture to expand up to three times its original volume during degassing (Fox, 2003).

Use a disposable stir stick (such as a tongue depressor) to mix thoroughly. Scrape the sides and bottom of the cup frequently to ensure that all material is fully incorporated. Incomplete mixing can result in improper curing.

To minimize air bubbles that may distort the tooth surface being replicated, the silicone mixture must be de-aired. Place the cup in a vacuum chamber with the air valve initially closed. Gradually draw out the air, allowing the material to rise and expand. Once the mixture reaches maximum expansion, it will collapse back to its original volume and continue bubbling. Let the mixture degas for 5–7 minutes to fully eliminate trapped air (Fox, 2003). To release the vacuum, slowly turn the chamber knob. A fast pressure release may cause the cup to tip or spill. Once the pressure has normalized, carefully remove the cup.

If you are using VPS (vinyl polysiloxane) instead of liquid silicone, start at one edge of the surface and slowly dispense the VPS using a controlled, continuous motion. Allow the material to flow evenly across the surface, minimizing air bubbles. Let it sit for 2–3 minutes. Once cured, lift the VPS off the surface without forcing or bending it.

Begin by rolling out a layer of sulfur-free clay to create a stable base for your mold. Next, use the clay to build a wall around the specific area of the fossil you want to sample. This “clay lock” should be tall enough to hold the silicone rubber when you pour it and ensure it fully covers the surface details you wish to capture. Essentially, you are isolating and “roping off” a defined section of the fossil so the molding material stays contained and precisely captures that area.

Pay special attention to the base of the clay walls: reinforce it well to avoid undercuts that can trap the silicone or damage the mold. For microwear studies, it may be helpful to build the clay outward horizontally first, then vertically upward, smoothing the corners and edges. This helps ensure that the resulting silicone mold will have built-in walls that are ideal for pouring in casting resin later, especially if you've added enough silicone to fully capture the surface depth.

For microwear analysis, it’s important to position the fossil so that the tooth surface lies as flat and horizontal as possible. This is important for getting high-quality images during later imaging work. Use additional clay beneath or around the fossil to adjust and level it. A horizontal orientation minimizes focus issues and reduces the risk of image distortion.

When pouring the silicone, hold the cup at least 5 inches above the clay lock and pour in a slow, thin stream at one corner of the mold area. This technique helps the silicone flow smoothly over the specimen and minimizes bubble formation.

Let the silicone cure according to the manufacturer's recommendations. While it cures, keep the mold in a clean, dust-free area to prevent debris from settling in the silicone. After the allotted curing time, gently touch the surface to test whether it has fully set. If the silicone still feels tacky or retains a fingerprint impression, it needs more time to cure. Check back after several hours and repeat as needed.

5.5 Demolding

Demolding is an important step that requires extreme caution: this is when there's the highest risk of breaking the fossil. After waiting for the silicone to fully cure, use a small spatula or your finger to gently tug around the entire edge. Next, carefully lift the edges of the mold and pull it away from the specimen evenly in all directions to avoid creating one-sided stress that could damage the fossil.

If the silicone is particularly stubborn and adheres to the specimen, you can remove the clay surrounding the mold so you can gently pull from the bottom edges of the silicone. Continue lifting at the top edges until you're able to completely remove the silicone from the fossil. However, be extremely careful when handling the bottom edges because you don't want to damage features that have captured the imprint of the tooth surface. Once finished with molding, remove any residue from clay or silicone that is on the fossil before starting the casting process.

5.6 Creating Clay Walls Around the Mold

To allow sufficient height for pouring the resin and creating a complete cast, the mold will require additional support along its sides in the form of clay walls. Use VPS putty (vinyl polysiloxane) to construct walls tall enough to contain the resin when poured over the tooth surface. VPS is chemically inert and does not bond with resin, making it ideal for this application. Prepare the putty by mixing the catalyst and base components according to the manufacturer's instructions. Be mindful of the working and setting times, as these materials typically cure quickly (often within one minute). Carefully inspect the putty walls for cracks or holes, and patch them with additional clay to prevent resin from leaking.

5.7 Casting

Since epoxy resin and its catalyst are noxious materials, always work in a fume hood and wear appropriate personal protective equipment, including rubber gloves and a lab coat. Inside the fume hood, set up the mold(s) and gather the necessary supplies: toothpicks, tongue depressors, disposable cups, and a scale.

Using a clear resin such as EPO-TEK® 301-2, follow the recommended mixing ratio by weight. In a disposable cup, combine the two components and mix thoroughly using a tongue depressor. Ensure the mixture is fully uniform; cloudiness or streaks might show incomplete mixing. Slowly pour the mixed resin into the mold, so it flows evenly over the surface, and reduces air entrapment. Inspect the casts for air bubbles, especially on the sampling surface. If bubbles appear, use a toothpick to carefully remove them without touching the mold walls.

Wait for the resin to cure according to the manufacturer's recommended time. After this period has passed, check the surface of the cast. If it still feels tacky, allow it to sit for several more hours and check again. Repeat this process until the resin is fully hardened. To remove the cast, gently flex the mold to loosen the edges. You should now have a complete replica of the tooth surface.

Chapter 6. Conclusion

This research evaluated the reliability of dental microwear replication through repeated molding and casting procedures using Eocene–Oligocene perissodactyl specimens from the Burke Museum of Natural History and Culture. By analyzing scratch and pit measurements across ten generations of casts, this research tested whether the replication process introduces variability that could affect microwear-based dietary reconstructions.

Results revealed statistically significant differences in scratch measurements across Brontotheriidae (UWBM #75645) casts (F (9, 203.79) = 2.01, $p = 0.039$), with post hoc analysis identifying a significant difference specifically between Generation 2 and Generation 8 (mean difference = 0.0334, 95% CI [0.0014, 0.0654], $p\_adj = 0.0330$). This suggests a threshold effect where surface degradation becomes detectable after multiple replication cycles. Further non-parametric analysis revealed significant distributional differences between the original cast (Generation 1) and subsequent generations (2, 5, 9, and 10), with p-values ranging from 0.0018 to 0.0129. Notably, comparisons between Generation 1 and Generations 3, 4, 6, 7, and 8 did not yield significant results ($p > 0.05$), indicating a non-linear pattern of variation throughout the casting sequence. In contrast, no significant differences were found for either scratch (F (9, 88.40) = 1.17, $p = 0.327$) or pit measurements (F (11, 54.10) = 1.740, $p = 0.089$) in the Subhyracodon (UWBM #35783) specimen.

The combination of parametric and non-parametric tests reveals a complex pattern of variation: while mean scratch lengths show significant differences only between specific generations (Gen 2 vs. Gen 8), the distribution of measurements shows significant differences at multiple points in the replication sequence. This suggests that the molding and casting process may introduce subtle alterations to microwear features that affect both their average dimensions and their distributional characteristics, with potential implications for paleodietary interpretations.

These outcomes have important implications for paleontological research. While dental microwear analysis provides a valuable look into the short-term dietary habits of species, even minor distortions in the molding and casting process can substantially impact interpretations of feeding ecology and habitat preference. This is especially important when studying taxa from periods of major ecological transition, where precise dietary information is important to understand adaptive responses.

For museums, this research shows the need for policies that limit repeated molding of the same fossil specimens while still providing research access. The standardized molding and casting guide provided in this thesis offers a starting point for best practices that can be adopted across museums. By employing consistent practices, researchers can minimize methodological error and strengthen data comparability between studies.

While this research focused specifically on perissodactyl fossils using polyvinyl siloxane (PVS) as the molding material, future research should expand to include comparative studies of different molding materials (e.g., platinum and tin-cure silicones) to determine which best capture microwear features. Additionally, research should focus on tracking changes in microwear features on fossils subjected to different cleaning, molding, and casting protocols.

Through continued refinement of molding and casting techniques and the establishment of standardized protocols, the paleontological community can balance research access and ensure the long-term preservation of irreplaceable fossil specimens. This work represents an important step toward more reliable paleodiet reconstructions and, ultimately, a more accurate understanding of ancient ecosystems and evolutionary patterns.

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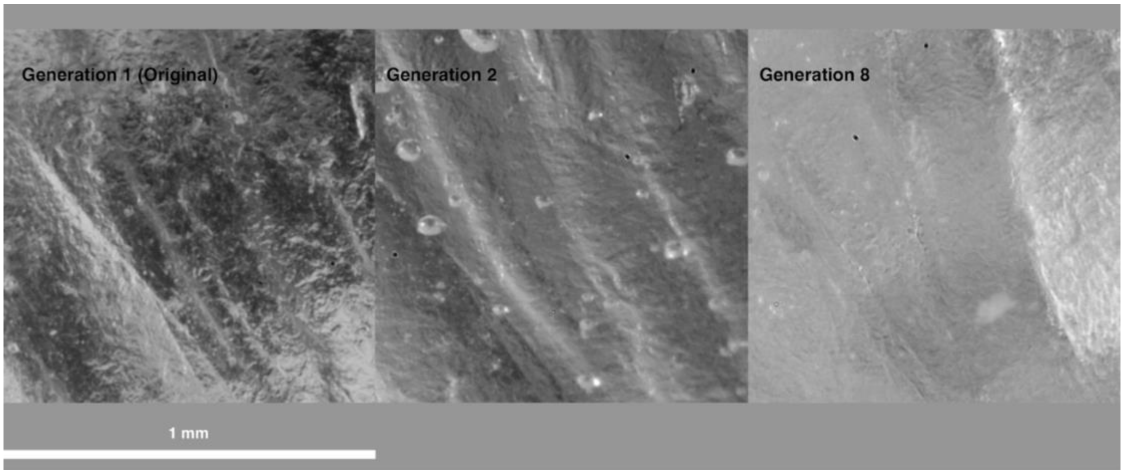
Appendix A

Cast Quality Across Statistically Significant Generations.



Appendix A. Representative stacked images of Brontotheriidae (UWBM #75645) specimen casts from statistically significant generations. Cast from Generation 1 shows minimal bubble interference and clear surface detail. Generation 2 exhibits a noticeable increase in bubble artifacts across the sampling area. By Generation 8, bubble presence is significantly reduced, but surface detail appears increasingly attenuated.

Appendix B

Data available upon request.