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Reconstructing the Sequence of Events Surrounding Body Disposition Based on Color Staining of Bone*

ABSTRACT: Literature regarding bone color is limited to determining location of primary and secondary dispositions. This research is the first to use bone color to interpret the sequence of events surrounding body disposition. Two scenarios were compared—bones buried and then exposed on the ground surface and bones exposed then buried. Forty juvenile pig humeri with minimal tissue were used in each scenario with an additional 20 controls to determine if decomposing tissue affects bone color. Munsell Color Charts were used to record bone color of surface and 2.5 cm cross-sections. Results reveal five main surface colors attributed to soil, sun, hemolysis, decomposition, and fungi. Fungi on buried bones suggests prior surface exposure. Cross-sections of strictly buried bones are identical to buried then exposed bone, stressing the importance of bone surface analysis. Cross-sectioning may help verify remains have been exposed then buried. Decomposition of excess tissue creates minimal color staining.

KEYWORDS: forensic science, forensic taphonomy, color staining, buried, exposed, disposition, fungi, *Aspergillus*, *Penicillium*

Although there has been a wide variety of taphonomic research into soft tissue decomposition (1–4), bone weathering (5), scavenging (6,7), the affects of buried (8,9), and wet environments (1,3,10,11), current literature contains little research into the effects of environmental conditions on the color of bone. In a general sense, it is known that bones exposed to the sun for long periods of time exhibit a less greasy appearance and are lighter in color (12), while bones buried in shallow graves tend to lose their greasy appearance over time (13). Prolonged contact with soil causes brown, tan, black, or other colored staining, depending on the organic and mineral content of the soil (14), while exposure to green algae, copper, or brass can stain bone various shades of green (11,14). Color differences have been used in case examples to reconstruct the position of bones on the ground surface (15) and distinguish perimortem trauma versus postmortem damage (16). Yet, the significance of bone color as a taphonomic indicator of body disposition is not well understood or investigated.

There are very few studies that exclusively examine bone color outside the context of cremation. Schafer (17) investigated potential correlations between skull color and age, as well as skull color and diabetes. No association was found between the color of the skull and diabetes; however, a positive correlation was identified with the yellowness of the skull and the age of the subjects (17). Similarly, tooth color has been used to estimate age at death since the creation of secondary dentin causes teeth to darken with age (18,19). In an archaeological context, López-Gonzáles et al. (20) conclusively determined the order of events surrounding deposit formation, carnivore activity, and orientation of the remains by examining the manganese staining found on animal bones.

The current study investigates the use of color by determining if it is possible to establish the sequence of events surrounding body disposition based on color staining of bone; specifically, to establish whether bone that has been buried then subsequently dug up and left on the ground's surface can be distinguished from bone that has been left on the surface and later buried. From a forensic perspective, it is important to reconstruct events surrounding the disposal of remains and subsequent disturbances to identify primary and secondary dispositions. Recognizing that a bone has been subjected to two separate environmental conditions can aid in the discovery of additional crime scenes, remains, and evidence. Reconstruction of the sequence of body disposition can also be used to corroborate or refute eyewitness statements as well as the defendant's testimony. Use of bone color in this way to interpret sequence of events has never been addressed in the literature.

Materials and Methods

A sample size of 80 juvenile pig (*Sus scrofa*) humeri was used for this study. Pigs are considered the second best animal models for comparative studies, especially for taphonomy and other forensic experiments (21,22). Long bones were selected because elements such as the humerus tend to have a high survivability rate in forensic cases (23,24). Each scenario involved 30 humeri, which is the minimum sample size recommended in statistics to achieve accurate results (25), plus an additional 10 to compensate for potential scavenging (n = 40) and 10 controls. The controls were used to determine whether or not decompositional processes affect the color of bone. In contrast to the test sample, the control bones retained their soft tissues.

A pilot study determined that 1 week of freezing for storage purposes prior to the start of the study did not change the color of the bone after the thawing process. Due to time constraints, measures were taken to accelerate soft tissue loss and maximize the degree

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of bleaching and soil staining. These measures included maximum removal of soft tissues prior to field exposure, shallow burials, and avoidance of shady areas during surface exposure. Color documentation took place before beginning the outdoor scenarios. Color was assessed using Munsell Color Charts under a natural daylight 18 W bulb.

Scenario 1 humeri were buried for 4 weeks then exposed on the surface for 4 weeks starting mid-June of 2007. Scenario 2 commenced mid-July of 2007, with a 4-week interval of surface exposure followed by a 4-week burial period. Thus both scenarios were exposed on the surface during the same 4 weeks in July to control for environmental conditions. During surface exposure, the humeri were divided evenly among 12 bottomless cages with the walls and lids constructed of chicken wire and lumber. The cages were staked into the ground as close together as possible to maintain the same environmental conditions and minimize the number of sides exposed to scavengers. A separate fenced location was chosen for the buried remains; however, the fenced perimeter did not provide protection from scavengers, resulting in the disturbance of 37 sample bones and all 10 controls, as well as the loss of two sample humeri and one control bone (Table 1). The placement of chicken wire over scenario 2 burials protected against further animal disturbances.

A burial depth of 0.30 m was selected because evidence suggests soil at this depth maintains the same temperature as the surface and continues to attract carrion insects (23). Ten graves were dug close together to limit the amount of soil variation. Each burial contained 10 humeri divided into two levels of five with 10 cm of soil separating the bottom from the top layer. As the controls contained more tissue than the sample bones, they remained in the primary disposition of each scenario until the equivalent amount of bone was exposed as the sample bones. The scenario commenced once the degree of exposed bone resembled that of the sample bones prior to placement in the field. The location of burial and surface exposure were identical and the amount of time subjected to each disposition remained the same as that of the sample bones.

Upon final recovery of bones from each scenario, surface color was documented using Munsell Color Charts and a natural daylight 18 W bulb. Tissues adhering to the bone were also documented. After scavenging of scenario 1 humeri (buried then exposed), the total sample size for scenario 1 was $n = 38$ test samples and $n = 9$ controls (Table 1). Scenario 2 humeri (exposed then buried) remained undisturbed by scavengers resulting in a total sample size of $n = 40$ test samples and $n = 10$ controls (Table 1).

Each bone that exhibited signs of bleaching after surface exposure was cross-sectioned. Sample sizes for cross-sectional analyses are recorded in Table 2. A miter saw was used to cut a 2.5 cm thick cross-section from the middle of each diaphysis so that color penetration could be analyzed. In addition, a sample of $n = 6$ pig femora and tibiae buried for 150 days in the same location as the burials of scenarios 1 and 2 humeri were obtained from a colleague and cross-sectioned. The six femora comprised of a readily available sample for comparative purposes to determine if bone color in scenarios 1 and 2 were qualitatively different from color observed in bones that are buried with no prior or subsequent disturbances. A dissecting microscope in conjunction with the daylight bulb was used to examine layers of color. The magnification remained constant at 100× magnification, while the location, color, staining depth, and additional features were documented. For cross-sectional color documentation, the outer cortex is defined as the cortical bone just beneath the periosteal surface. The inner cortex is the cortical bone immediately surrounding the trabecular bone. The middle cortex is the cortical bone between the outer and inner cortices.

TABLE 2—*Color patterns of cross-sectional analysis.*

Scenario	Sample Size (*n*)	% Demonstrated Pattern*	Outer Cortex	Middle Cortex	Inner Cortex
1-Sample	25	88	Light	Light	Dark
1-Controls	8	100	Light	Light	Dark†
2-Sample	13	31	Dark	Light	Light
		69	Dark	Light	Dark
2-Controls	2	100	Dark	Light	Light

*Distal end of cross-section is recorded here as this region exhibited thicker cortical bone, thus allowing the full extent of the pattern to be evaluated.

†Slightly paler in comparison to the other dark areas of the inner cortex.

Results

Five main color differences were observed on the surface of scenario 1 (buried then exposed) and scenario 2 (exposed then buried) bones: light yellowish brown (2.5Y 6/3, 2.5Y 6/4) designates soil staining, dark reddish brown (5YR 3/2, 5YR 3/3) denotes hemolysis, white (varying hues with 8/1) represents sun bleaching, dark reddish gray (2.5YR 3/1) indicates decompositional fluid staining, and greenish gray (GLEY 1 6/5GY) as well as olive (5Y 5/4) signify the most abundant types of fungi. Table 1 is a summary of the various factors contributing to the overall surface appearance of scenario 1 and scenario 2 sample and control humeri. With respect to the orientation of the bones in the grave, the inferior surfaces of the scenario 2 humeri display fungal growth, appearing as colored deposits on remaining tissues and exposed bone. The dominant types of fungi present include the genera *Aspergillus* and *Penicillium* (L Kohn, Mycologist, Per. Comm., April 7, 2008). Staining due to the decomposition of soft tissues of scenario 2 controls is restricted to small, localized areas rather than entire surface coverage.

Table 2 is a summary of the color patterns and frequencies observed in the cross-sectioned bones from scenarios 1 and 2

TABLE 1—*Results of surface analysis.*

	Sample Size	Bones Scavenged		Bones Recovered		Soil Coverage and Soil Staining		Hemolysis Staining		Bleaching		Decomposing Fluid Staining		Fungi	
Scenario	*n*	*n*	%	*n*	%	*n*	%	*n*	%	*n*	%	*n*	%	*n*	%
1-Sample	40	37	93	38	95	38	100	26	68	25	66	0	0	0	0
1-Controls	10	10	100	9	90	9	100	2	22	8	89	0	0	0	0
2-Sample	40	0	0	40	100	40	100	1	3	13	33	0	0	40	100
2-Controls	10	0	0	10	100	10	100	4	40	2	20	4	40	8	80

FIG. 1—*Cross-section of scenario 1 sample. Bone #15, distal end of section, caudal surface at the bottom.*

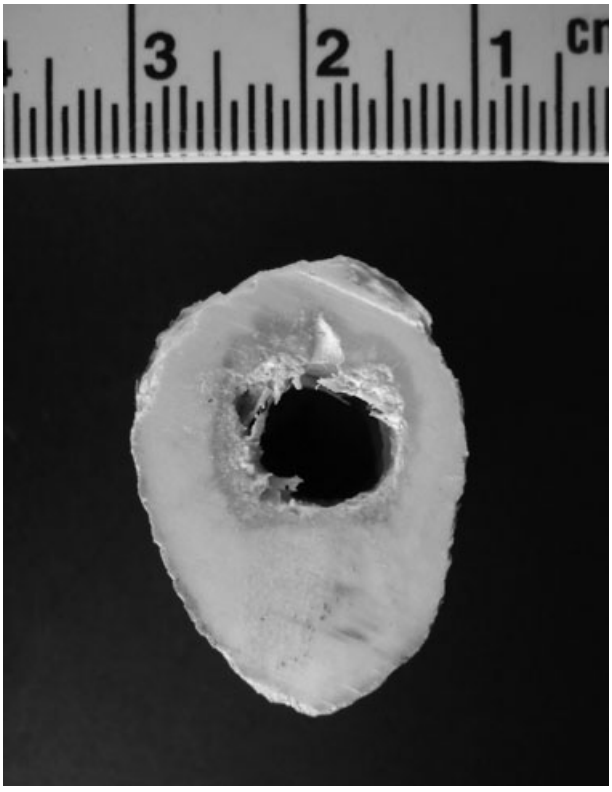

FIG. 2—*Cross-section of scenario 1 control. Bone #46, distal end of section, caudal surface at the bottom.*

sample and control humeri. Colors fall into two main groupings referred to as "light" and "dark." The light category corresponds to the Munsell colors white (varying hues with 8/1), pale yellow (5Y 8/2, 2.5Y 8/2, 2.5Y 7/4), yellow (2.5Y 7/6), and light gray (5Y 7/1). The dark category represents the Munsell colors grayish brown (2.5Y 5/2), light olive brown (2.5Y 5/3), and olive gray (5Y 5/2). The terms "light" and "dark" are used in Table 2 to highlight patterns. Figures 1–5 illustrate the cross-sectional patterns described in Table 2. The transition between light and dark regions is well-defined, not gradual.

Discussion

Surface Analysis

The factors contributing to the overall surface appearance of the sample bones are also visible on the control bones (Table 1). The main difference relates to the degree each factor is expressed. Scenario 1 controls (buried then exposed) produced a similar surface appearance as the sample bones, with the exception that there is less tissue present on the sample bones and thus more evidence of bleaching. Scenario 1 controls exhibit no signs of decompositional fluid staining, possibly due to the fact that a significant amount of tissue was removed during scavenging. As a result, the absence of staining due to the decomposition of the soft tissues cannot be attributed to the sequence of events (buried then exposed).

Scenario 2 controls (exposed then buried) yielded results similar to the sample bones (Table 1); however, the controls exhibit more soil staining, fungi is present in much smaller quantities, and staining due to the decomposition process is visible. Extensive soil staining of the scenario 2 controls is attributed to less tissue

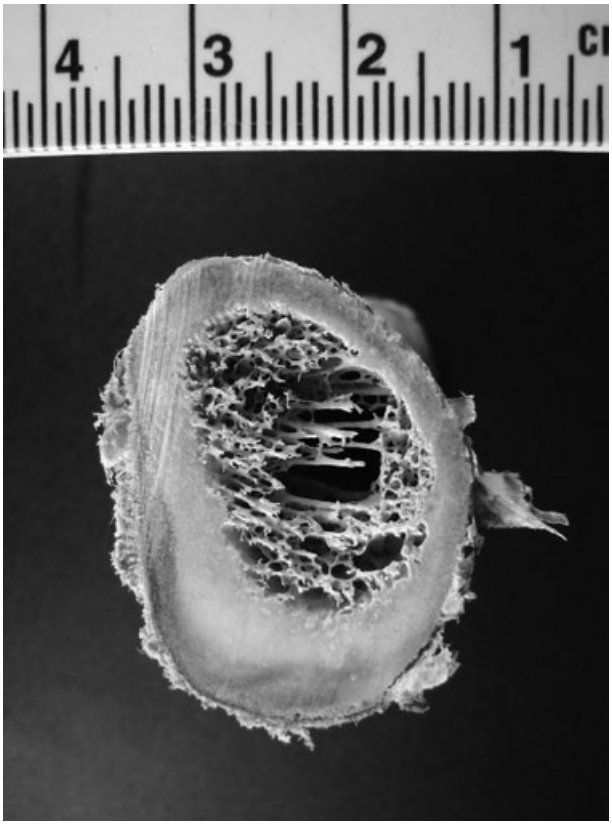

FIG. 3—*Cross-section of scenario 2 sample, Pattern 1. Bone #56, distal end of section, caudal surface at the bottom.*

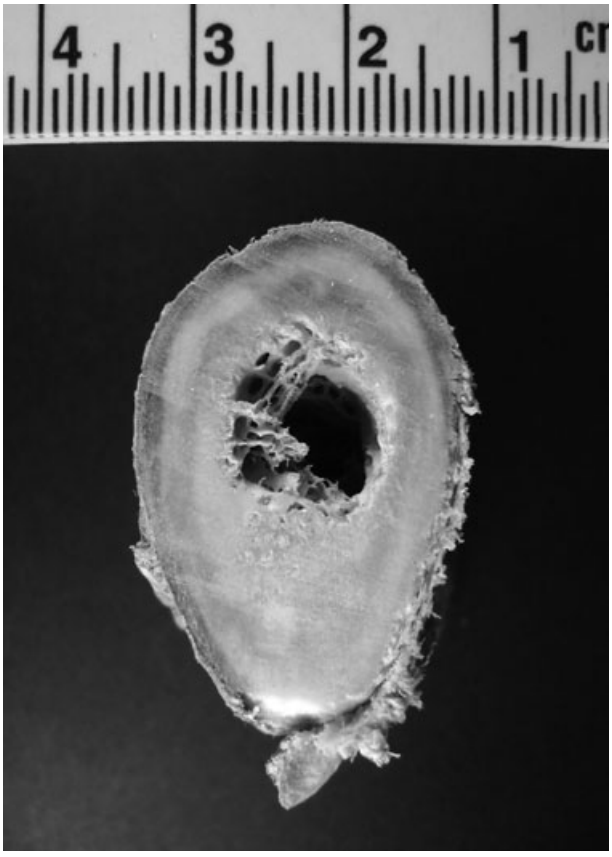

FIG. 4—*Cross-section of scenario 2 sample, Pattern 2. Bone #64, distal end of section, caudal surface at the bottom.*

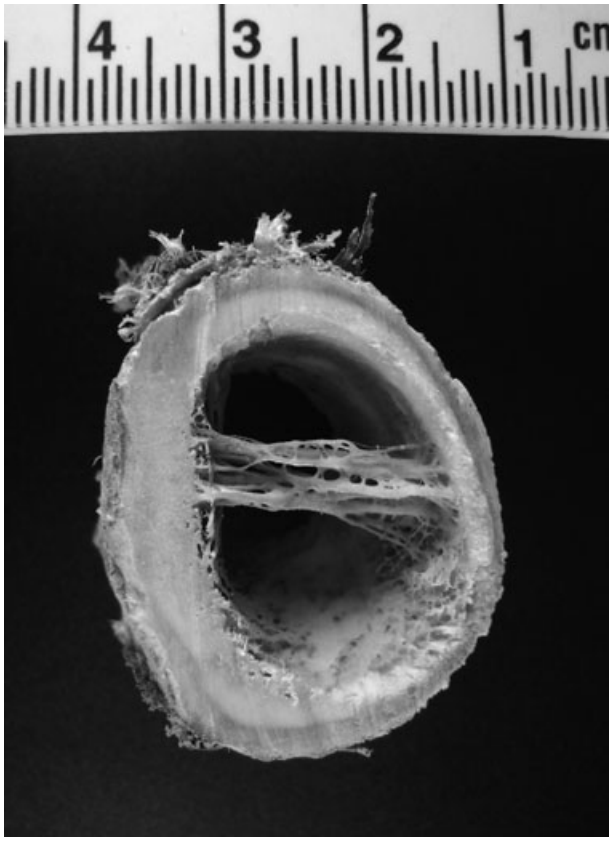

FIG. 5—*Cross-section of scenario 2 control, Pattern 1. Bone #95, distal end of section, caudal surface at the bottom.*

remaining prior to burial as a result of being left in the field for a longer period of time than the test sample. The presence of decompositional fluid staining on less than half of the scenario 2 controls suggests the process of tissue decomposition can affect the surface color of bone, but due to scavenging of scenario 1 bones the impact of burial and exposure sequence on decompositional staining cannot be determined.

There are notable differences between the two scenarios with respect to surface appearance. Scenario 1 shows evidence of bleaching, however fungal growth is absent. Scenario 2, in contrast, exhibits fungal growth but bleaching is undetectable. The genera *Aspergillus* and *Penicillium* are very common fungi that can grow on a variety of substrates under various conditions (L Kohn, Mycologist, Per. Comm., April 7, 2008). Although one might argue that scavenging of scenario 1 humeri could be responsible for the absence of fungi on these bones, none of the three undisturbed humeri from scenario 1 exhibit fungi. Similarly, none of the bones buried for 150 days and used in the cross-sectional analysis were associated with fungi. Thus, the ubiquitous presence of fungi on scenario 2 bones suggests that fungi are indicative of exposure on the surface followed by burial.

Cross-Sectional Analysis

The cross-sectional patterns of the controls mimic those of the sample humeri with the exception that the former exhibit slightly paler "dark" regions. The small localized distribution of staining due to the decomposition process is not extensive enough to penetrate the interior of the bone. The consistency between the controls and sample bones suggests that the presence of tissue does not affect cross-sectional analysis.

There are notable differences between the cross-sections of scenarios 1 and 2. After 4 weeks of sun exposure and burial, surface color of bone was minimal in both scenarios and did not penetrate the cortex. Despite this limitation, color differences produced by differential loss of bone grease were evident. Scenario 1 bones (buried then exposed) exhibit a pattern of light to dark color from the outer to inner cortex (Figs. 1 and 2, Table 2). The darker bone is light olive brown (2.5Y 5/3) and very greasy, in contrast to the lighter bone, which is pale yellow (5Y 8/2) and notably less greasy in texture. Scenario 2 bones (exposed then buried) exhibit a pattern of dark to light color, or dark/light/dark, from the outer to inner cortex (Figs. 3–5, Table 2). In this case, the darker bone is grayish brown (2.5Y 5/2) and its greasiness is difficult to determine, in contrast to the lighter bone, which is pale yellow (5Y 8/2) and similar in greasiness to the light bone observed in scenario 1.

At first glance, it would appear scenarios 1 and 2 can be differentiated on the basis of color, although not in the manner originally anticipated. Examination of the cross-sections of bones buried for 150 days complicates this interpretation. The pattern observed in the bones that were subjected to burial (only) is identical to the scenario 1 humeri (buried then exposed). In a forensic case, the inability to differentiate buried from buried/exposed bone would not depend upon color or cross-sectional analysis because the former would be found in a grave and the latter on the surface. Similarly, scenario 1 (buried then exposed) could be distinguished from scenario 2 (exposed then buried) because the former would be found on the surface and the latter in the ground. If the bones were discovered fairly recently after exposure in scenario 1, soil

would still be clinging to the bone. Analysis of context and the surface color of the bone becomes of utmost importance in this case.

In scenario 2, the bones would be found in the ground and the only way of knowing whether the body had been exposed prior to burial would be to check for: (1) correct anatomical position of bones; (2) missing elements with no evidence of dismemberment; (3) presence of fungi; and (4) cross-sectional color analysis of bone. If the bones in a grave are not in anatomical position, the body must have decomposed for some period under circumstances that would allow bone movement, i.e., above ground or in a container that prevented sediment infilling. Missing elements suggest the body was above ground or exposed during some stage of decomposition, allowing the bones to disarticulate, separate from the rest of the body, and for some reason not be included in the burial. If there is no evidence the bones are missing due to dismemberment, the most likely explanation is natural disarticulation due to decomposition above ground prior to burial. Fungi were found on all sample bones that were exposed and subsequently buried, and on 80% of the fleshed control bones. The current research supports the use of cross-sectional analysis of bone color pattern as a further line of evidence to confirm above ground decomposition prior to final burial.

Fungi on forensically significant human remains have been used to determine the prior location of a body and estimate postmortem interval (26). Both fungi genera observed in this analysis have been documented on cadavers in recent literature (26). Although the individual in the case report was found at the bottom of a well, results still indicate a possible connection between fungi and decomposing remains. The current analysis suggests the presence of fungi may be valuable for identifying the sequence of events surrounding body disposition and cross-sectional analysis of color can be used for verification if necessary.

This study has generated several avenues of research that require further exploration. First, it is necessary to include two additional scenarios, simple burial and surface deposition, for comparison. Analysis of four scenarios will help determine the uniqueness of the color patterns and may help explain their causes. Second, it is necessary to increase the time allotted for burial and surface exposure to discover: (1) how long it takes for bone to become sun bleached and surface stained; (2) if color staining is able to penetrate the cortex; and (3) whether the patterns observed in the limited time frame of 4 weeks for each form of disposition is maintained in bone that is exposed to extended periods of sun bleaching and soil staining. Third, the rate of grease loss in relatively fresh bone should be investigated as it may provide valuable information regarding time since death. Fourth, further research is necessary to verify the relationship between fungi growth and the sequence of events surrounding body disposition under controlled conditions. Fifth, bodies left or hidden above ground and allowed to decompose prior to burial may not be fully or even extensively skeletonized by the time the perpetrator returns to bury the body. Tests of fully fleshed bone exposed for short periods should be conducted to determine how long it takes before exposure of remains on the surface produces the effects observed in this study. Finally, as environmental conditions such as weather and soil composition vary geographically, staining patterns and color of bone may differ depending on the region. Experiments should be performed to account for such variables in the future.

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