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TECHNICAL NOTE

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Histological Determination of the Human Origin of Bone Fragments

ABSTRACT: A frequently encountered task in the forensic scenario is verification of the human origin of severely degraded fragments of bone. In these cases histological methods which consider osteon size and morphology can prove to be useful. The authors in the present study verify the applicability of published algorithms to flat and subadult bones from human, dog, cat, cow, rabbit, sheep, pig, chicken, quail, and turkey samples. Metric analysis was performed on 2031 Haversian canals. Analyses carried out on human samples confirmed a success rate of around 70% on long adult bones; however the percentage of wrong answers was particularly high in the case of newborns and older subadults as well as on flat bones in general. Results therefore suggest that such regression equations should be limited only to bone fragments from long adult bones.

KEYWORDS: forensic science, forensic anthropology, species specificity, regression analysis, flat bones, subadults

Species determination is crucial in the study of small bone fragments coming from a forensic scenario (1). Genetic and protein tests may not always be applicable to degraded bone fragments because of the limits related to difficult extraction procedures along with the problems related to decay (2–7); therefore, anthropological methods may be extremely useful, and sometimes the only ones capable of providing a solution.

Histological qualitative and quantitative differences in mammalian bone structure were discovered at the beginning of the 20th century (8). For example, plexiform bone structure is common in large and medium sized mammals, whereas it is rare in smaller sized animals and never observed in humans (9), although cases of plexiform bone structure in humans have been reported in children with rapid growth spurts (10). Differences in bone and osteon morphology also are reported as being important for species determination (11), although age, sex, and pathology have been pointed out (12) as factors affecting bone structure.

On the other hand, metric analysis of osteons has been carried out with different regression formulae and different statistical analyses (5,9,13) on different bone segments. Recent literature has reported the importance of using both morphological observations of bone structure and osteon measurements for the diagnosis of species (9). Among the different methods, one in particular has proven to be reliable even on cases of charred bone, with a correct diagnosis of species in 79% of cases. However, such formulae have mainly been developed on long bones, and frequently from adult subjects; their reliability on flat bones or on subadults has never been verified.

This study therefore aims at testing the performance of one of the few existing specific discriminant canonical equations, published almost 10 years ago (5), which claims to discriminate between human and nonhuman long bone in 79% of cases, on flat and juvenile bone.

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Materials and Methods

The test included 25 flat bones and 18 long bones of *Homo sapiens* (flat bones equally distributed among 5 adults, 12 subadults, 4 newborns; long bones among 3 adults, 12 subadults, and 3 newborns), 9 flat bones and 11 long bones of *Canis familiaris* (dog, equally distributed among 3 adults and 3 subadults), 5 flat bones and 6 long bones of *Felix catus* (cat, all from adult individuals), 6 flat bones and 7 long bones of *Bos taurus* (bovine, 1 adult and 11 subadults), 2 flat bones and 2 long bones of *Oryctolagus cuniculus* (rabbit, from an individual of unknown age), 4 flat bones and 3 long bones of *Ovis ammon aries* (sheep, 4 subadults), 2 flat bones and 2 long bones of *Sus scrofa domesticus* (pig, 3 subadults), 2 flat bones and 2 long bones of *Gallus gallus* (chicken, 2 subadults), 4 flat bones and 4 long bones of *Coturnix coturnix* (quail, 4 subadults), and 2 long bones of *Meleagris gallopavo* (turkey, 1 subadult).

The long bone samples were obtained from femur and humerus, the flat bone samples from the cranial vault bones and the scapula.

From every bone 2 cm thick cross-sections were cut with a hack-saw, and 140 undecalcified 100 μm sections were prepared by grinding on a Struers Dap 7 Lapping machine, using abrasive paper with an increasingly finer grain (150–2000 grain size); the sections were embedded in Perthex resin on a glass slide and examined by transmission light microscopy at 63× and 100×. Metric analysis considered 2031 Haversian canals (5). The sections were then observed under light microscopy with a magnification up to 400× in order to verify bone structure differences between different species. Measurements were taken on all mature osteons which were not in the resorption phase; osteons and Haversian canals were outlined manually by using the computer software program "Shion Image" on a scanned-in photograph at a standard magnification of 63×. The number of Haversian canals which

TABLE 1—*Number of Haversian canals which underwent statistical analysis.*

	No. of Haversian Canals
Homo sapiens	
Foetuses and newborns	
Flat bones	30
Long bones	76
Subadults	
Flat bones	60
Long bones	81
Adults	
Flat bones	104
Long bones	125
Canis familiaris	
Subadults	
Flat bones	60
Long bones	62
Adults	
Flat bones	152
Long bones	177
Felix catus	
Adults	
Flat bones	73
Long bones	181
Bos taurus	
Subadults	
Flat bones	86
Long bones	124
Adults	
Flat bones	60
Long bones	61
Oryctolagus cuniculus	
Unknown age	61
Ovis ammon aries	
Subadults	
Flat bones	125
Long bones	120
Sus scrofa domesticus	
Subadults	
Long bones	60
Gallus gallus	
Subadults	
Flat bones	30
Long bones	30
Coturnix coturnix	
Subadults	
Flat bones	30
Long bones	30
Meleagris gallopavo	
Subadults	
Long bones	33

underwent the statistical analysis is shown in Table 1. With the same software Haversian canal area (C Area), maximum Haversian canal diameter (Max DC) and minimum Haversian canal diameter (Min DC) were then calculated for every osteon which underwent metric analysis; a mean value of each indicator and corresponding median, standard error, minimum and maximum values, were then statistically evaluated for every sample (i.e., slide) and then inserted into the following equation, as reported in literature (5,13):

$$D = -3.99 - 0.07(\text{C Area}) + 0.04(\text{Max DC}) + 0.07(\text{Min DC})$$

A positive value of D should indicate a human origin; a negative result a nonhuman origin. The percentage of correct diagnoses was then grouped according to flat and long bone, as well as adults and subadults.

Results

Results obtained by the application of this specific equation to bone sections are shown in Fig. 1; the percentage of wrong answers, including positive D results in animal bone samples and negative D results in human samples were compared.

One can see that the type of bone examined did not influence results among nonhuman animal samples; in six species the test correctly identified animal samples as nonhuman (*Oryctolagus cuniculus*, *Meleagris gallopavo*, *Coturnix coturnix*, *Gallus gallus*, *Ovis aries*, *Felix catus*) regardless of whether the bone was flat or long; in three other species the percentage of wrong answers was between 1.3% and 4.7% (*Canis lupus*, *Bos taurus*, *Sus scrofa domesticus*), but in most cases a nonhuman origin was identified; using flat bones and bone samples of different age also did not seriously modify the test reliability.

Percentage of wrong answers was then calculated among human samples, particularly with respect to type of bone and age (adults vs. subadults). The metric analysis carried out on human samples confirmed a success rate of around 70% (slightly lower than that initially reported in literature) (5) on long adult bones but showed several limits in other cases (flat or juvenile bones) where the equation recognized human samples as nonhuman. Percentage of wrong answers was particularly high in the case of newborns (93.3% on long bones) and older subadults (56.1% on long bones) as well as on flat bones in general (68% in newborns, 60% in subadults, and 71.8% in adults).

Discussion

This brief but called for study has demonstrated the limits in the application of specific regression equations developed mainly on long bones for species determination, when applied to flat bones and to juvenile skeletons. Application of the regression formula in other animal species did not present differences in error rate between long bones, flat bones, and juvenile bones; species determination was in fact correct in most cases, with an average percentage of wrong answers amounting to 0.9%. On the other hand, in the case of human samples, age and type of bone influenced the results; the percentage of correct answers was higher in case of bone samples from adults (as expected), with results similar to data reported in literature (5), whereas bone fragments from newborns and children in most cases did not lead to a diagnosis of human origin, perhaps because juvenile human bone is less differentiated at this stage with respect to other animals as Zoetis et al. (10) have already mentioned; the same however seems to be true for flat bones.

Similarly, human flat bones of adults gave a percentage of wrong results close to 72%. Therefore, the use of the regression formulae appears reliable for a correct diagnosis of human material only when dealing with adult individuals and long bones. New regression formulae must therefore be developed and tested to fill in this gap.

The described study has shown the risk in using recommended regression equations to diagnose the human origin of bone fragments when samples are not provided from long bones and adult individuals. On the other hand the impact of increase in false positives in other animal species is negligible. Previously published regression equations should therefore be used only on bone fragments which appear to be mature (adult) and coming from long bones, and always with a cautious analysis of osteon morphology and distribution as well.

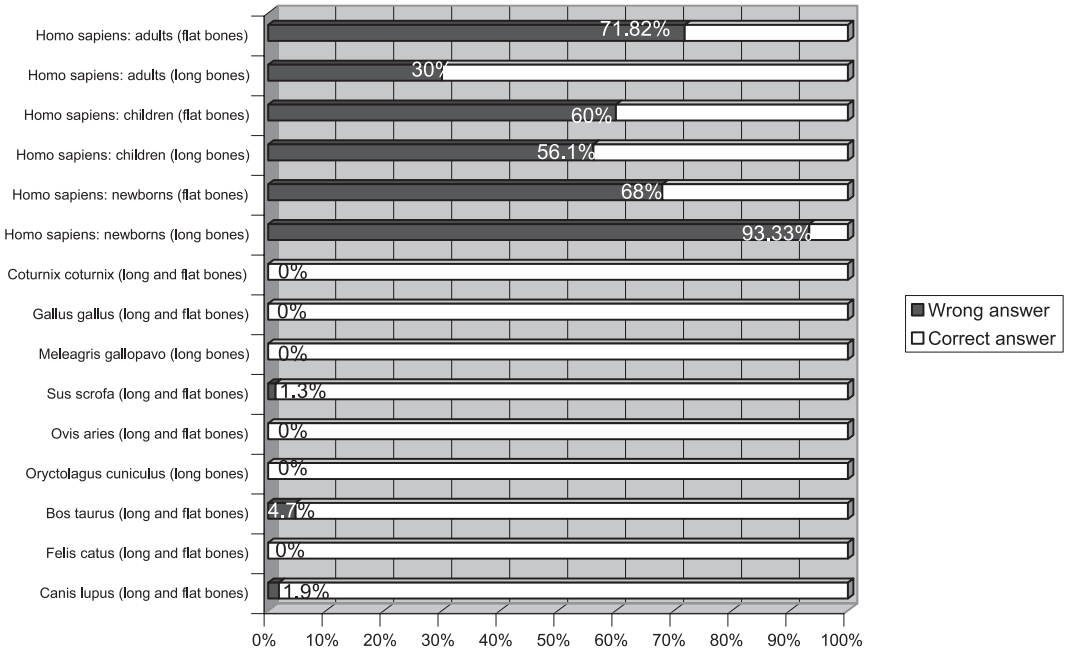

FIG. 1—*Percentage of wrong answers for different species and types of bone.*

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