

Improved sampling strategy for DNA typing of skeletal remains in missing persons casework

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Introduction

Traditionally, teeth and larger long bones are the preferred samples for DNA based identification of skeletal remains, whereas smaller bones are used only as a last resort. However, in the case of fragmented and commingled bodies, testing a variety of skeletal elements becomes a necessity to assist with the re-association of the remains. We present empirical data on DNA testing success rates from a variety of skeletal elements to allow better informed sample selection in missing persons case work when effective demineralization extraction techniques are used.

Material & Methods

Archaeology & Anthropology

Data from different excavation sites were compiled in order to assess the influence of the taphonomic conditions on the DNA typing success. Three types of sites were selected containing individuals buried between 1992 and 1995 and recovered between 1996 and 2010:

- (1) **Mass grave:** 9 large mass graves linked to a single event containing complete skeletons and commingled skeletal elements;
- (2) **Underwater site:** body parts recovered from the banks of a drained river;
- (3) **Surface site:** remains recovered from two similar surface sites.

DNA

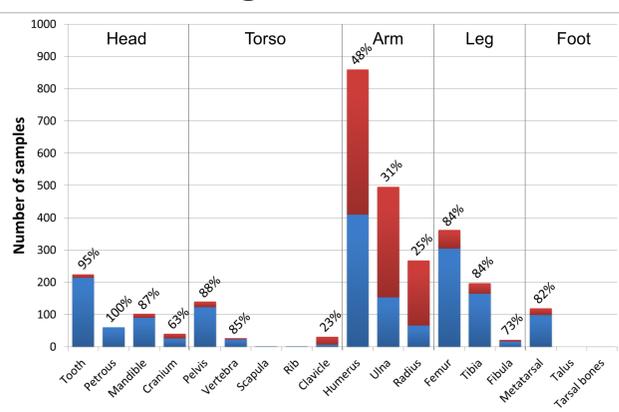
The samples were subjected to a decontamination procedure and were subsequently ground into fine powder. The powder then underwent a full demineralization DNA extraction process. All samples were at least amplified in duplicate using the PowerPlex 16 kit (Promega).

DNA profiles were analyzed using a full consensus approach (alleles called only if confirmed in multiple amplifications) and submitted to the ICMP database for matching if more than 11 loci + Amelogenin were successfully amplified. The entire process is described in *Huel et al., 2011, Methods in Molecular Biology, vol. 830*. Since ICMP's introduction of the full demineralization extraction protocol in 2009 over 11,000 samples have been analyzed.

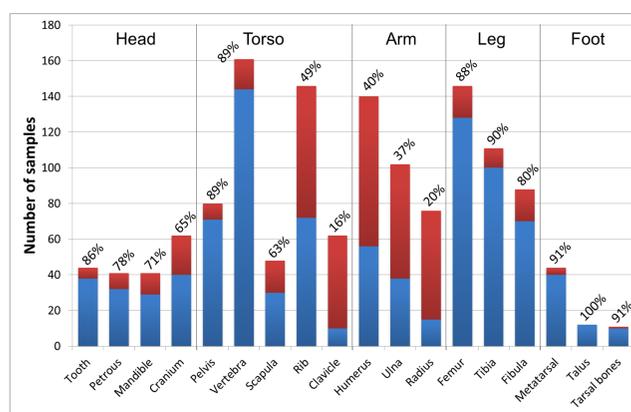
Results

Figures 1, 2, 3: Proportions of samples typed successfully vs. failed samples per skeletal element. The success rates per element are indicated above each bar.

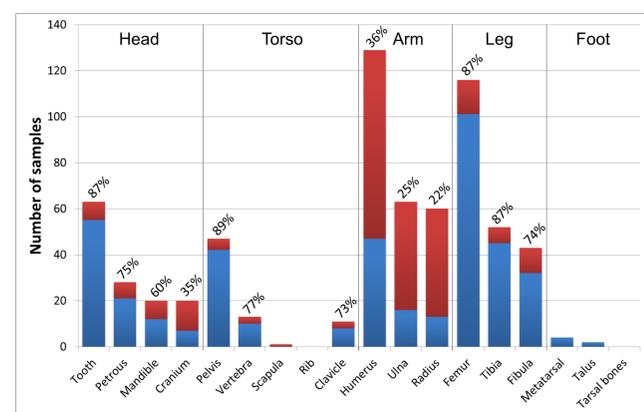
1. Mass grave – N = 2941



2. Underwater site – N = 1442



3. Surface site – N = 672

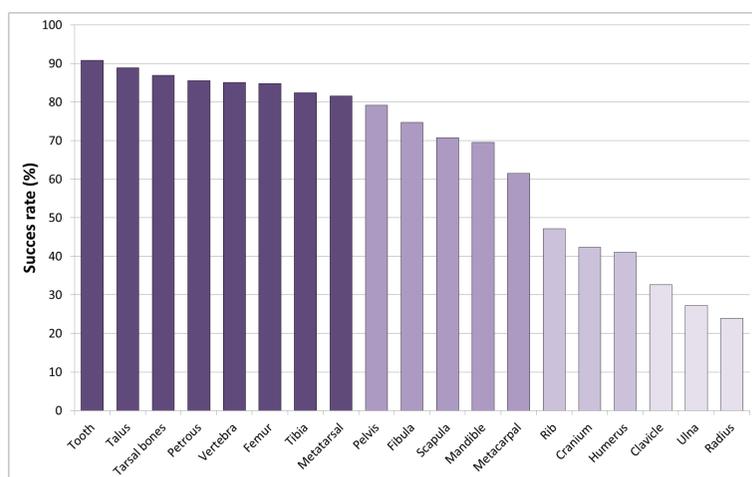


Success rates not shown for elements with N < 10

The large number of samples for arm bones, particularly the humerus, is explained by the fact that arm bones were originally considered the best choice when only upper torso remains were available. The taphonomic conditions had a lesser influence on the results than expected. For some elements, small variations were observed but the overall ranking was not significantly modified.

This data was combined with success rates obtained from hundreds of additional sites (Figure 4) and a ranking system applicable to most situations was generated (Figure 5). This priority system ranks elements from 1 (preferred) to 4 (least favored) and is further broken down by body part to assist pathologists and anthropologists with sample selection from fragmented or commingled remains.

4. Combined success rates – N = 11,651*



5. Sample Ranking*

1 > 80% 2 60%-80% 3 40%-60% 4 < 40%

Body part	Element	Rate	Rank
HEAD	Tooth	91%	1
	Petrous	86%	1
	Mandible	70%	2
	Cranium	42%	3
TORSO	Vertebra	85%	1
	Pelvis	79%	2
	Scapula	71%	2
	Rib	47%	3
	Clavicle	33%	4

Body part	Element	Rate	Rank
ARM	Humerus	41%	3
	Ulna	27%	4
	Radius	24%	4
HAND	Metacarpal	62%	2
LEG	Femur	85%	1
	Tibia	82%	1
	Fibula	75%	2
FOOT	Talus	89%	1
	Tarsal bones	87%	1
	Metatarsal	82%	1

* Other bones with a sample size below 10 were not included in the figures above. These bones include: patella, hand and foot phalanges, carpal bones, sacrum and sternum.

Discussion

The data indicates that smaller bones previously thought to be poor candidates for DNA testing, such as foot bones and vertebra, are actually good samples. Such information will greatly assist missing persons cases where forensic experts now have a larger choice of samples for analysis to help in the re-association of remains. It is important to note that success rates can vary depending on the DNA extraction protocol used.

The success rate should not be the only factor in sample selection of disarticulated remains. Many additional factors must be considered regarding a specific case. Sampling skeletal elements displaying forensically relevant information, such as evidence of trauma or other identifying features, should be avoided.

This data has been adopted into ICMP's standard operating procedure for DNA sampling (available upon request) which is applicable to most scenarios ranging from an isolated bone to a complete body. This strategy also improves cost efficiency by decreasing the number of tests required to obtain a DNA profile.