Elusive Enamel Protein:

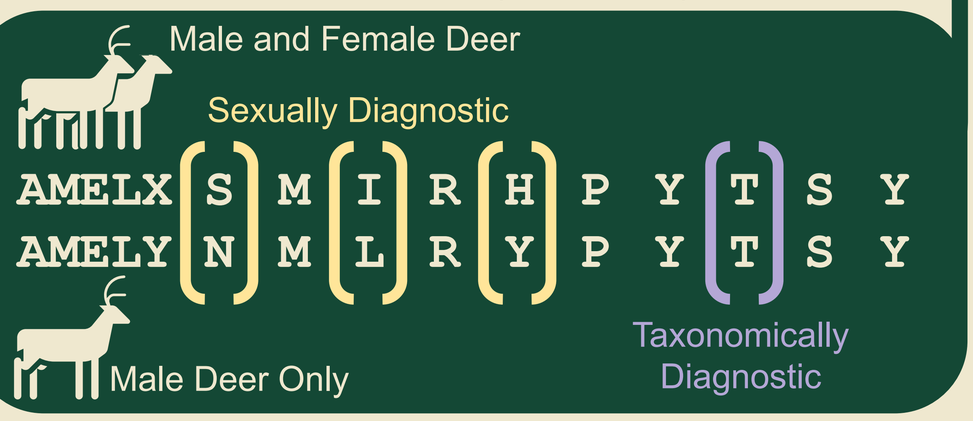
Developing a Method to Detect the Y Isoform of Amelogenin in Archaeological Cervids

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Proteomic Sex Estimation

The sex chromosome-specific protein amelogenin allows sex estimations to be made from tooth enamel alone[1,2]. This facilitates the estimation of sex for a range of ancient animal remains, including those which are juvenile or fragmentary[3]. Amelogenin-based sex estimations have been applied to archaeological humans[1,4,5] and Bovids[6,7,8,9] but could theoretically be applied to other Eutherian mammals. High throughput methods have also been developed allowing the sex of whole assemblages to be estimated[8,10,11]. Expanding amelogenin-based sex estimations to include deer species has the potential to provide insight into past human-environment interactions.

Initial attempts to estimate the sex of Cervids fail to consistently detect peptides diagnostic to the Y isoform, therefore further method development is required.



(2) Digestion

Current protocols used to analyse amelogenin in non-human animals do not perform an enzyme digestion[6,7,8,9], instead relying on the natural digestion of enamel proteins as part of amelogenesis[12].

The cleavage patterns of amelogenesis for deer species in archaeological contexts have not been investigated. If peptides are too large for LC-MS/MS analysis, then the addition of a digestion step to the method may be beneficial.

(3) LC-MS/MS Analysis

Study	Instrument	Detection Strategy
6	Quadrupole-Orbitrap	DDA
7	Quadrupole-Orbitrap	DDA
8	tims-ToF and Orbitrap	DDA
9	Tribrid (Likely Quadrupole-Orbitrap)	PRM

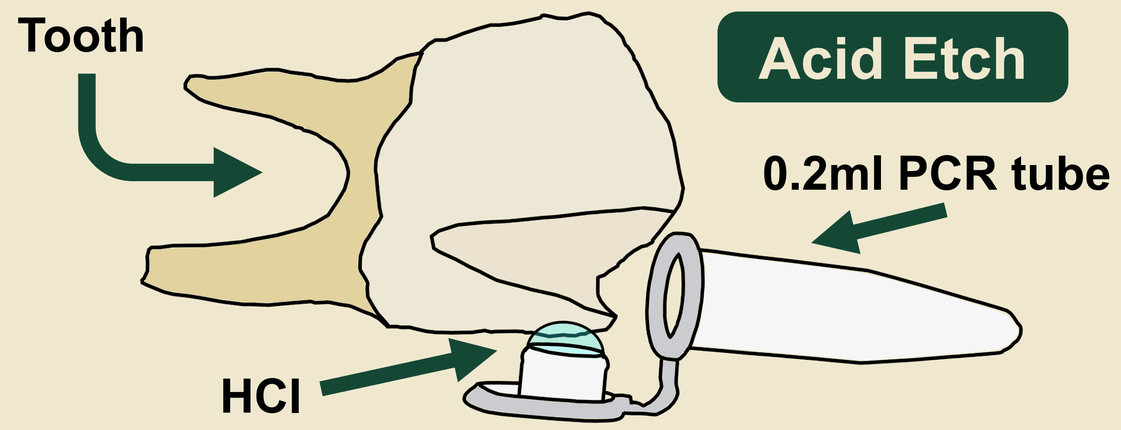
Deer teeth analysed by tims-ToF with a DDA strategy have so far only inconsistently yielded peptides diagnostic the Y isoform of amelogenin.

Instrument comparisons analysing archaeological amelogenin with a DDA strategy found no change in coverage when using faster tims-ToF settings compared to analyses with an Orbitrap, however there was a reduction in peptide count[8], highlighting the potential value of varying the instrument and search strategy applied.

Methods:

What is Currently Applied and What Could Change?

(1) Protein Extraction



Presently only a two minute surface etch with 10% HCl has been applied to deer teeth (as in study 8). The short surface etch has yielded good coverage of enamel proteins other than amelogenin Y isoform.

Given the variation in extraction techniques applied to non-human animals in other studies this is one area where the method could be modified.



Study	Species	Chemical	Method
6	*Bison bison*	5% HCl	10 minute surface etch
7	*Bos taurus*	18% EDTA	Powder dissolved
8	*Homo sapiens*	5% HCl	2 minute surface etch
8	*Bos taurus*	10% HCl	2 minute surface etch
9	Caprinae	5% HCl	Chip dissolved

Insight into Human-Animal-Environment Interactions

Once possible, sex estimations for deer species can be synthesised with other biomolecular and zooarchaeological information such as mobility data (from strontium stable isotope ratios) and age estimates (from tooth wear) to understand past herd structures and hunting strategies. Combining these analyses better facilitates the study of Human-Deer-Environment relationships throughout the past.



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