

ABSOLUTE AGEING OF CATTLE FROM TOOTH SECTIONS AND ITS RELEVANCE TO ARCHAEOLOGY

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From at least medieval times hunters, veterinarians, and farmers have recorded ways of assessing an animal's age. Many of these methods involve the hard parts studied today by archaeozoologists. Yet most of these methods are for assessing relative age only and in modern ecological studies in mammalogy and game management have either been replaced or calibrated by techniques for determining the absolute or real age in years of individual animals. Over the last 30 years these have concentrated to a large extent on incremental lines (also called 'growth lines', 'growth rings' and 'annuli') developing in tooth and bone tissue in mammals.

Papers in the early fifties such as that of Laws (1952) for elephant seal (*Mirounga leonina*, Linn.) described clear and beautiful rings for dentine deposition in marine mammals. These lines were likened to tree growth rings; growth rings in scales, otoliths, opercular bones and vertebrae of fish; and growth rings on oysters and other invertebrates.

As in many of these examples of growth rings, those in mammalian teeth are caused by a change in the rate of deposition (in this case of dentine or cementum) such as may occur on an annual or otherwise regular basis as a result of a check or slowing down in growth. Closer examination can show fine lines between the major ones. This paper describes an attempt to adapt these methods for use on cattle teeth from archaeological excavations and relates the methods used to earlier relevant work and possible future development of the techniques.

Incremental Growth in Ecological and Game Management Studies

A wide selection of species has been investigated for incremental growth in bones and teeth and anyone interested should certainly consult the works by Klevezal and Kleinenberg (1967) and Morris (1972).

In much recent ecological work on mammals tagging of young animals has in turn led to calibration and checking of tooth line data as a result of the presence of 'known age' animals in

populations of wild animals. Incremental data have thus been under even closer scrutiny and methods have evolved with some rapidity. But the influence of tagging has also meant that relative ageing techniques, like tooth wear, which can be used more easily and on live animals, have been improved and calibrated in some species. This could mean that in the future less research ^{on incremental growth} is likely to take place as the matter may have been developed as an adequate zoological tool for that species. This ^{is} unfortunate in two ways, first because the causes of the incremental lines are by no means fully understood, secondly the use of the techniques in archaeozoology has barely begun.

Archaeozoological Requirements

Some of the most useful incremental lines for mammalian ageing are those occurring as very clear rings in transverse sections, or cones in longitudinal sections, by the growth of dentine into the pulp cavity. These may show especially well in marine mammals. Better in many species is the deposition from the outside of extra layers of tooth cementum as, unlike the dentine, there is no spatial limitation on this deposition. Morris showed for hedgehog that there may also be appositional bone layers in the peripheral bone at the base of the mandible (Morris 1970).

These are therefore three possibilities for calibrating the mandibular tooth wear that archaeozoologists take so seriously. One advantage of incremental growth analysis over tooth wear analysis besides its 'absolute' quality might be greater accuracy in determinations from single teeth.

For the domestic ungulates cementum ^{sectioning} seems the most useful method of the three as its reliability is a common point of agreement in most work on modern ungulates (e.g. Gilbert 1966). The best results in modern studies are often obtained from canines, incisiform canines, or third incisors (I_3) but cheek tooth results would obviously be much more useful for archaeological work as these have had the major input of effort over recent years.

A second need is for the method of absolute ageing to cover as many individuals as possible and for this reason the first lower molar M_1 is probably the most suitable tooth although mechanically its position puts it under pressures which the other molars do not have to meet, especially during the eruption of the permanent premolars.

A third need is for any method of preparation to take into account the relative friability of archaeological material and it is suggested that, except where insufficient collagen remains, decalcification and the use of a freezing microtome offer advantages over the making of thin sections by grinding. This is discussed more fully in the section on methods.

As well as an ageing method, incremental growth has, from quite early days, been suggested as a way of determining season of death of the animal and obviously development of this aspect would be useful for archaeology and would result from further analyses into the cause of growth lines.

Incremental Growth in Domestic Species

With a few notable exceptions the work on incremental growth in mammal teeth has been for wild mammals. Modern domesticates are usually of known age or their ages can be adequately assessed on both live and dead animals using easier methods like incisor eruption and wear. A small amount of work on domesticates has occurred by people with an interest in archaeology. Saxon and Higham (1968, Fig. 1) showed on a sample of New Zealand ewes that transverse sections at the base of the crown viewed by phase contrast microscopy produced an alternation of 'white' layers and 'dark' layers. The former showed pale by what was presumably positive phase contrast and were regarded as representing Winter deposition. The 'dark' layers were thought to represent Summer deposition. Results are shown for an animal of 5 years 7 months (time of death presumably Autumn) which shows six dark layers corresponding it is believed to 6 summers and five white layers denoting lines of Winter retardation. There appears to be the beginning of a white edge after the last dark layer. The explanation for this apparent discrepancy is probably that according to Morris (1972), quoting a paper in Russian by Kleinenberg and Klevezal (1966) these bands are now known to have the reverse explanation to that given by Saxon and Higham. Low density active growth (probably the white band here) is likely to be the Summer one and the high density dark band (the one which takes up stain in serial sections) probably represents a slow accumulation over Winter. Turner's work on a wild sheep, the bighorn, Ovis canadensis, like that of several other writers showed clear Winter arrest resulting he suggested from both poor feeding and the rut. Desert sheep show a cessation of horn growth and cemental increment slowing during the Summer season (Turner 1977). This paper highlights another point of relevance to archaeology - so long as an approximate date of birth was known and

the date of death known the age of the modern bighorns could be estimated to the nearest month. This might be useful as a technique on seasonally occupied sites.

Saxon and Higham's work made the important point that thick cementum layers as at the root tip were not good for counting annuli, being too convoluted and interspersed with cells. Their explanation for the cementum rings as mirroring behaviour of the root in connections it makes with the periodontal membrane are difficult to understand as the picture used (Saxon & Higham 1968, Fig 1) seems to show crown cementum, however this may have come from below the gum even if not within the socket.

The use of transverse sections may also be misleading (Morris 1972, 88), although some workers point to disadvantages of longitudinal sections. The transverse/longitudinal controversy is well-aired by Gasaway (1978).

Saxon and Higham's ewes had been Winter fed and thus a physiological rather than a straight dietary reason was suggested for growth checks. This is reasonable as intake itself may drop in some species in spite of available food. They do not however say whether or not the animals were kept under cover during Winter. Finally this important work mentions that incremental lines had also been seen on pig and cattle teeth.

Both Klevezal and Kleinenberg (1967) and Grue and Jensen (1976) showed that farm-raised animals also developed incremental lines correlated with annual cycles and Grue went further in an investigation of the highly domestic and unnatural dog (Grue 1976). Specimens included a wide range of individuals from those kept as pets to Greenland sledge dogs kept in the open and seeking their own food for part of the year. Lines were found in all but a specimen of less than 12 months. ^{for the domestic dogs} The lines were generally fainter and more poorly defined than in similarly prepared sections from decalcified material of red fox, Vulpes vulpes, and a wolf, Canis lupus. The number of lines far exceeded the number of years the animal had lived but the results for the sledge dogs were more like those for wild carnivores suggesting that neither captivity nor seasonal uniformity of diet affect the lines (Grue's unpublished work on mink supports this view) but that highly domesticated indoor life may cause animals to lose the synchrony observed in wild species.

There is some evidence (Miles 1961) that this may have occurred in man as well.

Park red deer, Cervus elaphus, fed hay and root crops in Winter

showed significantly greater numbers of animals with difficult teeth in which bands were indistinct (Mitchell 1967, 284). If this phenomenon is generally recognised then it may indicate that a degree of physiological change has occurred during the evolution of park or domestic animals and that this and/or Winter feeding might boost growth, where a wild animal would suffer a longer period or one unbroken period of quiescence.

Incremental Evidence from Archaeological Material

Saxon and Higham included archaeological material in their sectioning, using the same method as used for the modern ewes, on sheep teeth from Iron Age Barley, England. A comparison of their two figures shows that the archaeological section is in some ways more difficult to interpret, possibly each 'dark' layer looks more uniform.

Grue (1976) had little luck with some Mesolithic dog teeth which had lost too much organic material to respond to her methods of decalcification but a section prepared by K. Rosenlund of a medieval cat canine was illustrated. Grue suggested that a more gentle decalcifying agent such as EDTA (ethylenedinitrilotetracetic acid) might give better results. This was successfully used on teeth of very young seals by Scheffer and Peterson (1967).

Movius () describes successful decalcification of 20-35,000 year old teeth from the Upper Palaeolithic in France whereas zebra and hartebeest teeth from Kenya only 18,000 years old had too low a collagen content (Gramly and Rightmire 1973). Bourque + Morris (1973) prefer thin sectioning.

Kay (1974) investigated incremental lines on the teeth of white-tailed deer, Odocoileus virginianus, from a central Missouri, Middle Woodland settlement using a modification of Ransom's (1966) method for thin-sectioning modern white-tailed deer. This was with a view to assessing both age and season of death - the latter by the colour of the final ring. Modern work on this species had been considerable. Lockard had already discarded the theory of 'rut lines', showing that these were 'split annuli' in the cementum occurring mostly at the sides of the tooth (Lockard 1972, 54). He had also usefully discussed various types of 'false annuli' - a topic highly relevant to sections of archaeological material. In spite of impregnation Kay's thin-sectioning methods produced microscopic cracks on the cementum in most sections which he suggests may have obscured lines in some teeth. Lines were used for age estimates and the time of death was assessed for most specimens as the first few months of the deer's annual cycle (probably late Spring and early Summer) as part of a broad light band formed at this period of rapid growth was visible.

Spiess (1976) made a general plea for the use of tooth evidence for determining season of death in archaeological material and

sectioned seal teeth from late 18th and 19th century and protohistoric eskimo sites to test whether time of occupation of the site could be confirmed. This was possible so long as the habits of the species concerned had been worked out on modern material - he compared the archaeological seal teeth with results on ringed seal, Phoca hispida, confirming occupation on one site as early to late Spring, the time of fasting and moult in these animals when a dark band had just formed. Decalcified, stained sections were used.

Methods used for Modern Cattle Teeth

Roger Jones experimented during 1976 with various methods of sectioning teeth from archaeological sites and decided that cattle would be the best species for an initial investigation. This established that incremental lines could indeed be seen (Plate 000) and that these showed up best by using decalcification of sawn 2-3mm thick longitudinal slices. Sectioning by freezing microtome was easier for orientation and more suitable for archaeological material with its increased friability. Permanent staining with haematoxylin gave good results. The timing of decalcification was crucial and this was monitored by means of X-ray photography in the initial experiments so that decalcification could be recognised immediately and overtreatment avoided. In the material used good lines were seen in the crown pad (Fig 000, Area A) and at the ends of the roots (Area B). It was decided that M1 would be the best tooth to use.

As there was no comparable work on modern cattle a feasibility study was needed which would also relate incremental growth to tooth wear. In 1978 Keith Turner undertook this as part of an undergraduate dissertation in the Department of Biology, University of Southampton. The account of the methodology which follows is taken from this work (Turner 1979).

Twenty-five lower jaws (i.e. right and left mandibles) of cattle were accumulated by a local knacker near Andover, Hampshire. Of these 18 were fresians and all but two were cows or heifers (Table 000). All were from local farms and were given an approximate age by the knacker based on information from the owner and his own experience. These ages were probably to the nearest month in the first year animals and the nearest year for older individuals. The boned-out mandibles were frozen until needed and cleaned by gentle cooking. Rotting may have been a better method but there was not time for this nor suitable accommodation and there was no evidence from the sections obtained that these stages, or the subsequent sodium perborate treatment of some mandibles, affected the cementum layers.

Both left and right first molars were then removed very carefully from the jaws after recording details of cheek tooth wear according to Grant (1975, 438) and incisor details according to a modified version of Andrews (1973). Removal of the tooth was most easily accomplished by removing the bone from one side of the tooth roots although Jones had shown that a better method with better equipment was to remove the whole tooth together with its surrounding area of mandible with the same saw used for cutting the tooth. Each M1 was then cut into 3 portions longitudinally with a diamond saw leaving the middle portion approximately 3 mm thick. This section was then decalcified using 5 % formol-nitric prepared as follows:

50 cm³ saturated formaldehyde solution

50 cm³ nitric acid (specific gravity 1.42)

This was made up to 1,000 cm³ with distilled water as needed. Concentrated formol-nitric should not be stored as it is spontaneously explosive. Each tooth was treated individually in a 3" x 1" specimen tube at room temperature the progress of decalcification being checked twice daily with ammonia solution. The formol-nitric was replaced every 24 hours. When the ammonia (0.880 solution) gave no white precipitate a more sensitive test using saturated aqueous ammonium oxalate was then ^{tried}. The decalcifying solution from around the tooth was allowed to stand ^{with ammonium oxalate} for 30 minutes and if no cloudiness appeared it was judged that decalcification was complete. This stage was too advanced and a balance was later struck during the experiments between allowing as much decalcification as possible but preventing damage to the tissue. This stage occurred at about the point that the gross section changed from being stiff to slightly flexible. This took 2-3 days.

The slab of tooth was then given two changes in 5% formalin (each for two hours) to remove the acid and stored in 5% formalin. Medial longitudinal sections were cut using a Leitz freezing microtome maintained at -20°C. The area chosen for counting lines and measuring the width of the cementum layer was the anterior region of the cementum in line with the dentine/cementum boundary of the molar pad (Fig. 000, Area C). Several sections were examined for each tooth from the most medial position within this area.

The staining procedure used was as follows:

1. 15 seconds in dilute sodium hydroxide solution (2, 5mm pellets in 50cm³ distilled water) to neutralize any remaining traces of acid.

2. 90 seconds in 0.1% toluidine blue.

3. Mount in water.

Thomas (1977) recommends the use of 0.01-0.03% toluidine blue and other water-soluble stains in tooth sectioning. Permanent mounts can be made by mounting direct in glycerine jelly and sealing with varnish.

Of the original 50 modern teeth, 41 produced good sections. Of the 9 failures, two were from the calf that died at birth and in the others the cementum separated from the dentine during preparation. The dark bands tended to be rather variable in appearance. Some stained very strongly and produced a well-defined line, others were considerably fainter and more difficult to discern. An apparently broad, diffuse band could generally be resolved into 2 or more distinct bands on closer scrutiny. They were regarded by Turner as separate bands if a region of lightly-staining cementum was visible between the bands for at least part of the way. Resolution at the periodontal edge was difficult. Discontinuity of lines was also far more of a problem at the tip of the root and the crown pad. Lines were only counted if they were visible for at least half the length of the cement visible in the field of view at a magnification of 100x. Lines like this were rare.

Results of the Study

The histological results on this basis showed a relative increase in the number of incremental lines acquired with age with both the number of dark-staining lines and the cementum width at Area C (Fig 000) being correlated both with the approximate ages of the cattle and with the Grant wear stage. The interpretation of the bands was not however easy and quite large discrepancies observed between results from left and right mandibles of the same animal for some individuals suggest that either the areas observed were not quite comparable or that this is still not the best place for reading the annuli. No similar discrepancies have been recorded elsewhere to our knowledge although one or two workers have used both jaws, sometimes with different methods. Only two of the animals studied (nos 21 and 22) showed a difference in tooth wear pattern in the two jaws and these did not have a very noticeable discrepancy in counts.

Results are shown in Table 000. Very high line counts may be a result of several methodological problems which need sorting out. One factor could be 'line splitting' already referred to and another could be overcounting as a result of counting all the constituent lines in what should be regarded as the 'growth check' band.

The time limits of an undergraduate project precluded further investigation of, for example, the areas less affected by functional stress, although Turner's preliminary studies seemed to suggest that these were equally or more difficult to interpret than the area used (Fig 000, Area C). All the material used in the study, including the remains of the gross sections, has been kept.

Methods used for Saxon Cattle Teeth

Cleaned mandibles from excavations at Melbourne Street and Chapel Road excavations (Saxon Southampton) were used. They came from Middle Saxon levels dating from between AD720 and AD 950 and each mandible contained M_1 and at least one other tooth (usually at least two) with eruption or wear data.

The methods used were similar to those described above for the modern teeth except that central gross sections sometimes had to be cut up to 6mm thick in friable specimens and decalcification took from 2 to 7 days.

Of the 87 mandible fragments supplied, only 37 yielded acceptable sections. Nineteen were considered to be too young to section using these techniques, 4 were already too broken and not used, 13 were decalcification or microtome failures, and in 14 the bands were not clearly visible in the cementum due to excessive cellularisation.

Results for the Saxon Teeth

The archaeological samples showed better correlation between the number of lines and the tooth wear stage, and the cementum width and wear stage than did the modern material. Values of 0.872 and 0.722, respectively, were obtained using Spearman's Rank Correlation Coefficient, giving highly significant results with a probability of less than $p = 0.01$. Results are shown in Table 000.

Modern and archaeological material demonstrated different relationships between the number of lines and cementum width as can be seen by the two very different regression lines in Fig. 000. Archaeological teeth had fewer lines with a mean of 35.00 bands/mm cementum whereas the modern teeth gave a mean of 81.24 bands/mm. There could be many factors involved in this difference, some of which have been discussed above - such as the possibility of overcounting the modern lines. If these are real differences, and obviously further work is needed, then the uses of incremental growth lines in cattle teeth from archaeological sites are even more interesting in their possibilities than was suspected.

Conclusions

As with many other archaeozoological methods the value of incremental growth lines on teeth is dependent upon results from modern comparative work. Only by understanding the causes of these lines in suitable modern parallels can real differences in archaeological teeth be pinpointed and interpreted.

More work on modern cattle is certainly needed. It would be useful to analyse a large modern sample of known age cattle where sex, nutritional history, and method of housing in Winter (if any) were known. More investigation into methods of decalcification, sectioning, and staining, especially for the younger age groups is also needed.

The preliminary work described here and that of others which has been discussed have shown, however, that the teeth of modern domesticates and those from archaeological sites do show incremental growth lines. But as yet these may be more difficult to interpret than those in many wild species.

On the basis of comparison with a modern sample (inadequate in that it was often unhealthy stock and from a distance away) the Saxon cattle teeth examined here produced incremental lines which, if not yet an absolute age indicator, at least could act for an assessment of relative age. Until it is understood whether lines are absolute age indicators in modern cattle it is not possible to assess the degree to which comparison of incremental lines on teeth from different archaeological sites could act as indicators of differences in, for example, climate or husbandry.

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TABLE 1

ERUPTION STAGE OF THE ANTERIOR TEETH IN CATTLE

(This is a modified version of Andrews (1973))

<u>Category</u>	<u>Stage of anterior tooth development</u>
1	Only deciduous teeth present
2	One of first permanent pair present and/or one/both of deciduous absent
3	Both first permanent pair erupting
4	Both first permanent pair at least $\frac{1}{2}$ up
5	One of second permanent pair present and/or one/both deciduous absent
6	Both second permanent pair erupting
7	Both second permanent pair at least $\frac{1}{2}$ up
8	One of third permanent pair present and/or one/both deciduous absent
9	Both third permanent pair erupting
10	Both third permanent pair at least $\frac{1}{2}$ up
11	One of fourth permanent pair present and/or one/both deciduous absent
12	Both fourth pair erupting
13	Both fourth pair at least $\frac{1}{2}$ up

TABLE 2

RESULTS FOR MODERN TEETH IN PROBABLE ORDER OF INCREASING AGE

Ref. no.	Grant value	Andrews I stage	knacker's estimate	Breed	Sex	RIGHT		LEFT	
						no. bands	cementum width	no. bands	cem. width
5	10	1	9 mo	Friesian x Hereford	Q	6	0.045	-	-
3	10	1	10 mo	Charolais x Hereford	Q	-	-	0	0.03
4	17	1	9-10mo	Hereford	Q	-	-	1	0.06
23	19	1	14 mo	Friesian	Q	6	0.09	8	0.08
8	26	4	3 yr	Friesian	Q	7	0.08	-	-
10	32	7	4-5 yr	Friesian	Q	-	-	8	0.08
20	33	10	3 yr	Friesian	Q	-	-	3	0.05
15	37	7	5 yr	Friesian	Q	12	0.12	10	0.135
19	39	10	3 yr	Friesian	Q	11	0.13	9	0.11
21	39R 41L	13	6-7 yr	Ayrshire	Q	9	0.085	6	0.075
22	39	13	5 yr	Ayrshire	Q	8	0.08	8	0.075
2	39	13	5-6 yr	Ayrshire	Q	9	0.13	5	0.10
13	39	13	?	Friesian	Q	10	0.105	13	0.12
9	40	13	5-6 yr	Friesian	Q	10	0.09	10	0.10
25	40	13	3 yr	Friesian	Q	9	0.06	8	0.09
16	41	13	6 yr	Guernsey	Q	14	0.16	7	0.08
12	42	13	7 yr	Friesian	Q	16	0.155	20	0.20
18	43	13	3 yr	Friesian	Q	9	0.085	10	0.075
1	44	13	5-6 yr	Friesian	Q	7	0.075	16	0.16
14	44	13	5 yr	Friesian	Q	12	0.17	14	0.20
17	44	13	5 yr	Friesian	Q	14	0.14	9	0.11
24	45	13	8 yr	Friesian	Q	14	0.135	14	0.145
7	45	13	6 yr	Friesian	Q	-	-	9	0.11
11	45	13	?	Friesian	Q	15	0.14	11	0.16

TABLE 3

RESULTS FOR ARCHAEOLOGICAL MATERIAL IN PROBABLE ORDER OF INCREASING AGE

e estimates made where teeth missing

S.A.R.C. site	Feature	layer	Grant Value	Side	No. bands	cementum width mm
XXIII	10	iv	18e	R	2	0.055
VII	53		19	L	1	0.05
VI	31	iii	22	L	1	0.04
VI	35	v	24e	R	4	0.08
IV	3521		24e	R	3	0.04
V	27		30e	L	2	0.06
VI	30	v	31	R	4	0.07
VI	36	ii	31e	L	4	0.09
XXIII	12	iv	31e	L	5	0.11
VII	43	iv	33e	R	6	0.07
IV	3520		34e	R	4	0.05
XXIII	7	i	34e	R	2	0.07
IV	19		36e	R	4	0.09
IV	15		37	R	4	0.105
IV	13		38	R	8	0.09
VII	53		39e	R	7	0.095
VIII	7	iii	40	R	3	0.09
IV	3523		41e	R	1	0.07
XX	114	i	41e	L	6	0.19
XI	24		41e	R	5	0.10
XXIII	2		41e	L	4	0.09
VI	?	x	42	R	5	0.095
VIII	54		42e	L	7	0.13
VI	4	x	42	L	10	0.14
XXIII	10		42e	R	5	0.16
V	17		43e	L	7	0.205
VI	30	viii	44	L	7	0.27
XI	72		44	R	7	0.165
I	4		44e	L	8	0.18
IV	15		45e	R	5	0.12
V	21		46e	L	3	0.10
VIII	7	v	46	L	8	0.16
IV	17		47	L	7	0.205
VI	39	iv	48e	R	17	0.30
V	34		48e	R	7	0.20
IV	3512		48e	L	8	0.23
VI	8	iii	49e	L	10	0.295