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Age assessment of red deer *(Cervus elaphus):* from a scoring scheme based on radiographs of developing permanent molariform teeth

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The stages of permanent tooth development observed in radiographs of the mandible are described and analysed for known-aged red deer *(Cervus elaphus}.* The ages by which the different stages of development were reached have been determined. By allocating scores for these different stages, the scores that may be expected for a particular age have been identified. Lastly, the predicted age was given, together with 95% prediction intervals obtained from a regression of age on total molariform scores. Tooth development in red deer was usually completed by 33 months. These data can be used to assess the ages of animals of unknown age.

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Introduction

Archaeozoologists and wildlife biologists in the past have assessed the ages of animals from the eruption status or by evaluating stages of tooth wear. Only rarely has the justification for assigning age in these ways been validated by examining the stages in animals of known ages. Though wear patterns are a useful guide to age, and are the only dental guide to age once the teeth are fully formed, it is likely that a more accurate assessment of age can be made from the several specific stages identifiable in the course of tooth development. This is well established for man (Demirjian, Goldstein & Tanner, 1973) and for cattle (Brown *et at.,* 1960). McCance, Ford & Brown (1961) reported from an experiment with undernourished pigs *(Sus scrofa)* that forming teeth were the least likely of the development processes to be disturbed. The only limitation on assessing age from tooth development in red deer is that the teeth are fully developed in just over three years. Though later changes due to age, as increased thickness of the cementum and narrowing of the pulp canals, can be seen, they proved too inconsistent to be relied upon. After three years, age may most easily be appraised from the wear of the teeth but, as has been shown (Brown & Chapman, 1990), in older animals, the range of possible age increased by + or — two years. Cementum incremental lines may be examined histologically and can give the best absolute age for many species in some environments (Stallibrass, 1982), but these procedures are lengthy and costly. The presence or clarity of the lines varies among different populations of red deer so their interpretation is not always easy. In ground, polished sections of red deer teeth from Richmond Park no lines were seen (Mitchell, 1980, pers. comm.). This is probably because the mild winter climate and year-round availability of food prevent a noticeable seasonal check in growth.

The scoring scheme that we devised for ageing fallow deer from the status of their developing teeth (Brown & Chapman, 199 la) has been applied to red deer.

Materials and methods

Sample

The sample consisted of 113 red deer, 60 males and 53 females of known age from 1 to 138 months (Table I). For convenience the sample was arranged in 8 age groups. A preliminary examination of the developing teeth showed no consistent differences between the sexes. Because of the small and uneven numbers of male

TABLE I

Red deer ***(Cervus elaphus),*** *sample size, age and sex*

Age (months)	Males	Females	Totals
1-11	12	6	18
13-19	4	20	24
26-33	19	9	28
38-42	13	2	15
50-55	4	7	11
63-66	4	2	6
75-78	1	5	6
87-138	3	2	5
	60	53	113

and female animals in the age groups, it was decided to analyse the teeth of both sexes together. The ranges of age for the groups were determined by the sample size that was available for any one age group. Altogether 82 animals, the oldest 40 months, were within the age range for tooth development to be taking place.

All the animals were pastured in the semi-wild conditions of Richmond Park, Surrey between the years 1965 and 1975. For 11 years during late May and June calves were ear-tagged, within a few days of birth, with plastic jumbo tags (Dalton Supplies Ltd., Nettlebed, Oxon. RG9 5AB). The tag in the left ear was numbered and colour coded for the year. The male and female halves of the right ear tag gave a colour combination

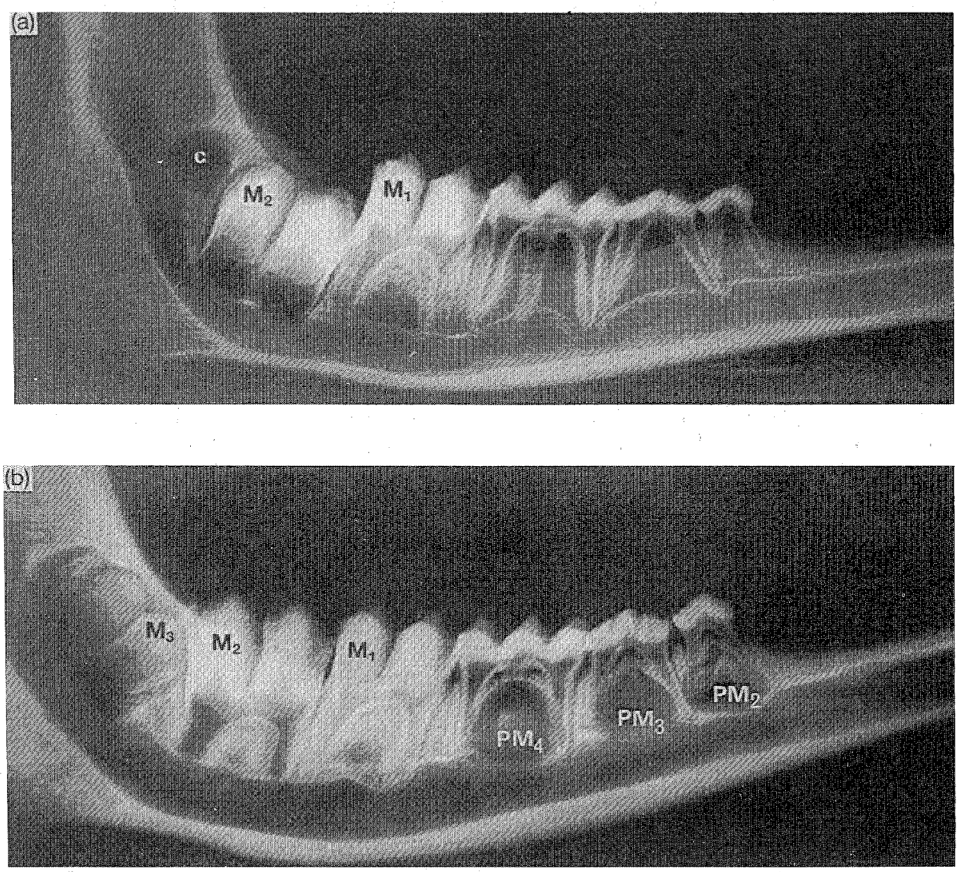

PLATE I. The radiographs show the various stages to which the molars and premolars have developed within the bone. (a) The first molar is at the late root formation stage (score 8); the second molar has its crown formation complete (stage 5), whilst only the crypt c (score 1) of the third molar is present. There is no evidence of the permanent premolars which will begin their development between the roots of the deciduous premolars. (b) Development of the molars has progressed. The first molar roots are at the full root length (score 9), the second molar is at the late root formation stage (score 8) and the third molar has all its cusps mineralizing (score 3). The developing permanent premolars, all with evidence of mineralization (score 3), can be seen below their deciduous precursors. The roots of the deciduous premolars are being resorbed.

specific to an individual animal within a year group. Upon the death of marked animals the skulls and mandibles were prepared by bleaching with sodium perborate tetrahydrate [NaBO^H^O] (Chapman & Chapman, 1969). As part of the normal culling programme 101 deer of the sample were shot within the appropriate legal seasons (males: mostly during August and September, plus some calves taken during the female cull, and females: mostly during November, December and January). Ten deer were the victims of a variety of accidents throughout the year and 2 were calves that died shortly after birth.

Radiographs

The right side of each mandible was radiographed. The lingual side was placed against the cassette in which the radiographs were held. All the premolars and molars were approximately at an equal distance from the film. A portable Watson x-ray apparatus type MX-2 was used. The x-ray source was maintained at a constant distance of 76-20 cm. The exposure time was 4 sec at 10 amp and 1 . 5 kv. Kodak MX5 industrex was used in a cassette. A x 10 lens was used to examine the radiographs in detail.

The radiographs showed the various stages to which the teeth have developed within the bone. Molars may be seen in varying stages of development (Plate la) and premolars may be seen developing beneath their deciduous precursors (Plate Ib). Drawings derived from tracings of radiographs of animals of different ages demonstrate the stages and sequential nature of tooth development (Fig. 1).

Teeth examined

Only the mandibular molariform teeth were examined in detail; but the same principles for analysis and interpretation apply equally to the incisors and the teeth of the maxilla. The deciduous teeth were excluded because, by the age of the youngest animal, all of these teeth had formed.

Tooth histology and development

A molariform tooth is made up of a crown and various roots. The crowns have cusps and in the case of the molars the paired mesial and paired distal cusps are separated by the infundibulum. All teeth are composed of a core of soft tissue, the pulp, which is completely surrounded by dentine, except for a foramen at the root apex for the transmission of blood vessels and nerves. The dentine of the crown is covered by enamel and of the root by cementum. The enamel of the Ruminantia is usually covered by a thin layer ofcementum, but this is not readily observable in red deer. The details of tooth development have already been described (Brown & Chapman, 1991a). A complete account of tooth development may be found in Osborn (1981) or Scott & Symons (1982).

FIG. 1. (a-g) Drawings derived from tracings of radiographs (*x* 0-6 original size) at different ages demonstrate the sequential nature of tooth development, (a) 5 months: the 1st molar had started development *in utvro* and here more than half the root length has formed representing stage 8. All the cusps of the 2nd molar are mineralizing, stage 3: the infundibula are not yet visible. There is no evidence of the permanent premolars. (b) 10 months: the 1st molar has achieved full root length with the apices open, stage 9. The 2nd molar is now at stage 5 with a completed crown. The crypt c is present lor the 3rd molar, stage 1. (c) 13 months: the 3rd molar is mineralizing, stage 2. Crypts c are evident for the 2nd, 3rd and 4th premolars, stage 1, whilst mineralization is just beginning for the 4th premolar, stage 2. (d) 15 months: all the premolars are mineralizing in their crypts beneath their deciduous precursors, stage 2. (e) 18 months: the crowns of the 3rd molar and all the premolars are formed, stage 5. The roots of the deciduous premolars are resorbing, implying that eruption of the underlying forming permanent teeth has begun, (f) 27 months: the 3rd molar is at the late root formation stage 8; the premolars are all erupting and will replace the lost deciduous precursors, (g) 50 months: the molariforms were completely formed sometime between 38 and 40 months.

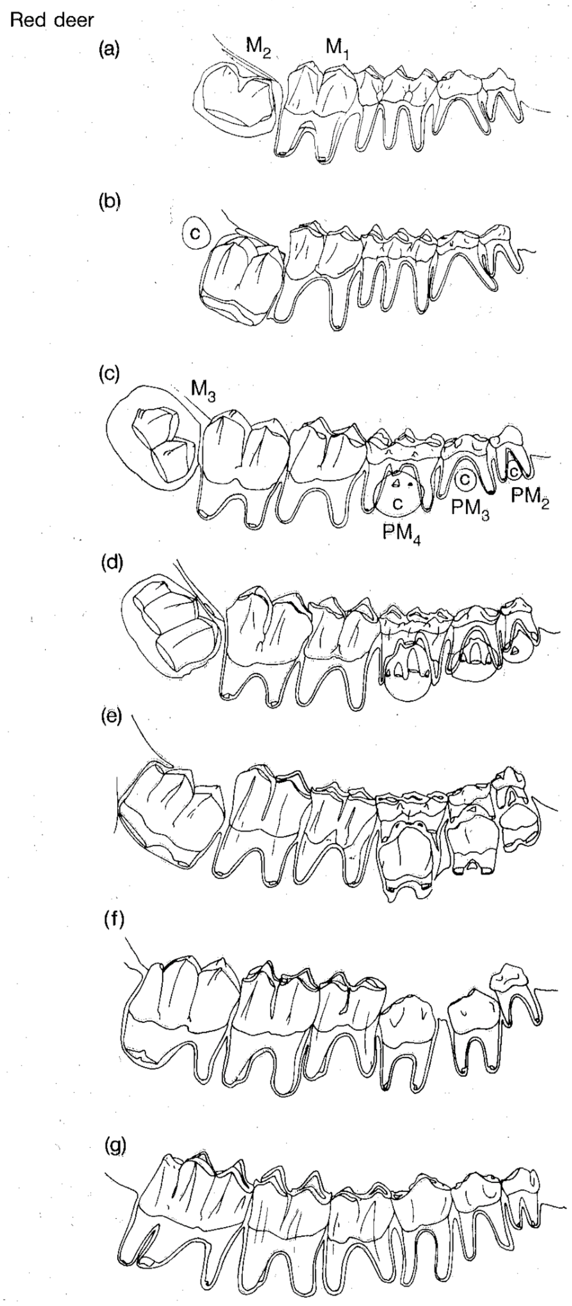

FIG. 1.

Tooth development stages and how they were scored

The stages used are identical to those described for fallow deer (Brown & Chapman, 1991a). From radiographs (Plate la & b and Fig. 1), it was possible to distinguish 10 distinct developmental stages. These stages were visible because of 2 distinct characteristics: first, as the tooth germ enlarged, the surrounding bone was resorbed by osteoclasts and the area, because of a decrease in the total mineralized tissue, became radiolucent, appearing as a dark zone; and secondly, as the tooth germ began to mineralize at the enamel dentine junction, the spread of this mineralization was observable as a zone of increased density which is radiopaque, appearing white on a radiograph. It should be noted that, whereas the permanent molars were forming in the mandible behind or distal to the deciduous molariform teeth, the premolars were developing between the roots of the deciduous premolar teeth. As the tooth germ and the crypt in which it lies form and the premolars develop and grow, osteoclasts are activated which resorb the surrounding bone and enlarge the crypt. When the roots of the premolars started to form, these teeth began to erupt and the osteoclasts began to destroy the roots and frequently parts of the crowns of the deciduous teeth, so that they eventually fell out. They were replaced by the underlying developing premolars, that erupt through the bone (Plate Ib). The stages that may be readily observed were as follows and are illustrated for the molars and premolars in drawings from radiographs (Fig. la-g): 1. Evidence of a crypt. 2. Evidence of mineralization. 3. All cusps mineralizing. 4. The infundibulum is formed. 5. Crown formation is complete. 6. Early root formation. 7. Half the root length formed. 8. Late root formation. 9. Full root length formed, apex opened. 10. Root apex closed.

1. *Evidence of a crypt.* The crypt is the darkened area in which the tooth germ is developing and growing. It was represented by a discrete area of bone resorption, defined at its periphery by a fine white encircling line, reflecting the contrasting levels of mineralization. It may only be a few millimetres in diameter. The earliest mineralization may have already begun, but is too slight to be distinguished against the bone that surrounds the crypt.

2. *Evidence of mineralization.* When the tooth germ reaches the bell stage of development, mineralization of the dentine and the enamel commences in the mesial cusps. This early mineralization may be detected within the crypt as a fine white radiopaque line locating the enamel dentine junction. It was recognizable because it followed the cuspal outline.

3. *All cusps mineralizing.* All the cusps were clearly outlined at different stages of mineralization. The mesial cusps started to mineralize before the distal ones.

4. *The infundibulum is formed.* At this stage the lingual and buccal cusps were formed and their mineralized fronts were continuous with each other in the centre of the tooth at the bottom of their infundibulum. There is no infundibulum in a premolar.

5. *Crown formation is complete.* All the crown was formed and the first evidence of root formation was observable at the mesial and/or distal ends of the tooth. The completion of crown formation can only confidently be determined when root formation has begun.

6. *Early root formation.* A fine inverted V-shaped line of mineralization was seen at the bifurcation of the mesial and distal roots.

7. *Half the root length formed.*

8. *Late root formation.* More than half but less than the full length of the root was visible.

9. *Full root length formed with apex open.* For some time after the full length of the root has been formed, the apex through which the blood and lymph vessels and nerves pass is wide open, but slowly closes.

10. *Root apex closed.* The fine canal through which the vessels and nerves pass was marked by continued mineralization of the dentine and cementum and could not be seen.

Where there was more than one root, the stage and the score was always related to the most advanced developing root. It was decided to score in this way because of the considerable variation in the age when the most distal apex of the third molar closed.

The score for an animal was determined by allocating the number given above for the development stage

TABLE II

*The ages (months) for red deer **(Cervus elaphus)** at which selected stages of tooth development were first present and consistently present. The symbol < is used for a stage that lakes place before a certain age*

	Ages (in months)					
	Molars			Premolars		
Stages	3rd	2nd	1st	4th	3rd	2nd
Crypt formation						
first present	5	1	*in utero*	11	6	5
consistent	10	< 4	*in utero*	11	11	11
Mineralization						
first present	9	**<3**	*in utero*	13	<13	< 14
consistent	13	4	*in utero*	14	14	< 14
Crown complete						
first present	18	<9	<4	<18	<18	<18
consistent	26	9	4	18	18	18
Late root formation						
first present	27	< 18	6	26	26	26
consistent	28	18	9	27	27	27
Closed root apex						
first present	38	27	18	<33	33	29
consistent	40	33	28	33	40	41

that a particular tooth had reached. These scores, from all the molariform teeth that were at some stage of development, were added together. As tooth development is a strictly sequential process, the low scores imply early and the high scores late development. A chronology of tooth development was constructed from the following selected events so that a quick appraisal could be made of an animal's age: crypt formation, first evidence of mineralization, crown completion, late root formation with the root apex open and final root formation when the root apex is closed and tooth development is complete. Because the animals of the sample were not evenly distributed throughout the different ages, the age at which a particular tooth development stage may have been reached is an approximation. For instance, for all the premolars crown completion had taken place at some age later than 15 months and less than 18 months (Tables II, III & IV).

Statistical analysis of the development scores

The analysis of the data for the molariform teeth is presented in three parts. First is the age at which selected development stages may be reached (Table II). Secondly, for each age group, the score that may be expected is given (Table IV). Lastly, the predicted age, together with 95% prediction intervals, obtained from a regression of total molariform development scores on age is given (Fig. 2). The calculations were performed using GLIM (Payne, 1986) and the fitted line was the logistic growth curve.

$$\textbf{Fitted scores} = \frac{60^{-2\ 3000+0.1638\ (ages)}}{1+e^{-2.300+0.1638\ (age)}}$$

(e is the mathematical constant 2-718 . . .).

From a calculation of the score of an animal of unknown age it is possible by reference to the regression plot to obtain an estimate of age (Fig. 2).

TABLE IV

*The age range within which a particular score may be found for molariform teeth of red deer (**Cervus elaphus**)*

Age Group (months)	Molars 3rd	Molars 2nd	Molars 1st	Premolars 4th	Premolars 3rd	Premolars 2nd	Totals
1–11 (n=18)	0–2	1–6	4–9	0–1	0–1	0–1	5–19
13–19 (n=24)	3–6	7–8	9–10	2–7	1–7	1–8	23–45
26–33 (n=28)	7–9	8–10	9–10	8–10	8–10	8–10	50–59
38–42 (n=15)	Tooth development completed*						60

* Within this last age group there were two animals that had two final stages to complete development (score 58), and two animals that had only one more stage to complete development (score 59)

The 1st premolar is absent in deer (Riney, 1951). For the 2[nd], 3[rd] and 4th premolars, crypt formation was seen at 5, 6 and 11 months, respectively, and for all the molariform teeth it was consistently present 3-6 months later.

Mineralization. The tooth germ started to be mineralized *in utero* for the 1st molar, at less than

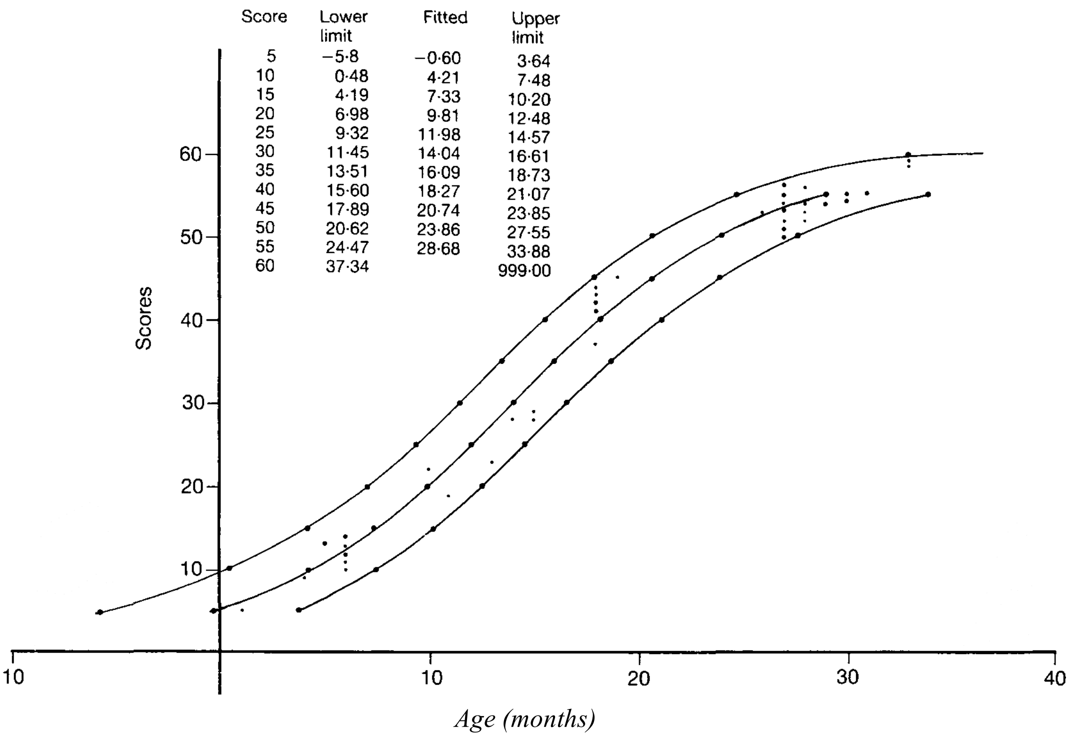

FIG. 2. The graph gives the predicted age, together with 95% prediction intervals obtained from a regression of total molariform development scores on age with plots at intervals of five. The scores for all the sample have been plotted and all come within the range of 95% prediction intervals. By using this graph, ages of animals of unknown provenance can be estimated.

three months for the 2nd molar and at nine months for the 3rd molar. For the 2nd and 3rd premolars, mineralization began before 13 and 14 months, respectively, and at 13 months for the 4th premolar. It was consistently present one month later for all the molariform teeth except the 3rd molar where mineralization was always found from 13 months.

Crown complete. For the molars, crown completion was consistently present for the 1st at four months, the 2nd at nine months and the 3rd at 26 months. The premolars had all their crowns completed by 18 months and so they would be found completed at an earlier age sometime after 15 months.

Late root formation. This stage with more than half the root formed was reached for the 1st, 2nd and 3rd molars by 9, 18 and 28 months, respectively, and for all the premolars by 27 months.

Closed root apex. The completion of tooth development was seen first at 18 months for the 1st molar, 27 months for the 2nd and 38 for the 3rd. The 2nd premolar was the first premolar to be observed with its development finished by 29 months and always complete at 41 months. The 3rd was completed between 33 and 41 months and the 4th premolar by 33 months.

All the development stages, except the formation of the infundibulum, were readily observable for the premolars in the drawings (Figs Ic-g).

Tooth development scores (Tables III & IV)

The scores for the individual molariform teeth for each animal that was young enough to have developing teeth are presented in Table III. There are three age groups 1-11 months, 13-19 months and 26-33 months within which the score attributable to tooth development defines the possible age range of the animal. Tooth development is, except for four animals, completed by 38 months. A 38-month-old animal was the youngest red deer with all its molariform teeth fully developed (Table III).

Regression analysis of developmental scores on age

The logistic growth curve was constructed by plotting total molar scores against age. The 95% inverse logistic prediction limits give the range of age variations for any specific score. For a score between 10 and 50 this is just over + or — three months. For lower or higher scores the range increases to + or — four months. The total molariform scores for all the sample, in which molariform teeth were developing, are recorded on the graph and all fall within the 95% intervals.

Prediction of age

By reference to Table II, using the stages of development that may have been reached, and Tables III and IV using the scores expected for a particular age, it is possible to give an estimate of age for an animal of unknown age. These tables can be used when a complete set ofmandibular molariform teeth are not available. When all the molariform teeth are available, the best estimate and range of age from the total score is given by the fitted line and its 95% prediction limits. This gives a complete spectrum of predicted ages from developing teeth for any total molariform score, from zero to 60 (Fig. 2).

Discussion

Sample (Table I)

As described for fallow deer (Brown & Chapman, 199la), the results have to be interpreted bearing in mind the distribution of ages within the selected age groups (Table I). Though it means that the exact range of ages for a particular development stage cannot always be given, it is still possible to make up a useable chronology of tooth development from our sample of known-age animals (Table II). The discontinuity of the ages leaves unavoidable gaps in the score to be expected for a particular age (Table IV). The discontinuity is overcome by the regression analysis and the graph can be used to assess any age during the period of tooth development.
All the molariform teeth can be included in building up a chronology of tooth development. It had been found that for the sample of red deer described here, assessment from wearing of the crowns could be made reliably only from molar teeth (Brown & Chapman, 1991 A). The formation of the crown and roots is an identical sequential process and normally takes place free from direct environmental influences. There were 113 red deer mandibles in the samples, but because tooth development was always completed by 41 months, only 82 animals have been used. In fact, the oldest animal which can be reliably used for interpreting chronology of tooth development was 33 months. After that age only the final stage (10) was left to be completed, and only at most for two teeth. The chronology and scoring tables have been calculated to form a database from which an assessment of age can be made of animals of unknown provenance.

Chronology of permanent tooth development (Table II)

The assessment of age from the chronology table can be made by deduction and elimination. If for instance no crypts are visible before five, six and 11 months for the premolars, respectively, an animal without crypts can be set below these ages. When all the premolars are at the late root stage of development with more than half of the root formed, the animal is at least 27 months old. The 3rd molars, because they are the last to form, give the most useful guide to age. If the 3rd molars are mineralizing the animal's age is at least 13 months old. When the 3rd molar crown is complete, the age may be assessed at 18 months and over.
In this sample, the youngest animal for which the 3rd molar's development was assessed as complete, was 38 months. So this age was taken to be the maximum age that tooth development assessment could be used for an appraisal of age. An examination of the detailed scores suggests a lower age, between 33 and 38 months, is more appropriate (Table III).

Tooth development scores (Table III & IV)

Each of the three age groups, from 1-11, 13-19 and 26-33 months, are associated with specific score ranged. This permits a ready method for identifying a likely age range. The data confirms the view that, for practical purposes, 33 months is the age by which tooth development is complete.

Regression analysis of developmental scores on age (Fig. 2)

Tooth development takes place in a steady incremental way, as implied by the regression line.

The graph enables an age to be read off against a calculated total molariform score. It is in our view the most accurate and the best way to assess age of relatively young animals in which there is a full complement of molariform teeth. It also defines the prediction intervals at a 95% interval, and gives an accuracy of plus or minus three months for any calculated score. The range is two months greater for the red than the fallow deer (Brown & Chapman 199 la); there is no obvious explanation for this difference.

A comparison of red and fallow deer tooth development

There are obvious differences of timing between the tooth development of the red and fallow deer. Overall all the development stages appear earlier in fallow than red deer, but crypt formation was found at the same age for the 3rd molar and earlier for the 2nd and 3rd premolars in the red deer. Yet, in spite of the larger teeth of the red deer, tooth development was generally completed at the same time, around 33 months for both species. Two 39-month-old animals had third molars which had not reached the final stage of tooth development (Table III).

Prediction of age

The narrow prediction intervals, of as little as + or — three months (Fig. 2), confirm that tooth development is an accurate method for assessing age of animals up to 33 months of age. After that age, rough assessments from radiographic appearances could be made from changes attributable to age. These are increased layers ofcementum, hypercementosis, with an irregular thickening of the root apices; reduction of the pulp size with the formation of irregular pulp canal outlines can also be observed. But it was thought that these assessments were too subjective and that the overlap of ages from these appraisals were too big. They were no more than crude indicators of age. For older animals, examining wear scores as described in our earlier papers would be more appropriate (Brown & Chapman, 1990, 1991*b*).

Summary

The right mandibles from the skulls of 113 red deer of known age were radiographed. Seventy-three of the animals had at least one molariform tooth incompletely developed. Most tooth development was completed by 33 months, with the third molar being delayed up to 39 months. In one animal the second and third premolars were still incompletely formed at 40 months. Ten tooth stages were identified. A selection of the most readily observable were used to construct a chronology of tooth development. All the stages were allocated a score and from these it was found that a count of all the scores for the permanent molariform teeth gave the best indication of age. The scores were used to obtain a regression of total molariform development scores on age. It is possible from the graph to determine what the age may be of an animal for which no data is available.

The calves were tagged by the late Donald Chapman who appreciated the value of known-age specimens and went to great efforts to obtain them: he also prepared many of the skulls. Many people assisted in searching for the calves, especially J. K. Fawcett and Diane Hughes. We also acknowledge gratefully the support of the former and present Superintendents of Richmond Park, the late G. J. Thomson and M. Baxter Brown, for allowing the deer to be marked and their staff who cooperated in saving material from culled deer. Our thanks are due to Pauline Howley for her assistance in radiographing the skulls and to Sandra Doyle for the drawings. We particularly appreciate the assistance from Dr Jim Burridge of the Department of Statistical Science, University College, London, who gave invaluable guidance on how to interpret and present our findings.

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