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Age assessment of fallow deer *(Dama dama):* 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 were described for known-aged fallow deer *Dama dama.* Ten stages were defined and, by allocating scores for these stages, the scores that may be expected for a particular age have been identified. Lastly, the predicted age was given for total molariform scores. These were obtained, with 95% prediction intervals, from a regression of age on total molariform scores. An age assessment from tooth development stages can be made up to three years, after which, no further development takes place.

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Introduction

Tooth development with its clearly recognizable stages is a valuable guide to the assessment of age. With radiographs these stages can be observed both before and after the eruption of the teeth into the mouth. Furthermore, the cells that form the teeth, and especially the ameloblasts which form the enamel of the crown, are very sensitive to any local ameloblastic upset or systemic ailment, causing macroscopic deficiencies on the tooth surface, Sisson (1953). It is thus possible to relate these episodes to a particular stage in tooth development and therefore the age when they occurred. An accurate knowledge of age and evidence of disease or environmental pollution as from excess fluoride are important aids for domestic and wildlife management. Brown *et al.* (1960), in an extensive study of eight breeds of cattle of known age, established a baseline for undertaking such analyses. Hillson (1986) has made a comprehensive review of the literature on tooth development and ageing for Man and other mammals.

In this paper, tooth development specifically refers to the stages through which a tooth passes from its initiation to form the tooth germ, a complex of embryonic tissues, followed by the formation of organic matrices and their mineralization, through to crown and root formation. Once a tooth has fully formed, there are changes which are ascribed to age; the external apical end of the root widens with deposition of cementum, while the internal pulpal canal is narrowed by irregular depositions of secondary dentine. We explored the possibility of using these changes to assess age, but found that they take place with too wide a degree of variation to be of any use. All the developmental stages are clearly visible in a radiograph (Plate I). Tooth development should be distinguished from tooth eruption, which in this paper only refers to the movements of the teeth through the bone, to enable them to occlude with the teeth in the opposite jaw. Determination of tooth development has the advantage, as reported by McCance, Ford & Brown (1961) who examined radiographically the developing teeth of undernourished pigs *Sus scrofa,* that, though it would be delayed, it was the least likely of the developmental processes to be disturbed by nutritional factors. Another advantage of assessing age from tooth development is that all the teeth can be assessed by the same radiographically identical criteria; whereas age assessment from tooth wear patterns is dependent on the morphological characteristics of the individual teeth. We had found, Brown & Chapman (1990), that molar but not premolar wear was a reliable indication of age. The mandibles of 56 fallow deer of known age from two days to 91 months have been radiographed. From these, the chronology of tooth development has been worked out. A simple scoring system to indicate stages of development was devised; from these scores the age was determined by which a particular score was attained. Using the scores for the different stages of tooth development, a regression of total molariform scores on age was calculated. It is possible from the curve that was derived to assess the age of animals of unknown age.

Materials and methods

Sample

The sample consisted of 56 fallow deer, 36 males and 20 females of known age from 2-3 days to 91 months (Table I). Altogether 40 animals, the oldest 35 months, were within the age range for tooth development to be taking place. For convenience, the sample was arranged in 6 age groups; because of the uneven numbers of male and female animals in the different 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.

TABLE I *Fallow deer* ***(Dama dama)*** *sample, number, age and sex*

Age groups	Males	Females	Totals
2 days-11 months	7	4	11
18-22 months	2	8	10
26-35 months	17	2	19
38-43 months	5	3	8
48-59 months	4	1	5
67-91 months	1	2	3
	36	20	56

All the animals were pastured between the years 1965-1975 in Richmond Park, Surrey, as described by Chapman & Chapman (1975). For 11 years during the month of June fawns were ear-tagged with plastic jumbo tags (Dalton Supplies Ltd., Nettlebed, Oxon. RG9 5AB) within a few days of birth. 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 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 [NaB03-4H20] (Chapman & Chapman, 1969). As part of the normal culling programme, 40 deer of the sample were shot within the appropriate legal seasons (males: mostly during August and September, and females: mostly during November, December and January). Sixteen deer were the victims of a variety of accidents throughout the year.

Radiographs

To observe the several stages of tooth development it is necessary to obtain radiographs of the jaws. Only the right side of each mandible was radiographed (Plate I). The lingual side was placed against the cassette in which the radiograph was held. In this way, 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. Details on the radiographs were examined with a x 10 lens.

Teeth examined

Only the mandibular molariform teeth have been examined in detail for this study; but the same principles for analysis and interpretation apply equally to the incisors as well as teeth of the maxilla. The deciduous teeth have been excluded because, by the age of the youngest animal in this example, all of these teeth would have been fully formed.

Tooth histology and development

A tooth is made up of a crown and a varying number of roots. The crowns have cusps and in the case of the molars the paired mesial and paired distal cusps are separated by the infundibulum. The tooth itself is composed of a core of soft tissue, the pulp, which is completely surrounded by dentine, save for a foramen at the root apex for the transmission of blood and lymph 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 of cementum, but this is not readily observable in fallow deer. Dentine and cementum are formed slowly throughout life by odontoblasts and cementoblasts, respectively; enamel is completely formed by ameloblasts

LOCATION FOR PAGE 170 WITH PLATE 1 (a-d)

Followed by

LOCATION FOR PAGE 171 WITH PLATE 1 (e-f)

before root formation begins; and once it has been formed no more enamel can be added as the cells lose their synthesizing function. Tooth development is a sequential and incremental process, with first dentine and then enamel forming along the enamel-dentine junction. The ameloblasts migrate to the future crown surface, while the odontoblasts retreat towards, and encroach on, the periphery of the pulp tissue. Cementum is also formed incrementally as soon as the dentine begins to form, but in smaller amounts. It adds to the thickness of the root, and forms small bulbous nodules at the root apices after development is complete.

Tooth development stages and how they were scored

From radiographs it is possible to distinguish 10 distinct developmental stages. These stages are visible because of 2 distinct characteristics: first, as the tooth germ enlarges, the surrounding bone is resorbed by osteoclasts and the area, because of a decrease in the total mineralized tissue, becomes radiolucent, appearing as a dark zone. The other distinguishing feature arises because, as the tooth germ begins to mineralize at the enamel dentine junction, the spread of this mineralization is observable as a zone of increased density, which is radiopaque, appearing white on a radiograph. It should be noted that, whereas the molars are forming in the mandible distal to the deciduous premolars, the permanent premolars are developing between the roots of their deciduous precursors. As the tooth germ and the crypt in which it lies form and the premolars develop and grow, osteoclasts, mineral resorbing cells are activated. These cells resorb the roots, and frequently parts of the crowns of the deciduous teeth, so that they eventually fall out to be replaced by the underlying developing premolars, that erupt through the bone. A selection of the stages, very similar to those described by Demirjian, Goldstein & Tanner (1973) have been used in this study. Complete details of tooth development may be found in Osborn (1981) or Scott & Symons (1982). The stages that may be readily observed are as follows and are illustrated for the molars and premolars in radiographs (Plate I). 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 formed. 8. Late root formation. 9. Full root length formed with apex open. 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 is 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 formation of the cellular tooth germ will be present and even the earliest mineralization may have already begun, but is too slight to be contrasted against the density of the bone that surrounds the crypt.

2. *Evidence of mineralization.* When the tooth germ has reached the bell stage of development, mineralization of the organic matrices of 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 is readily recognizable because it follows the cuspal outline.

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

4. *The infundibulum is formed.* At this stage the lingual and buccal cusps are formed and their mineralized fronts are continuous with each other in the centre of the tooth at the bottom of their infundibula.

5. *Crown formation is complete.* All the crown is formed and the first evidence of root formation is observable at the mesial and/or distal ends of the tooth. The evidence that root formation has begun must be present before it can be determined that crown formation is complete.

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

7. *Half the root formed.*

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

9. *Full root length 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 this aperture slowly narrows.

10. *Root apex closed.* The fine canal through which the vessels and nerves pass is masked by continued mineralization of the dentine and cementum and cannot be seen.
The development score for an animal was determined by allocating the number given above for the development stage 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, 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 are 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 formation will have taken place at some age later than 11 months and less than 18 months (Tables II and III).

Statistical analysis of the development scores

The analysis of the data is presented in three parts. First is the age at which a particular development stage may be attained. Secondly, for each age group the score that may be expected is given. Lastly, the predicted age together with 95% inverse prediction limits of age for a given score obtained from a logistic regression of total molariform developmental score on age is given. The calculations were performed using GLIM (Payne, 1986) and the fitted line was the logistic growth curve:

$$\text{Fitted score} = \frac{60e^{-2694+0.186\,(\text{age})}}{1+e^{2.694+0.198(\text{age})}}$$

(e is the mathematical constant 2-718 . . .).
Using this graph, having determined the development score for all the molariform teeth, it is possible to give an estimated age of the animal of unknown age.

TABLE II

The ages (months) for fallow deer ***(Dama dama)*** *at which selected stages of tooth development were first present, and consistently present. A stage that takes place before a certain age is indicated*

	Molars			Premolars		
Stages	3rd	2nd	1st	4th	3rd	2nd
Crypt formation						
first present	5	<3'5	*in utero*	9	8	7
consistent	9	<3'5	*in utero*	9	9	9
Mineralization						
first present	9	<3-5	*in utero*	9	11	< 18
consistent	11	4	*in utero*	11	< 18	< 18
Crown complete						
first present	< 18	8	<4	<18	<18	<18
consistent	<18	9	<4	<18	<18	<18
Late root formation						
first present	26	18	5	19	22	19
consistent	27	19	7	22	<26	22
Closed root apex						
first present	38	27	27	26	26	26
consistent	38	31	27	31	31	31

TABLE III

Fallow deer **(Dama dama)** of known-age tooth development scores of permanent molariform teeth. Only 41 animals have been included in this table because by 38 months tooth development is complete

Age		Molar		Premolar			Total scores
	3rd	2nd	1st	4th	3rd	2n	
Days							
2	0	0	2	0	0	0	2
2	0	1	2	0	0	0	3
Month							
3-5	0	2	4	0	0	0	6
4	0	3	7	0	0	0	10
5	1	3	8	0	0	0	12
7	1	4	8	0	0	1	14
8	0	5	8	0	1	0	14
8	1	3	8	0	0	0	12
9	1	5	8	1	1	1	17
9	2	6	8	2	1	1	20
11	2	6	9	2	2	1	22
18	6	8	9	7	6	6	42
18	7	8	9	7	7	7	45
18	6	8	9	7	7	7	44
19	6	8	9	8	7	7	45
19	6	8	9	7	6	6	42
19	6	8	9	7	7	8	45
19	6	8	9	7	7	8	45
19	6	8	8	8	7	7	44
19	6	8	9	7	7	8	45
22	7	9	9	8	8	8	49
26	8	9	9	10	10	10	56
27	8	9	9	9	9	9	53
27	8	9	10	9	9	9	54
27	8	10	10	10	9	9	56
27	8	9	10	9	10	10	56
27	8	10	10	9	9	9	55
27	9	10	10	10	10	9	58
27	8	10	10	9	9	9	55
27	8	10	10	9	9	9	55
27	8	9	10	10	9	9	55
27	8	10	10	10	10	10	58
27	8	10	10	10	10	9	57
27	8	10	10	10	10	10	58
27	8	10	10	9	9	9	55
27	8	10	10	9	9	9	55
27	8	10	10	10	10	10	58
27	9	10	10	10	10	10	59
31	8	10	10	10	10	10	58
35	9	10	10	10	10	10	59
38	10	10	10	10	10	10	60

Results

Chronology of permanent tooth development (Tables II & III)

Evidence of a crypt. This is the first visible stage in a radiograph of tooth development and the earliest evidence that it has begun. This stage was passed for the first molar before two days, the age of the youngest animal in the sample, and so development for this tooth had begun *in utero.* The crypt appears shortly after birth for the second molar, and is always present by 3-5 months. A crypt for the third molar was visible in the single five-month-old animal, and was always present from nine months. The crypts for the second, third and fourth premolars were first observed at seven, eight and nine months, respectively, and were consistently present from nine months.
Mineralization. The mineralization of the tooth germ begins *in utero* for the first molar and for the second molar before 3-5 months. It is always detectable by 11 months for the third molar. For the most anterior premolar which is the second premolar (Riney, 1951), mineralization is first observed sometime before 18 months, for the third at 11 months and the fourth at nine months. It is always present before 18 months for the second premolar and third premolars, and by 11 months for the fourth premolar.
Crown completely formed. This stage also showed the earliest evidence of root formation on the mesial and distal edges of the crown. It was completed some time before four months for the first molar, and first observed at eight months for the second and sometime before 18 months for the third molar. Likewise for the premolars it would have been observable some time before 18 months.
Late root formation. From the time the crown is completely formed, it takes for the molars between 8-10 months for the full length of the root to grow. The full length for the first, second and third molars is seen at 5, 18, and 26 months, respectively, and is consistently present a month or two later. The full root length for second and fourth premolars is first observed by 19 months and is always present at 22 months. The third premolar achieves this stage at 22 months and it will always be present earlier than 26 months.
Closed root apex. The length of time for the apex to close after the full length of the root has formed is 16 months for the first molar, 11 months for the second and third molars and only five months for the premolars. For the first, second and third molars tooth development is complete at 27, 31 and 38 months, respectively, and for all the premolars by 31 months.

Tooth development scores (Table IV)

These identify for each of the three early age groups, 4-11 months, 18-22 months and 26-35 months, the particular score which may be expected from calculating the tooth development score for all the molariform teeth. It was decided that, because there were no animals between 35 months when the third molar is still forming and 38 months when all teeth are fully formed, that tooth development should be regarded as being complete at 36 months (Table III).

Regression analysis of development scores on age

The fitted line (Fig. 1) was the logistic growth curve. The 95% inverse logistic prediction limits give the range of age variations. For a score between 10 and 50 this is less than three months. For lower or higher scores it increases to three months and above. The total molariform scores have been plotted on to the same graph and all fall within the prediction intervals.

TABLE IV

The age range within which a particular score may be found for fallow deer (Dama dama)

Age group (months)	Molars			Premolars			Totals
	3rd	2nd	1st	4th	3rd	2nd	
2 days-11 (n=ll)	0-1	1-6	2-9	0-2	0-2	0-1	2-22
18-22 (n =10)	6-7	8-10	8-9	7-8	6-8	6-8	42-49
26-3 5 (n= 19)	8-9	9-10	9-10	9-10	9-10	9-10	56-59

Tooth development completed at 36 months

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 an incomplete set of mandibular molariform teeth are available. When all the molariform teeth are available, the best estimate of age is given by the fitted line. This gives a complete spectrum of predicted ages for any total molariform score from zero to 60 (Fig. 1).

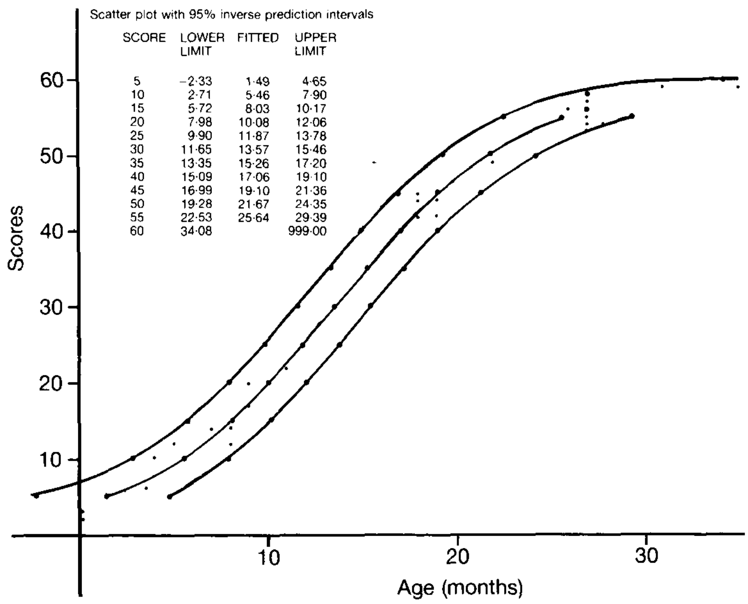

FIG. 1. The scatter plot of the data using a logistic regression of score on age (months). The limits given are the 95% inverse prediction limits of age for a given score at intervals of five. The inset table gives the age with the upper and lower limits for these scores.

Discussion

Sample (Table I)

The results have to be interpreted in relation to the nature of the sample which is described earlier in this paper (Table I). Because of the varying number of animals to be found at any particular age, and because some months are not represented at all, and others very well, 16 animals being 27 months old, some assumptions have had to be made. Nevertheless, a clear pattern of the sequential nature of tooth development emerges which can be used to assess age.

Chronology of permanent tooth development (Tables II & III)

A selection of the most readily recognizable stages has been given in the results indicating when they were first observed and the age by which they were consistently present: crypt formation, mineralization, crown complete, late root formation and closed root apex. The molars have a clear development sequence which is reflected in their times of eruption, but the premolars appear to develop in parallel with only a month or two distinguishing them in the earliest stages of development. Mineralization begins at the bell stage in tooth development, very soon after the crypt is first recognizable, as described in Osborn (1981) or Scott & Symons (1982). Because of the uneven spread of ages throughout this sample, it is not possible always to give exact timing for the several developmental stages, and approximations have been given. For instance, for the second premolar there are no animals to represent ages older than 11 and younger than 18 months, but it is apparent from the amount of the tooth that is formed, that mineralization of this tooth began soon after 11 months of age.

The time taken for the crowns to form fully, always confirmed by evidence of early root formation, takes place in less than eight months for the molars and probably the same time for the premolars. Approximately nine months later the full length of the root is formed. At this stage, the apex of the tooth, the pulp canal through which the vessels and nerves are transmitted, is wide open and visible. After this stage is reached there is a slow reduction of the pulpal canal width until the apical canal is no longer evident, being masked by the increasing density of the mineralizing dentine and cementum. The time it takes appears to be a reflection of size; for the molars it takes from 11-16 months, but only five months for the much smaller premolars. When this has taken place tooth development is complete. Any changes after this, like additions of cementum to the root surface or reduction in pulp width by secondary dentine depositions, are changes attributable to age.

Tooth development scores (Table IV)

From the data available for animals of known age, it is possible to allocate to a particular age group a range of scores. Therefore, if the total development scores were calculated for an animal of unknown age, it would be possible to place it within one of the three age groups, under two days-11 months, 18-22 months and 26-35 months. Where all the teeth are fully formed, alternative ageing techniques would have to be used. Within the youngest group measurements of the length and height of the mandible would give further support for allocation to an approximation age (Chapman & Chapman, 1970).

Regression analysis of developmental scores on age

The data for the regression graph are derived from the 37 animals whose radiographs would have shown tooth development in progress. One 38-month-old animal, the youngest animal whose molariform tooth development was complete with a maximum molariform score of 60, was included. The regression analysis gives the best prediction of age if all the mandibular molariform teeth are present. The derivation of this graph, in which plots can be made for scores selected at intervals of five, enables the unevenness of the sample to be compensated. For any particular score the 95% prediction shows a range of variation of from less than 2-3 months. Once tooth development is complete, at three years for fallow deer, this ageing technique is unsuitable. However, up to this age it is a very precise way of identifying the age of an animal. For instance, the range of variation at 12 months, with a score of 25, is only two months. For a tooth wear score at 12 months of 14, the 95% prediction intervals give a possible age range of 3-5 months (Brown & Chapman, 1990). There are ageing factors, hypercementosis and narrowing of the root canals which can be used to allocate an animal to a higher age group, but these will not be as precise as can be determined from tooth development or wear scores.

Validity of the scoring technique

An evaluation of the scoring technique as a means of assessing age is given by tooth development scores of 16 animals who were 27 months old. Their scores fell between a narrow range of between 53 and 59.

Tooth development scores compared with tooth wear scores

As was described in our paper on tooth wear. Brown & Chapman (1990), the premolars, because of their cusp morphology, were difficult to score consistently and were omitted from the final calculations. However, tooth development is taking place in a stable environment, and is not directly influenced by the nature of the pasturing or the food that is being eaten. Furthermore, as McCance *et al.* (1961) have noted, tooth development is the least likely of the mineralizing growth processes to be affected by adverse nutritional conditions. All teeth can be examined on an equal footing for the stage of development they may have reached. For animals up to 36 months, that had seriously damaged crowns, it would be a suitable means of appraising age. The statistical analysis of the data confirm that tooth development stages or scores are a very good indication of age, a finding supported for *Homo sapiens* by Hagg & Matsson (1985) who evaluated three different approaches. They concluded that the tooth development stages identified and used by Demirjian, Goldstein & Tanner (1973), the ones adopted in this paper, were the most reliable.

Summary

The right mandibles from the skulls of 56 fallow deer of known age were radiographed. Forty of the animals had molariform teeth at different stages of development. **By** 36 months all tooth development of the molariform teeth is complete, after which any changes taking place are attributable to changes with age. 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 molariform teeth

gave the best indication of age. The scores were used to calculate a regression of total molariform development scores on age.

Table II gives the ages by which selected development stages are initiated and completed. Table IV identifies the age range within which a particular score may be found. These two analyses enable a rough estimate of age to be made from the evidence of a single molariform tooth. The most accurate estimate of age, of an animal of unknown age with all the molariform teeth present, may be made from the scatter plot in the regression graph (Fig. 1).

The fawns 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 fawns, 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. We particularly appreciate the assistance of Dr Jim Burridge from the Department of Statistical Science, University College London, who gave us invaluable guidance on how to interpret and present our findings.

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