University of West Hungary

Theses of Doctoral (Ph.D.) Dissertation

A COMPARATIVE STUDY OF DIFFERENT AGE ESTIMATION METHODS AND AGE-RELATED MARKS IN ROE DEER (*CAPREOLUS CAPREOLUS* (L.) 1758)

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1. Aims

Roe-deer age concerns professional and amateur hunters, wildlife biologists and trophy judges as well. Knowledge related to methods of age estimation is essential for professional game management. An accurate determination of the years of age is obligatory during official trophy judgement, although age can be exactly determined only up to one year of age (PRIOR, 1994), after that age can only be estimated by methods of age estimation.

The aim of the thesis was to ascertain if there are any layers produced by the cement and secondary dentin stock suitable for estimating the age in roe deer living in Hungarian habitats. Further aims included studying the methods of age estimation and age-related marks formerly assessed by subjective views and subjecting their authenticity and characteristics to exact statistical (biometrical) analysis. This results in a determinable authenticity and accuracy of these methods, which provides an objective consideration of practical applicability.

2. Materials and methods

Study material included the collected roe-deer jaws, heads and the amputated skulls remaining after sawing off the so-called small skull, with all the related data. Jaws, heads and skulls were collected from different habitats of the country (Tolna, Győr-Moson-Sopron and Békés county) by the help of professional hunters and hunting supervisors. Study of the samples included recording the data of age-related marks and age estimation on the basis of the extent of tooth wear. Cement stock of the molars and secondary dentin and cement stock of the incisors were studied by the slide preparation technique and histotechnical methods. Age estimation methods and age-related marks were compared and assessed by means of the data obtained.

3. Results and discussion

3.1. Assessment of cement stock

Usual proposals in literature concern using the cementum of the mandibular M_1 tooth for age estimation purposes (MICHELL, 1963, 1967; SZABIK, 1973; AITKEN, 1975; KOVÁCS AND FELEK, 1991; CEDERLUND ET AL. 1991). By reason of our experiences the best layer to evaluate can be found similarly on the transversal slide of the M_1 tooth root curve, however, the zones are also clearly visible on the transversal slide of the M_2 tooth, while on M_3 they are generally faint. Sometimes there were no layers required for assessment in the root curve. In these cases layers were considered on a horizontal slide made near the lower quarter of the roots, primarily on the M_1 tooth, and on the other teeth as well if M_1 was not suitable for assessment. Our results show – in agreement with PRIOR (1968) – that in the year of tooth emerging the beginning of cement stock formation can be observed on the underdeveloped root. Occasionally there is also a visible white infiltration in the first yellow semi-transparent zone. The following years of cement stock thickening witness the forming of a thicker white opalescent zone in the vegetation period and a thinner yellowish semi-transparent zone in winter. Thus, on the basis of our observations we confirm the statement of AITKEN (1975) saying that counting the white zones yields the age in years.

Histological examination of the cementum showed that although hematoxilin-eosin dyeing proposed by KLEVEZAL AND KLEINENBERG (1967), SZABIK (1973) and KOVÁCS AND FELEK (1991) is an applicable procedure, excisions dyed with Giemsa can be more properly assessed and the technical implementation of preparation is not more complicated either. The Heidenhain azan and Goldner trichromium dyeing produces also a much more differentiated picture more advantageous to evaluate than hematoxilin-eosin, but these dyeing techniques are not available anymore in most medical histological laboratories, therefore their practical implementation is somewhat more complicated.

3.2. Assessment of the dentin

Histotechnical assessment of secondary dentin had similar results to the cementum. The number of growth lines in the samples studied agreed with the age estimated by the cementum of dental slides, which confirms the conclusion of KLEVEZAL AND KLEINENBERG (1967), that the number of secondary dentin zones equals the years of age in roe deer.

3.3. Tooth change

According to our observations tooth change in roe deer finishes by the age of 12 months. Consequently an accurate age estimation by tooth change can be performed only up to one year of age. This confirms the experimental results of PRIOR (1968) and BIEGER (cit.: KŐHALMY, 1999).

3.4. Tooth wear

Age estimation by tooth wear, if carried out precisely, shows very close, statistically verifiable correlation with the age estimated from cement zones. In the case of bucks correlation coefficient was r=0.9791, 0.9732 and 0.9691 on different reference sites, while r=0.9744 for does.

According to MEÁK AND SZEDERJEI (1957) age estimated on the basis of tooth abrasion can agree with actual age in as much as 70-80%. This is confirmed by our study, as the age of bucks estimated from the number of cement zones in dental slides agreed in 75.00-87.64% with the age estimated from tooth wear in different populations. In the case of the does the correspondence was 78.10%. These data are very near to the results of AITKEN (1975), since his study showed a 63,5% correspondence between the age estimated from cement zones and the age estimated from dental abrasion, and 90.5% of the difference between the two values was within one year There is neither any significant difference between our results and the data of CEDERLUND ET AL. (1991) obtained by examining individuals of a known age.

3.5. Age estimation by trophy judgement

Comparison of the age estimated from cement zones and trophy judgement revealed a middle-strength relation (r=0.6380, 0.4882). Data sets of the various reference sites revealed a slight disparity caused by the different training and experience of hunting inspectors.

The ages estimated from cementum and trophy judgement were in a 35.96% agreement on one reference site and in 39.28% on the other. Maximum difference was 6 and 8 years on the two sites, respectively. These data show a somewhat closer relationship, than data from KOVÁCS AND FELEK (1991), which is probably caused by the fact that they tested the accuracy and authenticity of the method by studying roe deer living in an enclosed environment.

Reasons for the inaccuracy of age estimation by trophy judgement:

1. During trophy judgement age is estimated by the character of the antler, the inclination of coronas and beams, the thickness of the skull's bone and the ossification of skull sutures. These methods are in weak or not more than moderate relationship with age.
2. The possibility of avoiding negative points (as evidenced by overestimated ages).

3. Age is often superficially determined during judgement (as indicated by both under- and overestimation).
4. Many times during small skull trophy judgements it is not required to present the chopped-off skull part, or it is not taken into consideration. Thus, taking the extent of tooth wear as a basis cannot specify the age estimated by trophy judgement.

3.6. Age estimation on site

In Békés county there was a very close, statistically justifiable relationship on the reference site between the ages coming from cement zones and the ones estimated in the field (r=0.9062). On the reference site in Tolna county the correlation coefficient was lower (r=0.5911), but the relationship between the two data sets was statistically verifiable. The observed disparity on the reference sites was caused by the different training of professional hunters working in the field.

3.7. Crown height of the molars

Examination of the age estimated from cement zones and the crown height of molars points out that the strongest relationship for all samples was found in the case of the total average of the molars (r=0.9519, 0.9423, 0.9347, 0.9323). A very strong relationship can be observed for M_1 (r=0.9438, 0.9310, 0.9289, 0.9093), M_2 (r=0.9444, 0.9248, 0.9207, 0.9059) and M_3 (r=0.9432, 0.9211, 0.9042, 0.9006). Correlation coefficients of the estimated age and the crown height of molars show somewhat higher values either for one or the other tooth, however, since the differences are negligible, their relation with the age estimated from cementum can be considered practically of the same strength. Correlation coefficients calculated for P_3 (r=0.8992, 0.8992, 0.8978, 0.8903), exceeded the ones for P_2 in every sample (r=0.8597, 0.8576, 0.8458, 0.7686). For one of the samples there was no clear relationship between the age estimated from cementum and the crown height of P_1 (r=0.1285). In the other three cases correlation coefficients exceeded the values of the critical coefficient (r=0.3266, 0.2412, 0.2394), but these coefficients are practically too low to give any information on age.

STUBBE AND LOCKOW (cit.: VARGA, 1996) found a close negative relation with an r=0,855 absolute value between the crown height of M_1 teeth and the estimated age. CEDERLUND ET AL. (1991) determined the absolute value of the correlation coefficient to be r=0.66 for the regression of the age and the crown height of M_1. During a study of roe deer of different sexes, ASHBY AND HENRY (1979) characterized the relation between the estimated ages and the crown height of mandibular M_1 teeth with linear regression analysis. According to their calculations the absolute value of the correlation coefficient was r=0.86 meaning a close, statistically proven relationship.

The present study demonstrated a somewhat closer relation when examining the crown height of M_1 teeth. During the experiments correlation coefficients for the bucks were 0.9093, 0.9289, and 0.9438 in different habitats, respectively. For does this value turned out to be 0.9310. Reasons for the closer relationship are summarized below.

1. We applied second-degree regression instead of linear regression, which in itself accounts for a higher correlation coefficient;
2. Sexes were assessed separately;
3. Examination of analyses was broken down into habitat-related figures.

3.8. Parameters of the incisors

The age estimated by cement zones and the outer crown height of I_1 teeth indicate a statistically proven relation. Calculated correlation coefficients (r=0.8564, 0.7747, 0.7323, 0.5847) showed a close relationship for does, and close, moderate and weak relations for bucks in different habitats, despite that data averages did not differ in effect from each other.

In the case of the estimated age and the tooth-neck length of I_1, correlation coefficients revealed a moderate or weak, but verifiable relationship (r=0.7046, 0.6007, 0.4921, 0.4629).

Correlation coefficients for the age estimated from dental slides and the length of the worn part formed on the lingual surface of I_1 teeth (r=0.8790, 0.7923, 0.7199, 0.6002) demonstrated a strong relation for does, and moderate and weak relationships for bucks. When dropping the data of half-year-olds, we get only a moderate correlation coefficient for does as well.

Correlation coefficients regarding the estimated age and the inner (lingual) height of I_1 crowns (r=0.8535, 0.7915, 0.7092, 0.5717) indicated a strong relation for does, and moderate and weak, statistically proven relationships for bucks. When dropping the data of half-year-olds, we get a moderate regression, similar to the data obtained for bucks.

Correlation coefficient calculated for the relation between the ages of bucks estimated from cementum and the tooth angle of I_1 exceeds the critical value only in the sample from Tolna county and only to a slight degree (r=0.2844), where element number was also very high. Correlation coefficients for the other two samples (r=0.1393, 0.1063) do not reach the critical value on a 95% confidence level, therefore there was no statistically proven relation for these cases. This means that the incisor angle of bucks practically does not increase after one year of age.

Data analysis of tooth angles for does pointed out that the calculated correlation coefficient (r=0.3755) slightly exceeds the critical value, yielding a weak, statistically verifiable relationship.

Additional calculations performed by dropping the half-year-olds lead to a correlation coefficient (r=0.2156) lower than the critical value. From this we can conclude that after one year the angle of I_1 teeth in does not increase in a statistically verifiable way.

BIEGER (cit.: KŐHALMY, 1999) reported data with regard to the growth of incisor angles with advancing years. We were unable to compare his results with our experimental data, since BIEGER (cit.: KŐHALMY, 1999) did not report any statistical analysis. His data indicate that the angle of incisors shows signs of monotonous growth with age. Our study does not support this finding, as in some populations the correlation coefficients barely exceeded the critical value, while others are below it, and the relative deviation of the data sets is high. Consequently there was either a very weak relation to show or there was no relationship at all. From this we come to the conclusion that there is no real relationship useful for practice between the age and the incisor, and this parameter technically cannot give information about age on an individual level.

3.9. Dry weight of the lens

After studying the connection between the estimated age and the dry weight of the lens, MARINGGELLE (1979) ascertains the growth of the lens dry weight, but omitted statistical analysis of the data. ANGIBAULT ET AL. (1993) found a remarkably close regression while studying the relationship of age and lens weight in roe-deer of known age. The correlation coefficient for bucks was r=0.969, while for does r=0.967.

With a correlation coefficient of r=0.1703 we were unable to disclose a statistically proven relation for bucks during our experiments. A probable reason for this is that in the case of bucks we could examine only one-year-old or older individuals and data indicate that actually there is no growth in lens weight above one year of age. Assessing the age of does and weight of the lens dried on 105°C revealed a correlation coefficient of r=0.8438. This significant difference was caused partly by the fact that we were able to examine a large number of half-year-old does, when lens growth is highly intensive. Moreover, there was a slight growth in the case of does up to an age of approximately 5 years, adding to the observed difference.

3.10. Ossification of the nasal septum

We can deduce the age from the ossification of the nasal septum by the method of RAJNIK (1977). Earlier literature contains only subjective assessment instead of references to statistical analysis of the method. However, on the basis of our experiments we support the views of RAJNIK (1977) asserting that the ossification of the nasal septum properly follows the advancing years of age. There was a close, statistically proven relation for does between the age estimated from cement zone numbers and from the nasal septum. Correlation coefficient turned out to be 0.8841.

3.11. Length of the mandibular set of teeth

Absolute values of correlation coefficients for the age of bucks estimated from dental slides and from the length of the mandibular set of teeth (r=0.5763, 0.5411, 0.4301) exceeded the critical value in all thee of the cases, thus within the samples examined there was a weak relationship for the age estimated from cement zones and the length of the set of teeth. Analysing the whole data set of the does resulted in a somewhat higher correlation coefficient (r=0,6492). Dropping data related to the half-year-olds yielded a value of only 0.4195.

3.12. Gap-toothed edge

When assessing the relation between the estimated age and the tooth gap the correlation coefficient calculated for does (r=0.3338, 0.2811) hardly exceeds the critical value. Thus there was a weak, statistically proven relation between the age estimated by cementum and tooth gap length. The correlation coefficient obtained from the whole data set of does (r=0.7337) suggest a moderate connection. The value calculated after omitting half-year-old individuals is much lower, just like for bucks (r=0.2866).

4. Theses

1. Age can be precisely estimated from the number of cement zones in stocks of roe deer living in Hungarian habitats. Growth zones of the cement stock can be assessed also on dental slides; however, it is more exact with histotechnical methods, first of all with the Goldner trichromium, Heidenhain azan and Giemsa dying.
2. In the case of roe deer, age estimation is not applicable with slide techniques on the basis of secondary dentin, since the prepared dental slides presenting the secondary dentin did not show any sing of age-related layers in the sample of analysis. On the other hand histological examinations can be successfully applied just like in the case of the cementum.
3. Age estimation by tooth wear yields very close, statistically verifiable correlation with the age estimated form cement zones.
4. Crown height of the molars exhibit strong, statistically proven relationship with the age estimated from cement zones, except the P_1 teeth. In all the samples the strongest relation appears for the total average of molars. Highly close relation can be observed in the case of M_1, az M_2 and M_3 teeth.

5. Length parameters of I_1 teeth indicate a statistically verifiable relation with the age estimated from cement zones.
6. The angle of I_1 teeth and the age estimated from cementum shows no proper relation, consequently this parameter is not indicative of age.
7. Dry matter content of the lens in bucks is intensive up to the first year of age, becoming statistically negligible in the succeeding years. On the other hand, lens weight of does keeps growing up to approximately 5 years of age.
8. According to RAJNIK (1977) there is a close, statistically verifiable relation between the estimated ages of does coming from cement zones and from the nasal septum. Examining the nasal septum of does gives appropriate information on age.
9. On-site age estimation of live roe-deer is an acceptable procedure complying with practical requirements, but the professional experience, training and precision of the person performing the estimation fundamentally determines the reliability of the method.
10. Considering that in a given case trophy judgement may result in imposing sanctions against a hunter or a person entitled to hunting, the accuracy of this method is not acceptable According to our study the age estimated from cement zones was only in an average agreement of 37.6% with age estimation coming from trophy judgement.

5. Publications on the subject

Publications issued in revised scientific journals:

1. **Marosán, M.** (2000): Korbecslési vizsgálatok gímszarvasnál. Soproni Egyetem Tudományos Közleményei, 46, 145-159 pp.

2. **Marosán, M.** and Gergátz, E. (2001): Az őz (Capreolus capreolus (L.) 1758) egyes korra utaló morfológiai bélyegeinek vizsgálata. Acta Agronomica Óváriensis 43/2, 113-126 pp.

3. **Marosán, M.** (2001): Az őz (Capreolus capreolus (L.) 1758) korbecslési módszereinek összehasonlító értékelése. Nyugat-Magyarországi Egyetem Tudományos Közleményei (in press)

4. **Marosán, M.** (2001): A gímszarvas (Cervus elaphus L. 1758) egyes korbecslési módszereinek értékelése. Vadbiológia 8, 43-48 pp.

5. **Marosán, M.**, Gál J. és Király I. (2002): Data Relating to the Body Measurements of Roe Deer in Hungary. Acta Agronomica Óváriensis, 44/2, 141-147 pp.

Publication issued in conference booklets:

6. **Marosán, M.**, Gál, J. and Náhlik, A. (2003): Életkorbecslési vizsgálatok tapasztalatai őznél. Kárpát-medencei Biológiai Szimpózium 2003. Budapest.

7. **Marosán M.**, Gál, J. and Király, I. (2003): Őzsuták testméreteinek vizsgálata. Kárpát-medencei Biológiai Szimpózium 2003. Budapest.

8. Szikossy, I., **Marosán, M.** and Papp, I. (2003): A fog cementállományának vizsgálata történeti embertani anyagon. Kárpát-medencei Biológiai Szimpózium 2003. Budapest.

9. **Marosán, M.** Gál, J. and Hargitai, G (2003): A dámszarvas fogkopásának vizsgálata. Wellmann Oszkár Tudományos Tanácskozás, 2003. Hódmezővásárhely.

Technical reports:

10. **Marosán, M.** and Gál, J. (2003): Age Estimation Methods of Red Deer. A gímszarvas életkorbecslési módszerei. A Vadgazda Vadászmagazin 2003/1. 38-41pp.

11. **Marosán, M.**, Náhlik, A. and Gál, J. (2003): Az őz (Capreolus capreolus (L.) 1758) korbecslési módszerei. A Vadgazda Vadászmagazin 2003/8.

12. **Marosán, M.**, Gál, J. and Náhlik, A. (2003): Életkorbecslési módszerek dámszarvasnál. A Vadgazda Vadászmagazin 2003/10.

13. **Marosán, M.**, Gál, J. and Náhlik, A. (2004): Életkorbecslési módszerek vaddisznónál. Erdélyi Nimród. (in press)