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TWO EXAMPLES SHOWING CONTRADICTORY RESULTS BY USING SKELETOCHRONOLOGY IN BIRDS

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ABSTRACT: Bone sections from two different bird species of known age (*Delichon urbica* and *Alectoris rufa*) have been analyzed by skeletochronology. Growth lines appeared in long as well as in flat bones, though only those observed in the ascendent branch of the lower mandible from *Delichon urbica* seemed to be related with the age of the animal. In the case of *Alectoris rufa* the number of lines present in bone sections did not always correspond with the age of the animal. These results cast doubts on the inferential value of incremental lines in bird bones for archaeozoological and wildlife management purposes.

KEYWORDS: SKELETOCHRONOLOGY, BIRDS, LINES OF ARRESTED GROWTH, MANDIBLE

RESUMEN: Utilizando la técnica de esqueletocronología, se han estudiado diferentes secciones óseas de dos especies de aves (*Delichon urbica* y *Alectoris rufa*) cuyas edades se conocían. Las líneas de crecimiento se observaron tanto en huesos largos como planos; sin embargo, únicamente las vistas a nivel de la rama ascendente de la mandíbula inferior de *Delichon urbica*, parecen estar relacionadas con la edad del animal. En el caso de *Alectoris rufa*, el número de líneas presentes en las secciones, no siempre correspondía con la edad del ejemplar. Los resultados generan dudas sobre la posibilidad de utilizar las líneas de incremento en aves y su aplicación en Arqueozoología y dinámica de poblaciones.

PALABRAS CLAVE: ESQUELETOCRONOLOGIA, AVES, LINEAS DE CRECIMIENTO, MANDIBULA

INTRODUCTION

Many methods have been developed for age estimation in vertebrates, including the increase in body dimensions or weight with time, fusion of epiphysis or the increase in dry weight of the eye lens (Lamotte & Bourliere, 1975). However, most of them are inaccurate and prone to errors (Halliday & Verrel, 1988). Skeletochronology seems to be a valuable technique that allows one to establish the age of most vertebrate groups with a high degree of accuracy (Klevezal & Kleinenberg, 1969; Halliday & Verrel, 1988; Castanet & Baez, 1991). The method is based on the presence of growth marks, lines of arrested growth (LAG), that can be identified on histological sections of certain bones. In birds, however, not much work using skeletochronology has been done (Koubert & Hrabe, 1984; Klevezal et al., 1972; Van Soet & Van Utrecht, 1971; Nelson & Boorkhout, 1980).

In this paper, a series of flat and long bones from two different samples of know age of the species *Delichon urbica* (House Martin) and *Alectoris rufa* (Red-Legged Partridge) have been analyzed in order to establish those most suitable for skeletochronological purposes.

MATERIAL AND METHODS

Ten *Delichon urbica* (House Martin) from Extremadura (South -West Spain), and fifteen *Alectoris rufa* (Red-legged partridge) from a farm (Center of Spain), have been used. All animals were of known age. Specimens were macerated in tap water at 370° C until total putrefaction took place. Several types of long (Tibiotasus, Tarsometatarsus and Phalanges) and flat (Mandibula and Sternum) bones were selected. The bones were cleaned from surrounding tissue and decalcified in aqueous solutions of either 3% nitric acid (NA, Panreac) or 5% ethylenediamine tetracetic acid (EDTA, Probus) and then washed in a constant flow of water for at least one hour. The central area of the diaphysis from long bones and specific areas (Figure 1) from the flat bones were selected, frozen in CO_2 and cross-sectioned in 10-20 m thick with a freezing microtome. Staining was performed using aqueous solutions of either Ehrlich's hematoxylin (EH, Merck) for 40-60 min or $5x10^{-4}$ M toluidine blue (TB, Merck), methylene blue (MB, Sigma) or acridine orange (AO, Allied chemical) for 10-15 min. Sections were afterwards washed for three succesive times in distilled water, mounted in glycerol gelatin (Merck) and protected with a cover slip.

The age of each animal was established by counting, under the microscope, the number of lines of arrested growth (LAG) present in the bone sections, considering the outer margin of the section as a LAG (Smirina, 1972).

RESULTS

It was difficult to observe lines at the diaphysis level of long bones, whereas these could be distinguished in most of the flat bones from both species. Since the number of lines in the sections from samples of the same individual was variable, it was difficult to estimate the age of the animal from the sections of all bones examined, except for the mandible. In the case of this bone we have found that some sections from *Delichon urbica* showed lines that seemed to correlate well with the animal's known age.

In order to select that portion of the mandible in which age-related lines were best seen, the bone was cut in several pieces (Figure 1) before sectioning it with the microtome. Cross-sections at level B of the ascendent branch of mandible turned out to be the best zone to observe reliable lines in both species.

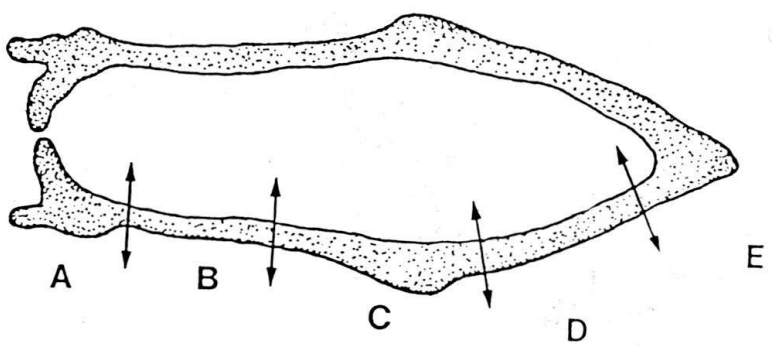

FIGURE 1 - Anatomical scheme of the mandible of *Delichon urbica*. The one belonging to *Alectoris rufa* is not represented. The mandibles were cut off at the levels indicated before being cross-sectioned.

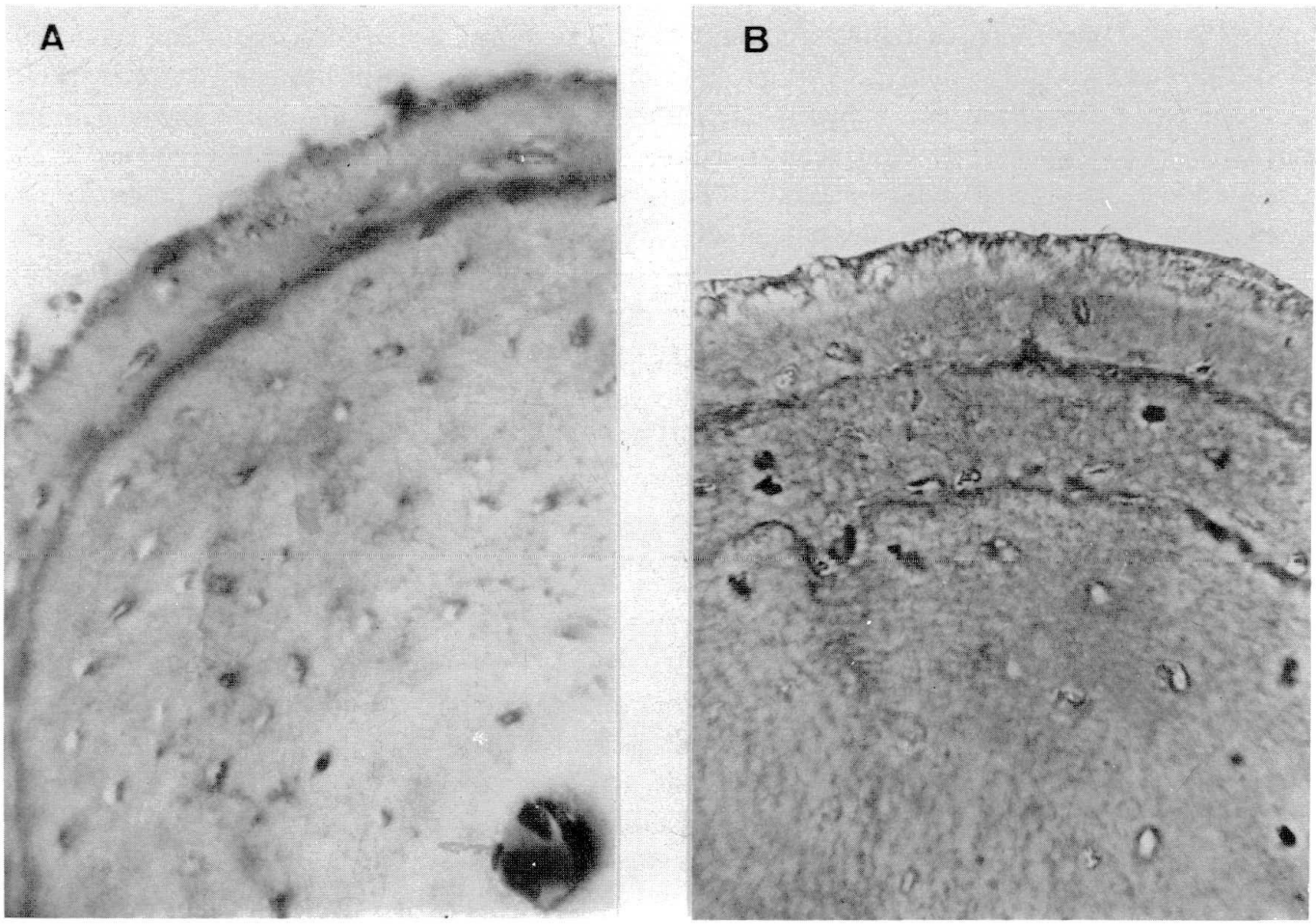

FIGURE 2 - Transverse sections at the ascendent branch of lower mandible (level B) of *Delichon urbica*. Sections were stained with Ehrlich's hematoxylin. A: one LAG is present on the section, since the animal was less than 2 years old. B: specimen with two LAGs. (x 40).

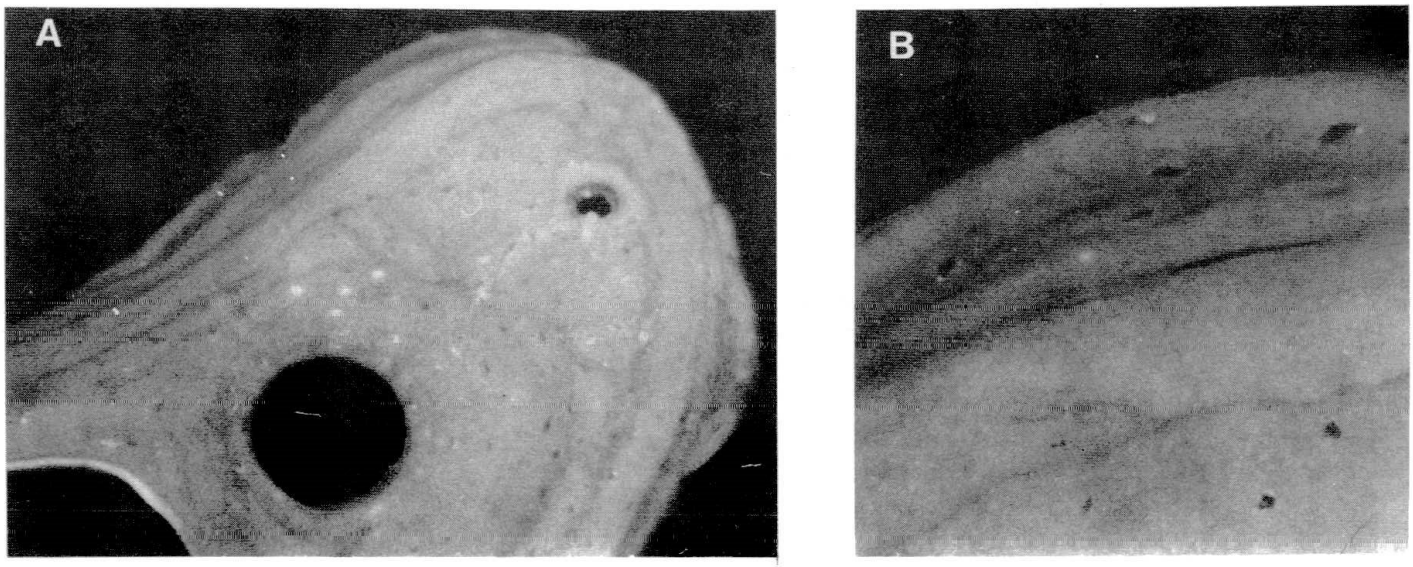

FIGURE 3 - Cross-sectioned mandible of a five years old specimen of *Delichon urbica* stained with 0.5 m acridine orange. Only three LAGs are clearly visible on the section, however, considerable bone remodelling is observed when compared with younger animals. A: (x 16); B: (x 40).

Figure 2 and 3 show mandible sections at level B from *Delichon urbica*. Assuming that a wide band followed by a narrow one (LAG) represents the bone tissue deposited in one year, this species shows a good correspondence between the number of lines and age for animals younger than four-five years. However, no more than four lines were observed in the mandible sections in any case. Old animals showed an high amount of bone resorption and remodelling which might account for inaccurate age assigments in later ontogenetic stages.

In the case of *Alectoris rufa* the number of lines present on the mandibular sections at level B did not correspond, in general, with the known age of the animal. Moreover, differences were observed in the number of lines in animals with the same age (data not shown).

Finally, no differences between either the various cationic dyes (EH, TB, and OA) or the decalcification procedures followed (NA and EDTA) have been found regarding the visualization of lines in the different samples studied.

DISCUSSION

It is tempting to consider that skeletochronology could be successfully applied for age estimation in *Delichon urbica*. Mandible, and, in particular, the ascendent branch, was found to be the best bone to this purpose. In young animals, the number of LAGs observed in the sections correlates well with age and it was possible to establish age-classes 1, 2, 3 and 4. When animals were older than four-five years, the number of lines counted were smaller than expected, and a strong bone remodelling was observed. It was, nevertheless, possible to create a new age-class corresponding to animals older than four years. Taking into account both factors, number of LAGs and bone remodelling, mandible analysis by skeletochronology could be a useful tool for age estimation of *Delichon urbica* and other bird species (Koubek & Hrabe, 1984; Nelson & Boohout, 1980; Van Soert & Van Utrech, 1971). On the other hand, no lines related with age have been observed on bones of *Alectoris rufa*, so that the method is perhaps not suitable for this species though the question still remains open as to wether this lack of positive correlation in the partridge relates to the hormonal imbalance which caged (i.e., farm) animals might eventually develop.

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