

## BONE PROFILER: A TOOL TO QUANTIFY, MODEL, AND STATISTICALLY COMPARE BONE-SECTION COMPACTNESS PROFILES

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Bone cross sections show many characteristics that have been used to infer various properties of the individual or taxon from which they were prepared. Life history traits (Castanet et al., 1993), locomotor patterns (Casinos, 1996), habitat (Laurin et al., 2000), metabolism (Riquès, 1983), and growth rate (Rimblot-Baly et al., 1995) are some examples. However, bone microstructure is very complex, and few objective, quantitative methods (other than very simple ones, such as the cortico-diaphyseal index, or the global compactness) are available to describe bone sections in a way that enables statistical inference or comparison.

Several cross-sectional properties, such as the cross-sectional area, centroid coordinates relative to reference axes, second moments of area about x and y axes, principal second moments of area and their orientation, section modulus, and polar moment of area have been obtained using SLICE (Nagurka and Hayes, 1980) by Ruff and Hayes (1983) and others, while Cubo and Casinos (1998) produced their own software for similar purposes. These parameters are especially useful for studying the stiffness, strength, or resistance of bones for animals of known weight and living in similar habitats. They do not describe how the bone tissue is organized.

To model bone tissue distribution in sections, we need to reduce its complexity to a limited number of variables that can be further compared using statistical tests. Among the various characteristics of bone sections that could be analyzed, we decided to work mainly on compactness (amount of bone present on a given surface) because this characteristic has previously been suspected to provide much information about the life style of taxa (Laurin et al., 2000). Furthermore, compactness can be measured relatively easily from a bone section (because it only requires knowledge about the presence or absence of bone on a given area). In contrast, bone mineral density requires the use of quantitative micro-radiography that is technically more demanding. An additional advantage of working with compactness is that it can be measured both on extant animals and on fossils. Bone mineral density can usually not be evaluated on fossil bones because of the variable amount of permineralization and other diagenetic phenomena that affect fossil tissues.

### SYSTEMS, METHODS AND ALGORITHMS

The standard procedure to analyze bone sections using our new software (Bone Profiler) is:

1. Digitize a histological section or a drawing thereof,
2. Open a bone section image,
3. Locate the center of the bone section,
4. Estimate bone compactness in concentric surfaces,
5. Model the bone compactness using a mathematical function,
6. Compare bone compactness from various bone sections.

The Bone Profiler computer program can handle all these steps automatically, making the comparison easy and reproducible overall. The Macintosh computer program (Power PC, Mac OS 8.x and 9.x) is freely available at URL: [http://www.ese.u-psud.fr/epc/conservation/Bone\\_profiler/Index.html](http://www.ese.u-psud.fr/epc/conservation/Bone_profiler/Index.html). It requires at least 8 Mb of free RAM. Digitizing images requires a flatbed scanner, a digital camera mounted on a microscope, or similar devices.

### Bone Center

Images of bone sections can be obtained using a video camera or a regular camera mounted on a microscope or a binocular lens, as well as by direct scanning (using flatbed scanners) for large sections. In some cases, bone (and calcified cartilage, if present) can be directly distinguished from the background (including non-mineralized spaces) using the color range. In other cases, the images have to be simplified to be analyzed by setting bone to one color and the background to another. Most standard graphic formats (JPEG, TIFF, BMP, PICT, PNG, GIF) can be read by the program. Next, the "bone center" must be determined. The user can position the center manually but an automatic procedure is also implemented. The center of the section (as defined by the outer surface of the bone) is not generally a correct estimate of the true center of the bone because bone deposition rate on the outer surface and bone resorption rate in the medullary cavity are usually anisotropic. The best estimate of the bone center is calculated as follows:

$$MbBc = MbMc \times SbMc/SbSc$$

where **Bc** is the bone center (the center we are searching for, using the outlines of the bone and of the medullary cavity), **Mc** is the medullary center, **Sc** is the section center (defined using only the outline of the bone surface), **Mb** is the point on the medullary border that is crossed by the axis that extends from the section center (Sc) to the medullary center (Mc) (in that direction), **Sb** is the point on the section border that is crossed by the axis that extends from the section center (Sc) to the medullary center (Mc) (in that direction), **MbBc** is the distance between the medullary border (Mb) and the bone center (Bc), **MbMc** is the distance between the medullary border (Mb) and the medullary center (Mc), **SbSc** is the distance between the section border (Sb) and the section center (Sc), and **SbMc** is the distance between the section border (Sb) and the medullary center (Mc). The bone center is located closer to the medullary center than to the section center, and outside the segment of the axis that links these two points (Fig. 1).

The program can also handle partial bone sections. This option is particularly useful when fossils are analyzed, because often only a part of the bone is adequately preserved. In this case, only the manual center-positioning option should be used and the program itself detects that a partial bone section is present.

### Compactness Profile

After the bone center has been located, the compactness profile of the bone section can be estimated. The program uses the bone center as a pivotal point, and compactness is estimated in all directions from that point by shifting the axis along which the pixels are read by one pixel at a time, at the edge of the bone, until the whole surface of the section has been covered. Then, the compactness is measured in 50 concentric zones, each of which measures 2% of the radius of the bone.

After considering several functions and looking at a few hundred bone sections, we found that the bone compactness **C** as a function of the distance to the center **d** was best described by a sigmoidal function:

$$C(d) = \frac{1}{1 + e^{(1/S)(P-d)}}(Max - Min) + Min \quad (1)$$

where: **P** is the relative distance from the center to the point where the

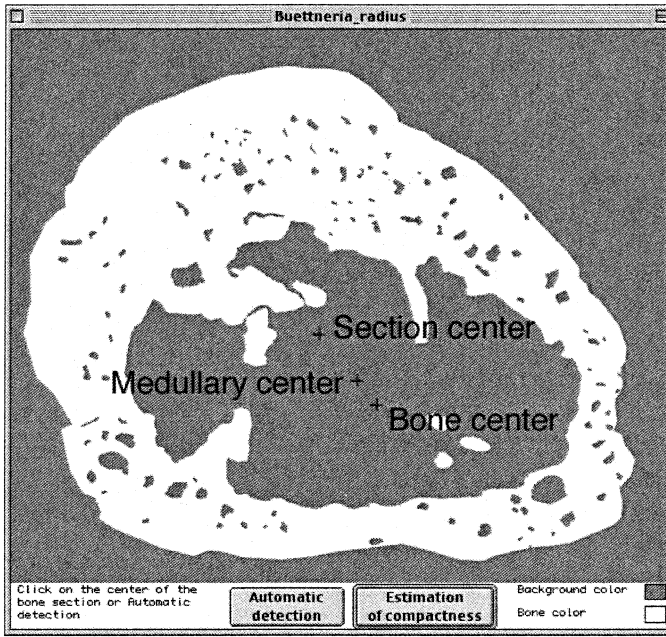


FIGURE 1. Automatic detection of the bone center using a section of a radius of the Upper Triassic (Carnian) stereospondyl *Buettneria*. Note that the program can also handle bone sections exhibiting color heterogeneity.

most abrupt change in compactness is observed. This relative distance is the ratio between the distance to the center and the radius. This generally corresponds with the position of the transition zone between the cortical compacta and the medullary cavity (or spongiosa). **S** is the reciprocal of the slope of the compactness change at point P. It usually reflects the width of the transition zone between the cortical compacta and the medullary cavity (or the medullary spongiosa). **Max** is the upper asymptote. It generally reflects the compactness in the outermost cortex. **Min** is the lower asymptote. It generally reflects the compactness in the center of the medulla.

The function is fitted by a Levenberg-Marquart algorithm using the sum of squared differences between observed and calculated compactness values as an adjustment quality measure (Press et al., 1992; Hilborn and Mangel, 1997).

A graph in the results window shows the observed compactness values as well as the fit by equation (1) (Fig. 2). The same window shows the estimates and standard deviations of the compactness profile parameters. It also gives R/t and CDI values that have been previously used to describe bone sections (Currey and Alexander, 1985; Castanet et al., 2000) when bones can be approximated by a tube (see Discussion).

**Statistical Comparison of Compactness Profiles**

When several sections have been analyzed, the program can test for within-group homogeneity as well as homogeneity between groups using the likelihood ratio test (LRT) approach. We first explain the theory.

Let  $P(d | m)$  represent the probability of the observed data given the model. Calculating  $P(d | m)$  requires estimating several parameters (P, S, Min, and Max for the most common case). Let  $L(m; d)$  represent the likelihood of the observed data given the model. It is a property of likelihood that  $L(m; d) \propto P(d | m)$  where the constant of proportionality is arbitrary (Edwards, 1972). The difference between the probability and likelihood approaches is that the probability approach describes the data for a given hypothesis, whereas the likelihood approach seeks the hypothesis that best describes the data.

The LRT tests the goodness of fit of two models using:

$$\delta = -2 \ln \left( \frac{ML_0}{ML_1} \right) \tag{2}$$

Where  $ML_0$  is the maximum likelihood under the null hypothesis (simple model) and  $ML_1$  is the maximum likelihood under the alternative

hypothesis (a more parameter-rich model). When the models are nested, the  $\delta$  statistic is asymptotically distributed as  $\chi^2$  with  $q$  degrees of freedom, where  $q$  is the difference in number of free parameters between the two models (Kendall and Stuart, 1979).

Let  $k$  profiles be compared. The null hypothesis is that all series are identical such that a single set of parameters (P, S, Min and Max) is sufficient to describe them. The alternative hypothesis is that the profiles are different from each other and that each profile  $j$  requires its own set of ( $P_j, S_j, Min_j$  and  $Max_j$ ) values.

We then need to construct a likelihood function that quantifies the quality of an adjustment of equation (1) to the data. For this, we will assume that the error of compactness in each zone (i.e., 2% of the total section) is normally distributed with a  $\sigma$  standard deviation. However, the  $\sigma$  values cannot be set constant according to the distance to the center of the bone section because, generally, bone structure in the cortex and in the medulla is less variable than in the transition zone (here measured by the P value). Therefore, for each zone  $i$  (1 to 50) which is located at a mean relative distance to P called  $d_i = |(2i - 1)/100 - P|$ , the corresponding  $\sigma^2$  values have been modeled as linear functions:

$$\sigma^2(d_i) = a \cdot d_i + b$$

where parameters  $a$  and  $b$  are estimated using least-square linear regression.

Then the likelihood of a series of  $n$  measurements from the same section fitted with equation (1) using a set of ML parameters (P, S, Min and Max) can be expressed by:

$$\begin{aligned} & -\ln L(d; P, S, Min, Max) \\ &= n \left[ \frac{1}{2} \ln(2\pi) \right] + \sum_{i=1}^n \left\{ \ln \sigma_i + \frac{[Co_i - Cc_i(P, S, Min, Max)]^2}{2\sigma_i^2} \right\} \end{aligned} \tag{3}$$

Where  $Co_i$  is the observed compactness on the surface  $i$  and  $Cc_i(P, S, Max, Min)$  is the compactness calculated using the values of P, S, Min and Max and the position of  $i$ .

For this test, we need to estimate (P, S, Min and Max) of the global profile of the group of  $k$  sections, and ( $P_j, S_j, Min_j$  and  $Max_j$ ) of the  $k$  individual profiles.

The  $\delta$  value can therefore be simplified as:

$$\begin{aligned} \delta &= 2 \sum_{j=1}^k \sum_{i=1}^n \left\{ \ln \sigma_i + \frac{[Co_{ij} - Cc_i(P, S, Min, Max)]^2}{2\sigma_i^2} \right\} \\ &\quad - 2 \sum_{j=1}^k \sum_{i=1}^n \left\{ \ln \sigma_{ij} + \frac{[Co_{ij} - Cc_i(P_j, S_j, Min_j, Max_j)]^2}{2\sigma_{ij}^2} \right\} \end{aligned} \tag{4}$$

If not all parameters (P, S, Min, Max) are selected to be tested, the unchecked parameters are unchanged in  $\delta$  estimates compared to the values calculated for individual series. Therefore, only the checked parameters contribute to changes in the likelihood function. The number of degrees of freedom is therefore  $(k - 1) \times$  (number of checked parameters).

Generally, the fit of equation (1) to the data is very good (see Fig. 2), and therefore the within-group likelihood ratio test detects significant differences between bones. A negative result implies that the fit is poor and that these profiles should be used with caution.

The theory for the test of homogeneity between groups is the same as for the within-group test, except that this time, the likelihoods of models for two or more groups of profiles are compared against the likelihood of a global model for a group that includes all the profiles. This test can be used to detect significant differences in the bone compactness profiles of various taxa or various bones in the same taxon (Fig. 3).

**DISCUSSION**

As for many biological objects, bone cross-sections are complex and can yield much data. The computer program proposed here has two advantages. First, it can standardize the method used to analyze bone sections. For example, no objective method has been described to determine where the cortical compacta ends and where the cancellous bone begins. Thus, cortical thickness and compactness measurements given by various authors are difficult to compare, unless there is no

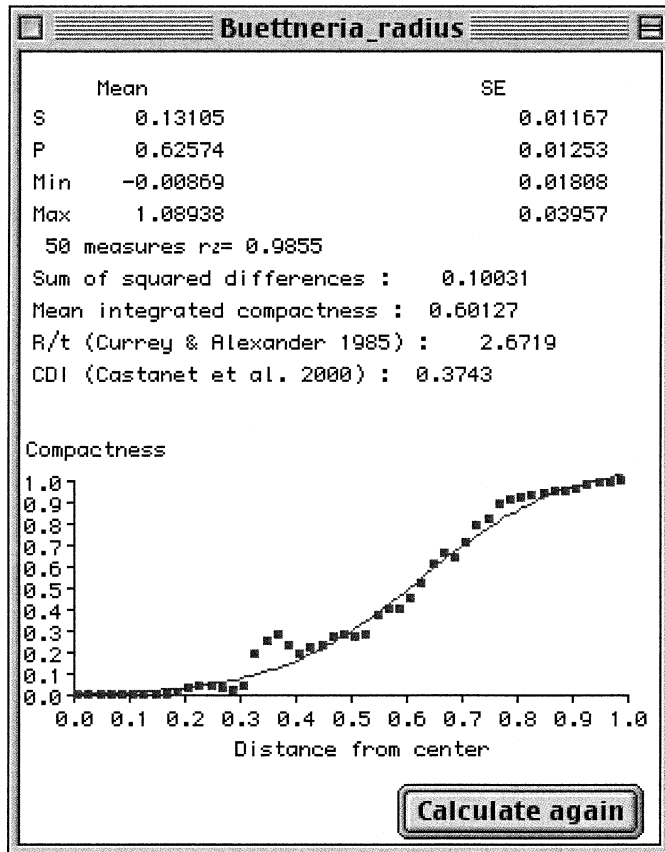


FIGURE 2. Fit by equation (1) (see text) of the compactness values of the bone image shown in Figure 1.

cancellous bone. Second, the program can model the bone compactness profile to test biological hypotheses.

The bone compactness profile can usually be satisfactorily described (Fig. 2) using only 4 parameters (S, P, Max and Min). These parameters share a relationship with parameters previously used to describe bones that show a simple tubular structure (in the absence of cancellous bone). The aspect of the compactness profile that has been quantified most often (Castanet et al., 2000) is the cortico-diaphyseal index or CDI (thickness of the cortex of the bone divided by the radius of the bone). Another index (K, the ratio between the internal and external diameter of the bone) has been used to compare the bones of tetrapods of various taxa and life styles (Currey and Alexander, 1985), to show how long-bone architecture was optimized to resist various types of physical constraints. Currey and Alexander (1985) have also used a ratio that they called R/t, where R is the outer radius of the bone, and t is the thickness of the wall.

Parameter P of our model has a simple relationship with the CDI, K, and R/t. P can be expressed as:

$$P = ID/ED.$$

where **ID** is the internal diameter of the bone (diameter of the medullary cavity), and **ED** is the external diameter of the bone.

The relationship between P and CDI can be expressed as:

$$CDI = (ED - ID)/ED = 1 - ID/ED = 1 - P.$$

K, as used by Currey and Alexander (1985) is exactly the same as P.

The R/t parameter is equal to  $ED/(ED - ID)$ . Hence,  $(R/t)^{-1} = (ED - ID)/ED = 1 - ID/ED = 1 - P$ . Therefore,  $R/t = (1 - P)^{-1}$ .

Moreover, Max often corresponds with cortical compactness and Min with the compactness of the medullary spongiosa, when they are relatively homogeneous.

Thus, the results obtained by our software are directly comparable to those of previous studies while being much more informative. This opens new possibilities of statistically comparing the long-bone micro-

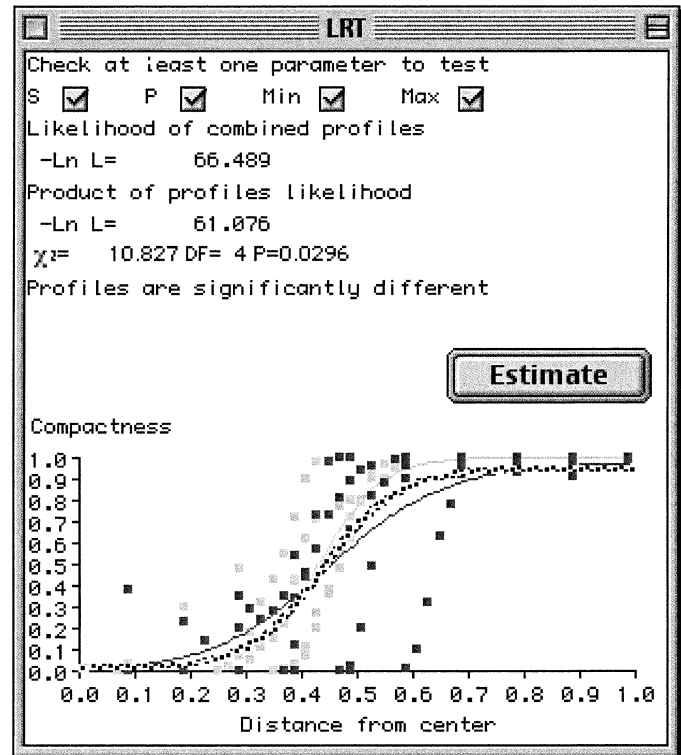


FIGURE 3. Likelihood ratio test for homogeneity between compactness profile parameters of two series of bone sections. The data and profiles of the two series are distinguished by the shading.

structure of various taxa. For example, it has been suggested (Ricqlès, 1977:fig. 26) that bone microstructure reflects the main habitat (from aquatic to terrestrial) of the organism. Analysis using Bone Profiler of an extensive database of femoral and humeral sections (Laurin et al., 2000) suggests that the parameters of the compactness profile model indeed reflect the habitat of extant tetrapods. For instance, the frequent presence of an extensive spongiosa that occludes the medullary cavity of aquatic taxa is reflected by a Min value higher than 0, whereas in terrestrial and amphibious taxa, the spongiosa is little developed or absent and Min is 0. The averages of this parameter are significantly different from each other in tetrapods of these two categories. In this case, our method confirms previously made qualitative observations (Ricqlès, 1977:fig. 26), but in other cases (S parameter, for example), qualitative observation is difficult or imprecise because visually describing the width of a transition zone between cortical compacta and medullary cavity or spongiosa is fairly subjective. Our method allows precise quantitative determination of this feature and statistical comparisons between groups. Thus, comparison of the compactness profile parameters of an extinct taxon with the distribution of these parameters in extant taxa of known habitat should enable us to infer the life style of the extinct taxon. Optimization of inferred habitat of several Paleozoic taxa on a phylogeny incorporating geological time could reveal the history and timing of the conquest of land by vertebrates (Laurin et al., 2000).

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