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History of sciences

Fifty years after Enlow and Brown's *Comparative histological study of fossil and recent bone tissues* (1956–1958): A review of Professor Donald H. Enlow's contribution to palaeohistology and comparative histology of bone

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Abstract

Fifty years after the publication of Enlow and Brown's seminal paper (1956–1958), this historical analysis of Professor Donald H. Enlow's works emphasizes their influence on the evolution of palaeohistology, comparative bone histology, and bone biology in general. Comparative analysis of recent and fossil bone tissues has shown the great variability of bone at the tissue level (histodiversity). Historically, Enlow's works have greatly influenced a shift in the reinterpretation of the causes of bone histodiversity, from phylogenetic to more functional (ontogenetic) explanations. This allows us to consider the issue of complex causalities in evolutionary biology. ***To cite this article: A.J. de Ricqlès, C. R. Palevol 6 (2007).***

Résumé

Cinquante ans après la publication de l'article d'Enlow et Brown sur l'histologie comparée des tissues osseux actuels et fossiles (1956–1958) : une revue de la contribution du professeur Donald Enlow à la paléohistologie et à l'histologie comparative de l'os. À l'occasion du cinquantenaire de la publication d'Enlow et Brown (1956–1958), cet article constitue une analyse historique des travaux du professeur Donald Enlow et de leur influence sur la paléohistologie, l'histologie osseuse comparative, et sur la biologie du tissus osseux en général. L'analyse comparative des tissus osseux actuels et fossiles a démontré la grande variabilité tissulaire de l'os (histodiversité osseuse). Historiquement, les travaux d'Enlow ont largement contribué à réorienter la compréhension des causes de l'histodiversité osseuse vers des explications de nature fonctionnelle (ontogénétique) plutôt que phylogénétique. Cela conduit à évoquer le problème des causalités complexes en biologie évolutionniste. ***Pour citer cet article : A.J. de Ricqlès, C. R. Palevol 6 (2007).***

Keywords: History of sciences; Bone histology; Palaeohistology; Bone biology; Evolution

Mots clés : Histoire des sciences ; Histologie osseuse ; Paléohistologie ; Biologie de l'os ; Évolution

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Le professeur Donald H. Enlow est une autorité de la seconde moitié du XX^{e} siècle dans le domaine de l'étude du tissu osseux (histologie). Au cours de sa carrière, il a publié de nombreux travaux en histologie osseuse, en anthropologie physique et en orthodontique. Les recherches qu'il a poursuivies sur le développement post-natal de la face et de la mandibule humaine ont conduit à des retombées pratiques considérables sur les plans biomédical et odontologique, en particulier en orthodontique et en pédodontique [14,15,18].

Plutôt que d'établir une revue des conséquences pratiques importantes des travaux du professeur Enlow découlant de ses études remarquables de l'histologie osseuse humaine, nous concentrons ici notre attention sur les aspects fondamentaux de ses travaux scientifiques. Ceux-ci sont liés à son implication initiale en paléohistologie et en histologie osseuse comparative, et à leur retentissement sur des problèmes généraux concernant la biologie et l'évolution de l'os en tant que tissu. Ces travaux correspondent aux premières phases de sa carrière.

Le premier travail d'Enlow débute par une tentative de classification et de nomenclature de l'os en tant que tissu [9 (pp. 408–412; Planches 1–3)]. Il semble que cette classification, dans laquelle la vascularisation du tissu osseux apparaît comme un critère très important, ait été fortement inspirée par celle de Foote [20] ; la terminologie de cet auteur est elle-même adoptée en partie. Cette tentative de classification s'est révélée très utile, dans la mesure où elle a aidé à se rendre compte à quel point la description traditionnelle du tissu osseux (recopiée de traités en précis) était manifestement inadéquate, mais elle a elle-même posé certains problèmes [42].

Ce travail de paléohistologie et d'histologie comparative d'Enlow et Brown [9] demeure un exemple d'enquête étendue, soigneuse et objective, accompagnée d'une remarquable discussion critique. Cependant, dans les conclusions de cette étude, on ressent comme une certaine déception des auteurs eux-mêmes vis-à-vis de l'approche comparative « extensive » qu'ils ont suivie. Pour ne citer qu'un exemple, à propos de la discussion sur les relations entre structure histologique et taille somatique, leur entrée en matière est la suivante (notre traduction) : « Les généralisations concernant des relations entre taille individuelle et patrons tissulaires ne sont pas manifestes », et beaucoup d'autres considérations des conclusions [9] présentent ces mêmes connotations, quelque peu négatives.

Il est donc intéressant de constater un changement d'approche complet de la part d'Enlow dans ses deux articles suivants [10,11], qui peuvent être regardés comme les « prototypes » de son fameux livre de 1963 : *Principles of bone remodeling* [12]. Dans ce maître livre et d'autres publications de la même période, on constate un abandon de l'approche comparative « extensive » au profit de l'analyse intensive de quelques os pris comme « modèles » dans un tout petit nombre d'espèces : *Homo* et *Macaca* [14,15]. C'est ce changement méthodologique qui va conduire Enlow à ses grandes synthèses interprétatives. Une coupe dans un os est aussi toujours une section dans le temps du développement ontogénique individuel. Les types de tissus et leurs associations dans une section expriment fondamentalement la succession d'événements qui eurent lieu précisément à ce niveau au cours du développement de l'os [12]. On ne peut les interpréter pleinement qu'en les intégrant à l'histoire totale de la croissance osseuse prise dans son ensemble, c'est-à-dire dans ses trois dimensions spatiales et sa dimension temporelle. En bref, Enlow est parvenu à décrypter les règles d'une « stratigraphie » du tissu osseux ayant une signification fonctionnelle [12]. La croissance, le modelage et le remodelage de l'os, avec toutes les contraintes topologiques intrinsèques à la biologie de ce tissu, sont exprimés par sa diversité histologique. En fait, les différences histologiques observées localement ne sont pas seulement les conséquences – et l'expression – de la croissance, du modelage et du remodelage osseux. Elles nous montrent plutôt en action *les causes biologiques elles-mêmes* de la spécificité de la croissance et de la mise en place de la forme locale au cours du développement. En d'autres termes, la diversité histologique de l'os exprime à nos yeux les facteurs biologiques agissant dans le temps et l'espace en tant que causes proximales, efficientes, des différences de forme et de taille observées entre organes et entre espèces [42–44].

Il est remarquable de constater que bien peu de chercheurs entraînés initialement à une approche expérimentale « intensive » du tissu osseux surent interpréter la signification de l'histodiversité osseuse comme le fit Enlow, dès lors que lui-même se tourna vers l'approche « intensive » sur des modèles. La longue familiarité préalable d'Enlow avec l'histologie comparée et la paléohistologie [9,13,17] ne fut donc pas, à cet égard, une perte de temps. Bien au contraire, c'est elle qui lui donna, avant tant d'autres, une compréhension profonde et globale des problèmes généraux de la biologie osseuse, ce qui lui permit de déchiffrer l'histodiversité osseuse de façon significative. Il est également remarquable de constater qu'un autre pionnier de la signification biologique de l'histodiversité osseuse, le professeur Rodolfo Amprino, suivit un cheminement intellectuel quelque peu comparable au début de sa carrière [1,2].

Pour résumer, on peut considérer que les publications d'Enlow à partir de 1962 ont été fondamentales, après celles d'Amprino, pour susciter un changement d'interprétation presque complet de la signification de l'histodiversité osseuse [47]. Plutôt que de mettre l'accent sur une causalité de nature phylogénétique et taxonomique, le point de vue traditionnel des paléontologues et des histologistes comparatistes avant lui, Enlow a insisté au contraire sur le rôle des facteurs immédiats, d'ordre fonctionnel, qui contrôlent les variations générales et locales du dépôt osseux au cours du développement [10–13]. Bien qu'il ait reconnu et pris explicitement en compte la causalité complexe et pluraliste de l'histodiversité osseuse, en particulier le rôle de facteurs mécaniques (voir ci-dessous), il a vraiment mis en exergue le rôle majeur des facteurs ontogéniques, ceux qui sont liés au déterminisme immédiat de la croissance locale et générale et de l'édification de la forme, pour rendre compte de la variabilité du tissu osseux. Cette approche a été extrêmement fructueuse et porteuse d'influence [42,43,44].

Enlow a, par ailleurs, mis l'accent (1968, 1973) sur l'importance particulière de la compréhension du *contrôle local* de l'activité des cellules productrices (ostéoblastes) et destructrices (ostéoclastes) de l'os [16,18]. C'est en effet à ces niveaux locaux que les activités de ces cellules s'intègrent de façon à produire les “remises en place séquentielles” en cours de croissance (*growth relocations*), qui rendent possible la croissance tout en conservant la forme et la fonction de l'os [12].

Le *factor of achitectonic circumstance* peut être compris comme exprimant la « remise en place séquentielle » de toutes les différentes régions de l'os, au fur et à mesure de la croissance de celui-ci [16]. Il est la conséquence des mécanismes de contrôle locaux, quels que soient ceux-ci, et doit être envisagé comme une caractéristique majeure de la biologie normale de la croissance osseuse [16]. Il y a chez Enlow une perception précise de la distinction logique et sémantique qui doit être faite entre des causes biologiques immédiates, efficientes, au sens physiologique ou biomécanique, et leurs conséquences ou expressions structurales. En d'autres termes, il distingue clairement entre *process* (mécanismes) et *pattern*s (résultats) [18]. Cela est clairement démontré par la distinction qu'il fait entre *bone relocation* (remise en position séquentielle), une conséquence morphologique (= résultat) et *bone remodeling* (remaniement osseux) = un mécanisme biologique.

Enlow a pris ses distances avec la notion généralement admise alors (et toujours largement acceptée [50,51]), selon laquelle les contraintes mécaniques appliquées à l'os modulent très largement sa biologie et peuvent donc être considérées comme un agent causal très important de ses caractéristiques structurales. En d'autres termes, la biomécanique serait le facteur capital, peut-être unique, contrôlant le comportement de l'os et ses caractéristiques tissulaires associées. Grâce à une liste précise de cas concrets, Enlow montre dans quelle mesure l'action présumée sur l'os des pressions et tensions, envisagées dans des limites très variées, y compris à des valeurs biologiquement peu plausibles, peut *ou non* être acceptée comme facteur explicatif de la structure et de la biologie osseuse [16]. Il discute la logique critiquable des conclusions de tant de travaux expérimentaux proposant que, puisque l'application de contraintes artificielles (ou la suppression de contraintes naturelles) peut modifier le cours normal du développement osseux in vivo, ces contraintes mécaniques constituent, par elles-mêmes, le facteur principal régulant la croissance et le remaniement osseux.

Pour avoir eu le privilège d'observer et de discuter les préparations au microscope avec Donald Enlow, j'en retiens l'expérience non seulement d'une « lecture », mais aussi d'un véritable déchiffrement. Au-delà de la description statique et objective des divers types de tissus, de leurs positions et proportions relatives, etc., ce qui ressortait était une compréhension dynamique de la section, en termes d'interprétation fonctionnelle. Autrement dit, on apprenait à « comprendre » des structures statiques, muettes, en termes de dynamique fonctionnelle. Ces interprétations fonctionnelles se concentraient sur la dynamique de croissance et sur les contraintes géométriques et topologiques du modelage et du remodelage exprimées dans les structures tissulaires, en tenant compte des particularités biologiques spécifiques de l'os en tant que tissu dur vivant [12]. La meilleure comparaison que l'on puisse offrir est celle avec la stratigraphie et la géologie historique. Parce qu'elle est formée de matériaux durs, une falaise, tout comme une tranche d'os, constitue un enregistrement spatio-temporel. Le géologue doit apprendre à déchiffrer les dépôts, les failles, les plissements, l'érosion, les discordances, les dépôts secondaires, etc. dans ses coupes stratigraphiques pour pouvoir reconstituer l'histoire passée et l'évolution d'une région. De même, l'histologiste des tissus durs doit apprendre à reconnaître des structures analogues pour interpréter convenablement ses lames minces dans le contexte tridimensionnel et temporel de l'os qu'il analyse.

Certes, ces interprétations histomorphogénétiques de l'os n'épuisent pas toutes les informations enregistrées dans sa structure. Au-delà de l'enregistrement de sa propre dynamique de croissance, le tissu osseux enregistre bien d'autres « messages », qu'à la suite d'Enlow on s'emploie aujourd'hui à déchiffrer : quelques exemples en sont donnés ci-dessous [5,6,19,22,31–34,38]. Il apparaît ainsi que l'histodiversité osseuse exprime une causalité biologique très complexe, dans laquelle les facteurs immédiats (ontogéniques), bien mis en exergue par Enlow, sont combinés avec d'autres (biomécaniques, phylogénétiques. . .), d'où résulte la très grande richesse de l'information biologique susceptible d'être extraite du tissu osseux.

1. Introduction

Professor Donald H. Enlow (Fig. 1) was a leading authority of the second half of 20th century in bone histology: the study of bone at the tissue level, using the compound microscope. During his career, he has published extensively in the domains of comparative histology of bone, physical anthropology, and orthodontics. Various studies that he conducted on the postnatal development of the human face and lower jaw [14,15] led to considerable practical breakthroughs in medical and dental care, notably in pedodontics and orthodontics. Rather than exploring those practically important consequences of Professor Enlow's masterful analyses of human bone histology, we review here the origins and more general aspects of Enlow's scientific accomplishments. They are linked to his early involvement with comparative bone histology and palaeohistology, and their bearing on the general issues of biology and evolution of bone as a tissue. Those contributions cover the first part of Professor Enlow's career. They start with the renowned three-part paper by Enlow and Brown [9] published from 1956 to 1958 and end with Enlow's contribution to the first volume of Carl Gans' famous series *Biology of the Reptilia*, published in 1969 [17], and with some general papers published later on [18]. During this period, covering roughly 15 years and ending with the early seventies, it is possible to follow a very interesting intellectual evolution.

Fig. 1. Donald H. Enlow during his early career, when he was involved in palaeohistology and comparative histology of bone. (Photo via Prof. Mary Schweitzer).

Fig. 1. Donald H. Enlow au début de sa carrière, quand il se consacrait à la paléohistologie et à l'histologie comparative de l'os. (Photo obtenue par l'intermédiaire du Pr. Mary Schweitzer).

2. Enlow's approaches and methods in comparative bone histology (Fig. 1)

For Enlow and Brown (1956–1958), the scientific approach is obviously a "natural history" one, using a broad comparative methodology [9]. Under the influence of Hod Sawain, his mentor on field trips in the Permian Texas red beds who conveyed to him the spirit of the great Harvard vertebrate palaeontologist Professor Alfred Romer, it is clear that the young Donald Enlow was first deeply inclined toward palaeontology, and this is why the description of fossil material is so prevalent in his early works. One can also notice in them a deep influence of an earlier student of bone comparative histology in the United States, Dr. Foote, whose *opus magnus* was published in 1916 [20]. One cannot overemphasize the clarity, usefulness, and everlasting value of Enlow and Brown's work, which has been my own direct inspiration [42]. Nevertheless, in their conclusions (1958), one can feel some discomfort toward the broad comparative approach that they took [9]. To quote only one example, when discussing possible relationships between body size and bone histology, Enlow and Brown begin (p. 214) with the following sentence: "generalizations concerning relationships between body size of an individual and bone tissue pattern are not apparent." Similar sentences can be found repeatedly in their conclusions about several other important issues regarding the significance of bone tissue variability, notably about Haversian replacement. It is clear that the 'program' once proposed by John Queckett [40] at the dawn of palaeohistology in 1849, namely to use bone microscopical structures “in determining the affinities”, in other words, to use bone histology for taxonomic determination, had proved impractical and had essentially failed.

So it is interesting to find a complete metamorphosis in the intellectual and practical approach taken by Enlow in his next two main papers, published in 1962 [10,11], which may be viewed as ‘prototypes’ of his famous book of 1963, *Principles of Bone Remodeling*. In this masterpiece [12], and his other papers of the period, there is a complete change from the extensive comparative approach to the intensive analysis of model bones in a few selected species [14,15]. Indeed, in order to decipher the biological significance of the great diversity and complexity that characterize bone histology, one has, as a first step, to reduce as much as possible the potential causal factors of tissue variability. Hence, selecting a few well-defined models in order to study them intensively was a prerequisite to understand bone histodiversity [12].

This was the new way that Enlow took, using some model bones in *Homo* and *Macaca*. From this came his great breakthroughs. A bone section is always also a slice in time of ontogeny. The actual tissue types and their associations in a section first and foremost express the succession of events that took place at that very level during bone development. They can be fully understood only if they are integrated into the total history of the bone as a whole, viewed in its three dimensions of space plus the time dimension of its full ontogeny [12]. With his concepts of ‘sequential relocation’ and ‘V principle’, Enlow thus ended the century-old approach of sharply dividing bone studies between issues of endochondral ossification, studied in longitudinal sections in epiphyses [26], on the one hand, and of periosteal ossification studied in shaft cross sections, on the other one [45]. He emphasized instead the structural and functional relationships between the two, and integrated them into a fuller explanation of growth remodelling. In short, Enlow deciphered the rules of ‘bone tissue stratigraphy’ in a functionally meaningful way. Growth, modelling, and remodelling, with all their built-in topological constraints on bone, are expressed in their histological diversity [12] (Fig. 2). Actually, the histological differences observed locally are not merely the *consequences* – and expression – of bone growth, modelling and remodelling. Rather they show at work the real *biological causes* of bone local-specific growth and shaping during ontogeny. In other words, bone histological variability expresses for us the *biological factors that act in time and space as the actual causes* of species and organ-specific shape and size differences [42–44].

With this view, Enlow documented and built upon some earlier approaches put forward by the great Italian histologist Rodolfo Amprino [1,2]. In his masterful 1947

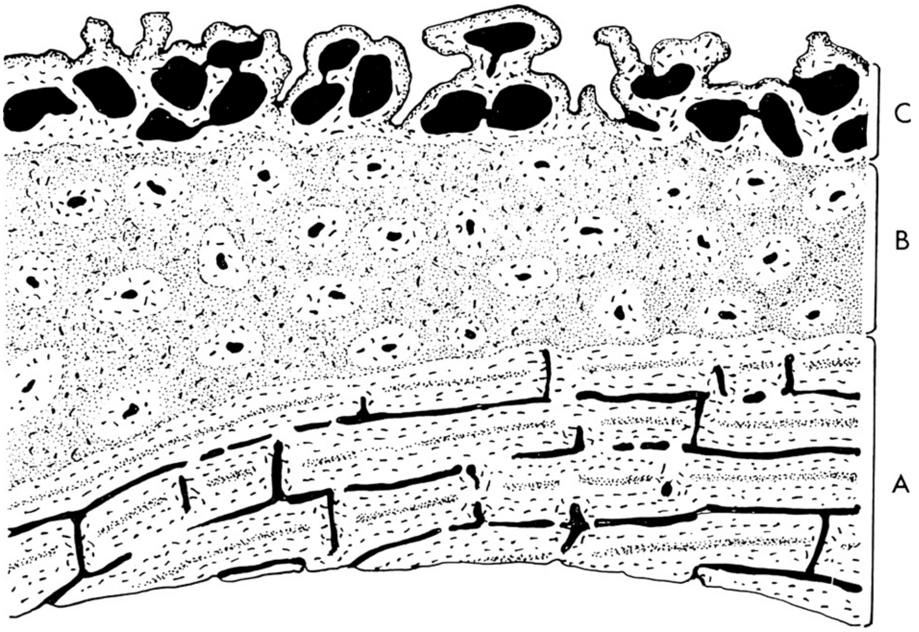

Fig. 2. A sketch drawing from Donald Enlow (1966) to illustrate bone cortex overall stratification by three types of primary bone tissues expressing fast bone growth. From [13].

Fig. 2. Un dessin par Donald H. Enlow (1966) illustrant la stratification du cortex d’origine périostique par trois types successifs de tissus d’apposition rapide (semi-schématique). D’après [13].

paper, of which the original French title may be translated as *The structure of bone tissue considered as the expression of differences in growth speeds* [1], Amprino anticipated many of Enlow's findings of the 1960s. In turn, Enlow integrated and generalized Amprino's ideas into his own interpretative scheme.

Two great papers end this era: Enlow's *Evaluation of the use of bone histology in forensic medicine and anthropology* (1966), on the one hand [13], and *The bone of Reptiles* (1969), on the other hand [17]. In both papers, one can observe a synthesis between Enlow's explanations of bone histodiversity in terms of ontogenetic factors of local growth and remodelling dynamics and their various consequences, with the wider dataset brought forward by the comparative approach.

A general remark may be appropriate to conclude this section. Many bone histologists, actually the vast majority of them, were trained in the realm of human anatomy, physiology, and biomedical approaches. By training and tradition, they were thus at once inclined toward the intensive, experimental approaches of bone biology (notably biomechanics) on selected models. Nevertheless, very few of them ever discovered or even really grasped the full significance of bone histodiversity as Enlow did when he turned to the intensive approach using models. Thus, in retrospect, Enlow's long early involvement in the realm of comparative histology and palaeohistology [9,12,17] was not a waste of time. It gave him, long before most others, a broad and unique insight into the general issues at hand in bone biology, which allowed him to decipher histodiversity meaningfully. It is very interesting to note that another great pioneer of the biological interpretation of bone tissue diversity, Prof. Rodolfo Amprino [1,2] followed a rather similar intellectual pathway during his early career.

3. Growth, structure, form and function. On some prevalent themes in Enlow's comparative evolutionary work

This section could well combine the titles of two famous books of everlasting interest: *On Growth and Form* by D'Arcy Thompson (1917) and E.S. Russell's *Form and Function* (1916), as well as those of many of their followers (see [35]). Indeed, during his career, Donald Enlow worked extensively, from various points of view and approaches, on fundamental and perennial issues common to developmental biology and evolutionary biology: *the origination, significance, and evolution of form*, using bone tissue as a model [45]. Here are some selected focal points of his interests and thoughts, spread among many of his works.

3.1. Enlow's influence on the classification of bone tissues types

Enlow and Brown's first paper (1956) begins with a classificatory scheme of bone tissues (pp. 408–412; Plates 1–3) [9]. It appears that this scheme, in which bone vascularity is a very important character, relies heavily on earlier works by Foote (1916, etc.) and even some of Foote's vocabulary is adopted [20]. This approach proved useful, insofar as it helped to realize that the traditional (or textbook) descriptions of bone as a tissue were grossly inadequate, but it also raised some problems. Enlow came back to this issue, notably in his 1966 paper on the use of bone histology in forensic medicine and anthropology [13] (Fig. 2). In this paper, he introduced much new vocabulary and many concepts. For instance, an important category of vascular canals, the simple primary canals (*not* primary osteons), is clearly introduced for the first time. On the other hand, some issues, such as the significance of laminar tissue *sensu* Enlow, still conflicted with former, well-rooted practices [25]. Accordingly, I could not keep some of Don Enlow's schemes and terminology in my own efforts toward a general classification of bone tissues [42–44,46, etc.]. It is obvious, however, that my own debt (and that of later authors such as Reid [41], etc.) to the Enlowian approach to bone tissue classification is extensive.

3.2. Enlow's influence on the interpretation of bone histovariability: functions (notably growth) versus phylogeny

Since Cuvier and the rise of comparative anatomy and palaeontology, a traditional use of anatomical/morphological characters (and of differences among them) was for taxonomic/systematic purposes. Characters (and their associations and subordinations) were used first to create taxonomic entities and, later on, to diagnose actual specimens as pertaining to such entities. After 1859, Darwinian evolutionism popularized another dimension of taxonomic practices by interpreting classificatory schemes as more or less successful attempts to reconstruct and formalize the tree of life, namely its phylogeny. According to such views, interpretation of bone histodiversity by comparative histologists and palaeontologists of the late 19th century was a straightforward transposition to the tissue level of the taxonomic approaches and practices of morphologists who classified organisms [45]. This 'program' was already explicit in the title of Queckett's early dissertation [40], and was more or less implicitly followed by later authors [47], often within the intellectual framework of Haeckelian recapitulationism. However, this 'program' proved difficult to implement. Apart from the dermal skeleton of lower vertebrates, in which taxon-specific combinations of bone and dental tissues commonly allow taxonomic diagnoses [29], the use of variations of bone tissues to assess taxonomic determinations generally proved poorly successful and even deceptive. This is clear from many comparative works from the late 19th and early 20th centuries [2,20,25,30,36,39,48], and even those by Enlow and Brown themselves [9]. As is evident from Enlow's later approaches of the early 1960s, the ontogenetic factors linked to bone growth and remodelling appear as the actual, immediate (or proximate) factors (or causes) that modify bone as a tissue. Conversely, it also appeared at last that the (so far) all-important 'phylogenetic factors' could at best be viewed as medial (or distal) causal factors of bone tissue diversity, imposed by a more or less remote ancestry [42–44].

To sum up, it can be said that Enlow's publications from 1962 on were instrumental (after those of Amprino [1,2]), in stirring up a nearly complete change in the interpretation of bone histodiversity [47]. Rather than focusing on a phylogenetic-taxonomic causality, the traditional view of comparative histologists and palaeontologists before him, Enlow focused instead on the immediate functional factors that control local and general variations of bone as a tissue during ontogeny. Although he fully recognized the pluralistic causality of bone diversity, notably the influence of biomechanical factors – more on that below –, he definitely stressed the major significance of ontogenetic factors, those linked to local and general growth and the shaping of form, in order to make sense of bone tissue variability. This approach has been immensely successful and influential [19,28,41,43].

3.3. Functional factors: general and local, Wolff's law and the factor of architectonic circumstance

One of the most intellectually challenging and deeply reasoned early papers by Don Enlow is his *Wolff's Law and the factor of architectonic circumstance*, published in 1968 [16] and summarized in 1973 [18]. In his earlier works, we saw that Enlow had conclusively demonstrated that a great part of the histological variability of bone could be satisfactorily understood in terms of local shape modelling and remodelling dynamics, linked to the general topological constraints of growth introduced by the peculiar biology of a living – but hard – tissue: namely sequential relocation [12]. Those considerations, in turn, led to the problem of the *biological control* of the living cells (mainly osteoblasts and osteoclasts) that are actually responsible for the processes of bone deposition and erosion. This control should be understood in time and space, and at all integrative levels. For instance, it works both at the general systemic level of the whole organism (hormones), at the level of whole bones as organs and at the very localized bone levels where each tiny bone region must keep, while growing, its specific (and functional) shape and structure.

In his Wolff's law paper, Enlow clearly analyzes this situation [16]. He begins with a precise hierarchical distinction between the various biological factors, from molecular and local to systemic and general, that have been proposed as actual controls on bone biology and structure. He keeps a highly open, pluralistic view, but wisely discusses whether each and every proposed control factor of bone biology may be understood as (1) necessary and sufficient, and hence primarily causal, or (2) merely as ancillary, triggering, or forming a threshold, or a link within a causal cascade, or (3) only as an accessory, associated, supportive or catalyst, or (4) sometimes even as an effect rather than a causative agent. He stresses the special importance of understanding the *local control* of bone-forming and bone-destroying cells, because it is at such local levels that cell activities integrate to produce the all-important growth relocations that allow growth with maintenance of bone shape and functions [18]. The 'factor of architectonic circumstance' may be understood as concerned with the progressive relocation of all of a bone's different parts as the whole bone grows. It is a basic effect of local control mechanisms, whatever they are, and should be regarded as a major feature of normal bone growth biology.

Second, Enlow [16] takes issue with the then prevalent (and still widely accepted: e.g., [50,51]) notion that mechanical strains applied to bone modify its biology to a great degree and, accordingly, are the main causal factors of bone structure [16]. In other words, biomechanics would be the all-important, perhaps unique factor controlling bone behaviour and its related histological characteristics. Through a precise list of indisputable case studies, Enlow shows to what extent the assumed functions of tensions and pressures, even set within highly variable and unlikely thresholds, may or *may not* be accepted as explanatory factors of bone structure and biology. He argues against the flawed logic of the conclusions of many experimental works that submit that, because artificially applied stress (or removal of normal stress) can change the normal course of growth in vivo, stress thereby represents the main influence that regulates all bone growth and remodelling. Third, he proposes that the all-important process of growth relocation by remodelling may be expressed by a descriptive concept of 'growth fields' that are generally very distinct from the actual anatomical bone limits, but instead can extend over parts of several adjacent but anatomically distinct bones [16,44,45]. One can find here seeds of many new approaches, which paved the way for bone biology, fundamental and applied, up to the present.

3.4. *Enlow's early distinction between patterns and processes*

In the same papers [16,18]) appears an original reflection that has perhaps remained unnoticed, but that ultimately received extensive coverage and fame, perhaps not so much in bone histology itself, but in more or less closely related fields, such as systematics and developmental biology. This reflection deals with the logic and semantic distinctions between terms that are merely *descriptions* of structures or events, but that are rather often mistakenly understood, or accepted, as *explanations* of how they take place. Enlow asks [18 (p. 404)] if some factors proposed as biological controls of bone behaviour are merely titles for some biologic process or events that do not however explain the operation of the mechanisms involved. Are they, in effect, just synonyms for 'control processes', without explaining how the processes themselves actually work? There is a sharp perception of the logical distinction between actual biological causes, in a physiological and mechanistic sense, and of their structural outcomes and expressions, in other words, between process and patterns. It can be said that this concept is clearly expressed by the Enlowian distinction between *bone relocation* (a resulting *pattern*) because of *bone remodelling* (a biological *process*). It was in the famous book by Stephen Jay Gould, *Ontogeny and Phylogeny* (1977) that the Enlowian request for a clear semantic distinction between descriptive patterns and explanatory processes later found an explicit treatment, applied to the issue of developmental heterochronies [23], Later on, the development of cladistic methods in systematics prompted the general distinction between evolutionary patterns (the description of phylogeny) and evolutionary processes (the study of the mechanisms from which the pattern results). This approach, exemplified in the titles of Eldredge and Cracraft's [8] and Carroll's [4] books, alleviated some intellectual confusion brought by the semantics of the traditional evolutionary synthesis. There too, Enlow has been a pioneer.

4. Some current developments of Enlowian themes in palaeohistology and comparative studies of bone as a tissue

On the issue of the value and significance of the comparative approach to bone histology, as practiced by Enlow and Brown (1956–1958), it is clear that it falls into the realm of the general comparative method in evolutionary biology. This method, as opposed to the experimental method, has been critically reviewed from an epistemological and operational point of view by Harvey and Pagel [27]. Stemming from this theoretical approach and others (e.g., Blomberg and Garland, 2002) [3], a recent trend has been to implement more quantitative approaches to comparative evolutionary research, including much more refined and reliable statistical treatments, notably taking into account the special constraints introduced by explicit phylogenetic analyses (cladistics).

One of the tenets of these analyses is that the comparative method can at best suggest or support inferences because it brings evidence for correlations, but cannot bring by itself demonstrative evidences about causality, this last aspect being restricted to the experimental approach [27]. This echoes Ernst Mayr's well-known distinction (1961) between two scientific regimes in biology: biology of proximate causes and biology of ultimate causes [37]. Proximate causes are those that act within the living organism. Ultimate causes explain the data by reference to the organism's evolutionary history. Biology of proximal causes is a functional biology; biology of ultimate causes is an evolutionary biology. Both are useful to explain particular biological structures or phenomena, but their epistemological regimes differ. Functional biology (notably physiology) is experimental and akin to physicochemical sciences (i.e., nomothetic: seeking general laws), whereas evolutionary biology (notably palaeontology) is a historical science [21]. The very nature of 'causes' (and of their explanations) differs within the two scientific regimes, although they are complementary rather than contradictory [21]. This has been also expressed under the concept of 'Seilacher's triangle' (1970), a concept also used by Gould [24]. According to such views, any biological structure integrates three fundamental aspects (or causal agents): historical (phylogenetic, conveyed by the genetic background), functional (adaptive, physiological), and constructional or structural (linked to the materials used, e.g., skeletons, with the intrinsic constraints (notably topological) that they introduce) [24]. According to Cubo [5] and Cubo et al. [6], one has to try to integrate historicism and functionalism in our explanations rather than antagonize them. Indeed, Darwin noted (1859) that adaptations inherited from ancestors (phylogenetic signal) originated in the past by natural selection, and that they should, therefore, be considered ultimately as functional effects. However, if students of bone as a tissue often acknowledged the complex, multivariate causation of bone structure in a qualitative way [7,9,43–45], how do we quantify the relative "weight" of the various components? If functional 'ontogenetic factors' are all important causes of bone histodiversity, what about the role left to biomechanical factors and to the possible occurrence of a 'phylogenetic signal'?

Recent works in comparative bone histology have fully taken into account various aspects of the above considerations, using up-to-date, highly sophisticated statistical controls, and thus they offer a quantitative follow-up to Enlowian earlier comparative/evolutionary works. For instance, on the issue of the significance of microanatomy of bones (development of compacta versus spongiosa, and the contrast between them, etc.), it has been shown that it contains both an ecological (aquatic vs. terrestrial) and a phylogenetic signal [22,31].

The issue of different longitudinal growth at the various epiphyses, in connection with the structures actually preserved among fossils, have started receiving quantitative answers, thanks to some experimental studies in birds [38]. It may be expected that such studies will provide quantitative estimates of longitudinal growth rates among various fossil tetrapods, notably dinosaurs [38].

Comparative analyses of compact bone and the precise body localization of different tissue types among birds gave interesting quantitative results. First they offer a statistical validation of 'Amprino's rule' (linking primary bone tissues typology to growth rates, [33]), but, interestingly, they also demonstrate that growth rates alone cannot account for the local distribution of tissue types [32]. Tissue segregation among various bones in the skeleton (and among different birds, depending on their taxonomic position and flight styles) appears to be highly correlated with the biomechanical strains experienced by various parts of the skeleton. For instance, the laminar pattern of bone vascularity would be adapted to shearing strains experienced by bones under high torsional loading (main wing bones and femora) [32,34].

Finally, new statistical developments (partition analysis of variance) allow us to sort out phylogenetic versus body-size signals from the bone microanatomical and histological data set into their proper phylogenetic context [6].

It thus appears, from a general perspective, that bone histodiversity expresses a very complex causality, in which immediate factors (such as the ontogenetic causes analyzed by Enlow) are integrated with other ones (biomechanical, phylogenetic...). The result is the extraordinary wealth of biological information that may be ultimately deciphered form bone as a tissue.

Obviously, many further developments in comparative bone histology are forthcoming, with fascinating applications for vertebrate palaeobiology [19,28,49].

5. Concluding remarks: preserving thin sections collections

It is necessary to end this review with a few comments about the thin section collections and their fate. Prof. Bromage informs me that it would have been only a matter of hours before Prof. Enlow's unique collection of thin sections (approximately 100 000 preparations!) were thrown into the trash, when he fortunately arrived to salvage it. It is, however, not very difficult to understand the scientific, patrimonial and historical value of histological collections, especially when they form the factual basis of important publications, either descriptive or experimental. This is all the more relevant for the palaeohistological collections of thin sections because of the rarity, sometimes uniqueness, of the fossils they come from, and because of the enormous investment in time and technical know how their creation implies. Professor Bromage and the Dental School of the New York University should be congratulated for initiating the permanent conservation and digitalization project of Prof. Enlow's histological collection. This is exactly the way to go. The time is ripe to build large histological reference libraries, which can now benefit from all the tremendous possibilities of computerization, both for curation and exploitation on the spot and on line. This enhances the practical and patrimonial values of such collections, in fact data banks, by a factor of thousands [47]. I propose now that the issue of conservation of histological collections in general, and of fossil thin sections in particular, be taken up seriously by the international scientific community.

Acknowledgments

This paper expands a talk given at the College of Dentistry of the New York University. I thank Professor Tim Bromage and the New York University College of Dentistry for the excellent idea to convene a seminar in New York, around Professor Donald H. Enlow (6 & 7 November 2006) in order to celebrate his career for his forthcoming eightieth birthday, and fifty years after the publication of Enlow and Brown's (1956–1958) seminal work. This has been a splendid opportunity for the community of bone and tooth histologists and biologists

at large to pay a well-deserved intellectual tribute to the work of a scientist and scholar who has greatly influenced almost three generations of hard tissue specialists. Beyond this intellectual tribute, there is also a deep personal implication for many of us and it is fitting now, in retrospect, to try to acknowledge all that Professor Enlow gave us, and accordingly our debt towards him.

Observing and discussing thin sections under the microscope with Don Enlow was an extremely rewarding experience: not only 'reading', but also even deciphering them. Beyond the objective but static structural description of tissue types (their relative proportions, positions, and so on), what emerged was a dynamic interpretative understanding of the section in terms of its functional significance. In other words, Enlow helped us to learn to interpret mute structures in terms of function. Those functional interpretations focused on growth dynamics and on the geometrical/topological constraints of modelling and remodelling linked to tissue structures, taking into account the biological peculiarities of bone as a living hard tissue. Thank you Don.

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References

[1] R. Amprino, La structure du tissu osseux envisagée comme expression de différences dans la vitesse de l'acroissement, Arch. Biol. 58 (1947) 315–330.

[2] R. Amprino, G. Godina, La struttura delle ossa nei vertebrati: ricerche comparative negli anfibi e negli amnioti, Comment. Pontif. Acad. Sci. 11 (1947) 329–464.

[3] S.P. Blomberg, T.J. Garland, Tempo and mode in evolution: phylogenetic inertia, adaptation and comparative method, J. Evol. Biol. 15 (2002) 899–910.

[4] R.L. Carroll, Patterns and processes of vertebrate evolution, Cambridge Paleobiol. Ser., Cambridge University Press, Cambridge, UK, 1997.

[5] J. Cubo, Patterns and process in constructional morphology, Evol. Dev. 6 (2004) 131–133.

[6] J. Cubo, F. Ponton, M. Laurin, E. de Margerie, J. Castanet, Phylogenetic signal in bone microstructure of Sauropsids, Syst. Biol. 54 (4) (2005) 562–574.

[7] J.D. Currey, The many adaptations of bone, J. Biomech. 36 (2003) 1487–1495.

[8] N. Eldredge, J. Cracraft, Phylogenetic patterns and the evolutionary processes, Columbia University Press, NY, USA, 1980.

[9] D.H. Enlow, S.O. Brown, A comparative histological study of fossil and recent bone tissues. Part 1, Tex. J. Sci. 8 (1956) 405-443; part 2, ibid 9 (1957) 186-214; part 3, ibid 10 (1958) 187-230.

[10] D.H. Enlow, A study of post natal growth and remodeling of bone, Am. J. Anat. 110 (2) (1962) 79–102.

[11] D.H. Enlow, Functions of the Haversian systems, Am. J. Anat. 110 (3) (1962) 269–306.

[12] D.H. Enlow, Principles of bone remodelling, Charles C. Thomas, Springfield, IL, USA, 1963.

[13] D.H. Enlow, An evaluation of the use of bone histology in forensic medicine and anthropology, in G. Evans (Ed.), Symposium on joints and bone, III Int. Congr. Anat., Springer, Berlin, 1966, pp. 92–112.

[14] D.H. Enlow, A morphogenetical analysis of facial growth, Am. J. Orthod. 52 (4) (1966) 283–299.

[15] D.H. Enlow, A comparative study of Facial growth in *Homo* and *Macaca*, Am. J. Phys. Anthropol. 24 (3) (1966) 293–307.

[16] D.H. Enlow, Wolff's law and the factor of architectonic circumstance, Am. J. Orthod. 54 (11) (1968) 803–822.

[17] D.H. Enlow, The bone of Reptiles, in: C. Gans (Ed.), Biology of the Reptilia, 1, Academic Press, London, 1969, pp. 45–80.

[18] D.H. Enlow, Growth and the problem of the local control mechanism (Editorial), Am. J. Anat. 136 (4) (1973) 403–405.

[19] G. Erickson, Assessing dinosaur growth patterns: a microscopic revolution, Trends Ecol. Evol. 20 (12) (2005) 677–684.

[20] J.S. Foote, A contribution to the comparative histology of the femur, Smiths. Contrib. Knowl., 1916, Publ No. 2382, Washington DC, USA.

[21] J. Gayon, Do biologists need the concept of function? A philosophical perspective, C. R. Palevol 5 (3–4) (2006) 479–487.

[22] D. Germain, M. Laurin, Microanatomy of the radius and lifestyle in amniotes (Vertebrata, Tetrapoda), Zool. Scripta 34 (2005) 335–350.

[23] S.J. Gould, Ontogeny and phylogeny, Belknap Press, Harvard University, Cambridge, MA, USA, 1975.

[24] S.J. Gould, The structure of evolutionary theory, Belknap Press, Harvard University, Cambridge, MA, 2002.

[25] W. Gross, Die Typen des mikroskopischen Knochenbaues bei fossilen Stegocephalen und Reptilien, Z. Anat. 103 (1934) 731–764.

[26] W.R. Haines, The evolution of epiphyses and of endochondral bone, Biol. Rev. 17 (1942) 267–292.

[27] P.H. Harvey, M.D. Pagel, The comparative method in evolutionary Biology, Oxford University Press, 1991.

[28] J.R. Horner, K. Padian, A. de Ricqlès, How dinosaurs grew so large and so small, Sci. Am. 293 (2005), French version: Pour la Science 334 (2005) 82–88.

[29] P. Janvier, Early Vertebrates, Clarendon Press, Oxford, UK, 1996.

[30] V. Kiprijanoff, Studien über die fossilien Reptilien Russlands, Mem. Acad. Imp. Sci. St. Petersb., Ser. 7 (1881–1883) 28–31.

[31] M. Laurin, M. Girondot, M.-M. Loth, The evolution of long bone microanatomy and lifestyle in lissamphibians, Paleobiology 30 (2004) 589–613.

[32] E. de Margerie, Laminar bone as an adaptation to torsional loads in flapping flight, J. Anat. 201 (2002) 521–526.

[33] E. de Margerie, J.-P. Robin, D. Verrier, J. Cubo, R. Groscolas, J. Castanet, Assessing a relationship between bone microstructure and growth rate: a fluorescent labeling study in the King Penguin chick (*Aptenodytes patagonicus*), J. Exp. Biol. 207 (2004) 869–879.

[34] E. de Margerie, S. Sanchez, J. Cubo, J. Castanet, Torsional resistance as a principal component of the structural design of long

bones: Comparative multivariate evidence in birds, Anat. Rec. 282 A (2005)49–66.

[35] G.R. McGhee Jr., Theoretical morphology – The concept and its applications, Columbia University Press, NY, USA, 1999.

[36] J. Matyas, Die mikroanatomische Knochenstruktur und die Abstammungsgeschichte eizelner Wierbeltieregruppen, in Xth Int. Zool. Congress, Budapest, 1929, pp. 666–704.

[37] E. Mayr, Cause and effect in Biology, Science 134 (1961) 1501–1506.

[38] L. Montes, E. de Margerie, J. Castanet, A. de Ricqlès, J. Cubo, Relationship between bone growth rate and the thickness of calcified cartilage in the long bones of Galloanserae (Aves), J. Anat. 206 (2005) 445–452.

[39] F. Nopcsa, E. Heidsieck, Über eine pachyostotische Rippe aus der Kreide Rügens, Acta Zool. 15 (1934) 431–455.

[40] J. Queckett, On the intimate structure of bone as composing the skeleton of the four great classes of vertebrates viz. Mammals, birds, reptiles and fish, with some remarks on the great value of such structures to assess affinities of minute fragments of organic remains Trans, R. Microsc. Soc. Lond. 2 (1849) 46–58.

[41] R.E.H. Reid, Bone histology of the Cleveland–Lloyd dinosaurs, and of dinosaurs in general. Part 1: Introduction: introduction to bone tissues, Brigham Young Univ. Geol. Inst. Stud. 41 (1996) 25–71.

[42] A. de Ricqlès, Recherches paléohistologiques sur les os longs des tétrapodes ; VII – Sur la classification, la signification fonctionnelle et l'histoire des tissus osseux de tétrapodes. Première partie: structures, Ann. Paleontol. 61 (1975) 51–129.

[43] A. de Ricqlès, Recherches paléohistologiques sur les os longs des tétrapodes ; VII – Sur la classification, la signification fonctionnelle et l'histoire des tissus osseux de tétrapodes. Deuxième partie : fonctions, Ann. Paleontol. 62 (1) (1976) 71–126, ibid 63 (1) (1977)33-56; ibid 63 (2) (1977) 133–160.

[44] A. de Ricqlès, Recherches paléohistologiques sur les os longs des tétrapodes; VII – Sur la classification, la signification fonctionnelle et l'histoire des tissus osseux de tétrapodes. Troisième partie : evolution, Ann. Paleontol. 64 (1) (1978) 85–111, ibid 64 (2) (1978) 153–184.

[45] A. de Ricqlès, Some remarks on paleohistology from a comparative-evolutionary point of view, in: G. Grupe, A.N. Garland (Eds.), Histology of ancient human bone: Methods and diagnosis, Springer Verlag, Berlin, 1993, pp. 37–77.

[46] A. de Ricqlès, F.-J. Meunier, J. Castanet, H. Francillon-Vieillot, Comparative microstructure of bone, in: B.K. Hall (Ed.), Bone. Vol. 3: Bone matrix and bone specific products, CRC Press, Boca Raton, FL, USA, 1991, pp. 1–78.

[47] A. de Ricqlès, J. Castanet, H. Francillon-Vieillot, The 'message' of bone tissue in Paleoherpetology, Ital. J. Zool. (suppl. 1) (2004) 3–12.

[48] A.L. Seitz, Vergleichenden Studien über den mikroskopischen Knochenbau fossiler und rezenter Reptilien, Nov. Acta Abh. Kaiser Leop-Carol. Deutsch Akad. Naturforsch. 37 (1907) 230–370.

[49] E. Stockstad, Dinosaurs under the knife, Science 306 (2004) 962–965.

[50] M.C.H. Van der Meulen, G.S. Beaupre, D.R. Carter, Mechanobiological influences in long bone cross-sectional growth, Bone 14 (1993) 635–642.

[51] M.C.H. Van der Meulen, D.R. Carter, Developmental mechanics determine long bone allometry, J. Theor Biol. 172 (1995) 323–327.