Ontological classification of bones and skeletal tissues

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1 Introduction

This document outlines some issues for the development of a pan-vertebrate skeletal anatomy ontology intended for devo-evo research. This document draws partially on a very cursory treatment of the issues outlined in Hall & Witten (H&W) [1].

The reader is assumed to have passing familiarity with some of the existing Anatomy Ontologies (AOs):

1. **ZFA** Zebrafish

2. **TAO** Teleost [2]

3. **XAO** Xenopus [3]

4. **FMA** Foundational Model of Anatomy (adult human) [4]

5. **MA** Adult Mouse [5]

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6. **EMAP** Developing Mouse [6]

7. **CL** Cell (multi-species) [7]

8. **CARO** Common Anatomy Reference Ontology (upper, multi-species) [8]

9. **Uberon** Gross anatomy (multi-species) [9]

The other ontology of relevance is **GO**, the Gene Ontology [10], primarily for its developmental terms.

The reader is assumed to have been exposed to either the Relations Ontology [11], or be familiar with OWL and OWL axioms for making class-level statements involving relationships such as partOf.

1.1 Outline

First I will sketch out some of the issues in developing the upper levels of anatomy ontologies. I will focus on CARO and FMA and briefly overview some existing issues with these strict single-inheritance structure-based hierarchies, and how their application differs in individual AOs. I will then propose a simplistic binary distinction that should hopefully be good enough for making progress during the meeting.

I will very briefly review some principles of ontology design, and hopefully lay to rest any concerns about single-inheritance hierarchies. The core message here is that ontology authors should focus on listing properties of the individual anatomical entities, and the let the reasoner do the work of classification.

Next I will provide an overview of how the two major skeletal tissue types (cartilage and bone tissue) are handled in existing AOs, followed by some relevant cell types in CL. After that I will tackle the classification of the main skeletal elements themselves.

Finally I will describe the use of a skeletal anatomy ontology for making evolutionary phenotype statements and homology statements.

Some of the recommendations in this paper come from attempts to unify different species-specific AOs in the Uberon ontology. They may differ in places from recommendations made by others, nothing should be regarded as written in stone.

2 Upper Level Classification

I will first summarize briefly some of the extensive work that went into CARO (based on the FMA) and its implications for vertebrate skeletal anatomy ontologies.

2.1 Tissues and organs in CARO and FMA

CARO adopts a 3-level distinction between tissues, multi-tissue structures and organs:

1. **portion of tissue** Anatomical structure, that consists of similar cells and intercellular matrix, aggregated according to genetically determined spatial relationships.

2. **multi-tissue structure** Anatomical structure that has as its parts two or more portions of tissue of at least two different types and which through specific morphogenetic processes forms a single distinct structural unit demarcated by bona-fide boundaries from other distinct structural units of different types.

3. **compound organ** Anatomical structure that has as its parts two or more multi-tissue structures of at least two different types and which through specific morphogenetic processes forms a single distinct structural unit demarcated by bona fide boundaries from other distinct anatomical structures of different types.

We spent some time crafting the definitions, but found that it was difficult to come up with satisfactory definitions that applied across species whilst adhering to a strict FMA-based structural paradigm. In practice applying the resulting definitions can be difficult. What does "similar cells" in the above mean? Are neurons and glia similar? What is a "specific morphogenetic process"?

Figure 1 summarizes some of the relevant classes in CARO and FMA. Classes are implicitly disjoint (i.e. no class can be a subclass of two disjoint classes, or, equivalently, nothing can be an instance of two disjoint classes simulatenously).

Note that FMA does not have *multi-tissue structure*, but does have *organ component*.
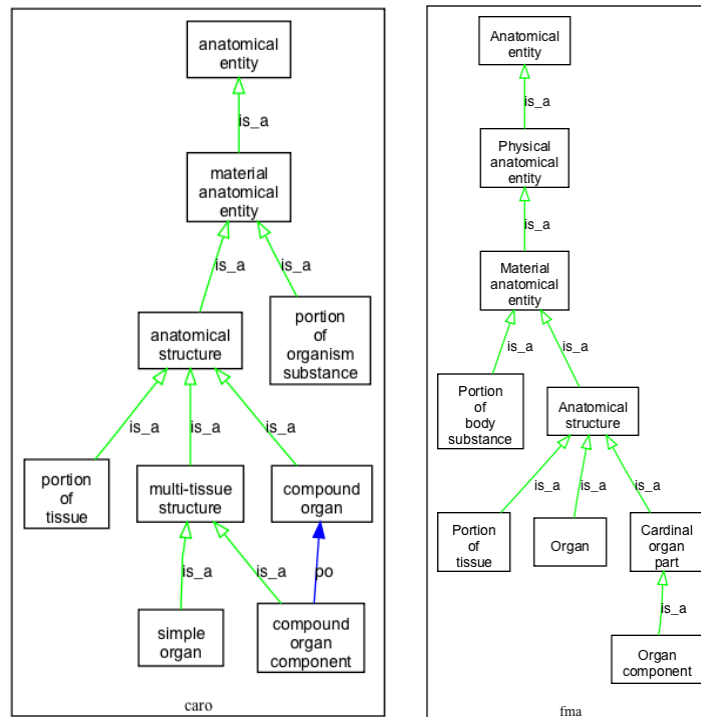
Figure 1: Tissues in CARO (left) and FMA (right). Classes are implicitly disjoint - i.e. no single entity is both an anatomical structure or a portion of organism substance.

2.2 Is a bone a tissue or an organ?

In the Teleost Anatomy Ontology (TAO) are currently subtypes of portion of tissue but in reality individual bones are composed of bone tissue. In contrast, the Foundational Model of Anatomy defines individual bones as subtypes of bone organ. How should individual bones be classified? Should all bones be subtypes of organ with relationships to tissues, cell types, and mode of development?

Discussions on upper-level structural classifications in anatomical ontologies rarely reach a satisfactory conclusion – in particular, discussions of what an "organ" is. My opinion is that distinctions between organ and non-organ is often historical, accidental, arbitrary, and not of much use for reasoning.

I'd urge you not to spend too much of the meeting getting into discussions of what constitutes an organ. Making fine-grained distinctions should serve some useful purpose. Does distinguishing organs from organ components serve a useful purpose? Are people likely to query one and not the other? Is the distinction useful for hypothesis generation.

Taking a use-case driven approach, I would argue that here there is an important distinction to be made between (a) the whole bone and (b) the a (portion / blob / lump) of bone tissue. This is because bones may consist of different types of tissue, and homologous bones may be made from different tissues. By distinguishing bones from bone tissue we give ourselves more flexibility in that we can "make" different bones from different tissue types. Furthermore, we might want to classify whole bones in different ways from tissue.

It might be illustrative to show how existing ontologies differ here. Figure 2 shows if and where bone and bone tissue is distinsguished in three vertebrate AOs. Both FMA and MA make the distinction between bone and the tissue that constitutes bone, whereas in ZFA these are not distinguished. MA is *isa-incomplete*[1], and in this case does not make a commitment to whether or not bone is a organ. However, we can probably assume that it is not a subtype of tissue, since this would be equivalent to "bone tissue".

The crucial part of the CARO definition for compound organ seems to be *distinct structural unit demarcated by bona fide boundaries.* By this criteria, most of the subclasses of bone in ZFA (tripus, rib, etc) would be more like organs than portions of tissue. In addition, in CARO portions of tissue are of presumably intended to be homogeneous (TODO: check), yet some bones may actually be composed of different tissue types.

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[1]Not every class has an isa parent
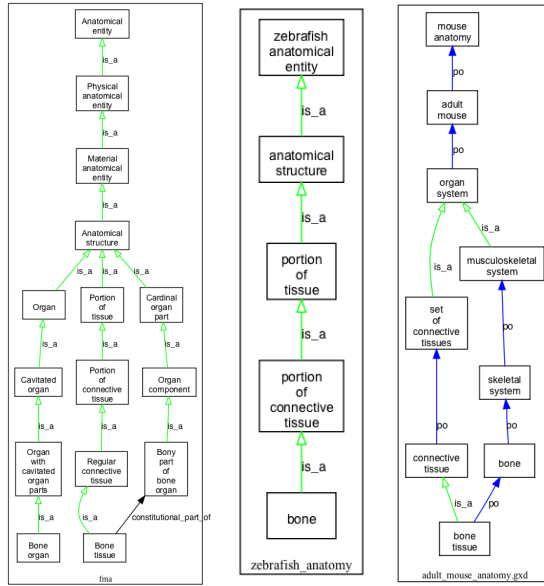
Figure 2: Bone in FMA (left), ZFA (middle) and MA (right). Both FMA
and MA make the distinction between bone and the tissue that constitutes
bone, whereas in ZFA these are not distinguished (Note that FMA makes
the distinction between the bony part of a bone and the bone itself, but does
not create a relationship between them – this kind of thing is not uncommon
in the FMA).

I would recommend treating all bone names as denoting some kind of structural unit / element with a defined boundary, i.e. as a bone. There may be a historic or terminological objection to calling bones organs (TODO: check), but I do think the crucial distinction is between distinct units and the stuff they are made from. Perhaps a more neutral term such as *element* would be better than *organ*. I will treat specific bones as bone organs in the rest of this document, but the actual terminology is not so important.

There is a valid argument for simplifying further and treating all named bones as subtypes of tissue, as in ZFA/TAO. The argument in favour is that bones with defined boundaries in one species may be partially or wholly fused, eliminating the defined boundaries. There is also the question of symmetry with treatment of cartilage - should we have both cartiliginous elements and cartiliginious tissue, analagous to bone and bone tissue? The arguments here seem perhaps less compelling than for bone. There is a compelling simplicity in treating everything as tissue.

I'm not sure how easy it is to distinguish between portions of tissue and multi-tissue structures. I think it would be OK to avoid any major commitments here during the meeting, and to focus on the distinction between named bones and stuff/substances. With that in mind, there is another FMA legacy issue with CARO, the distinction between substances and tissues.

2.3   Substance vs Tissue

Both CARO and FMA distinguish between portions of organism substance and tissues (see figure 1).

The definition of organism substance is: *Material anatomical entity in a gaseous, liquid, semisolid or solid state; produced by anatomical structures or derived from inhaled and ingested substances that have been modified by anatomical structures as they pass through the body.*

We can see that this was derived from the very similar FMA definition: *Material anatomical entity in a gaseous, liquid, semisolid or solid state, with or without the admixture of cells and biological macromolecules; produced by anatomical structures or derived from inhaled and ingested substances that have been modified by anatomical structures. Examples: saliva, semen, cerebrospinal fluid, respiratory air, urine, feces, blood, plasma, lymph.*

We can see some difficulties here when we consider teeth. ZFA, MA and FMA all treat enamel and dentine as portions of organism substances (see figure 3). Portions of organism substances are implicitly disjoint from portions of tissue in CARO and FMA. Yet in H&W the treatment of enamel
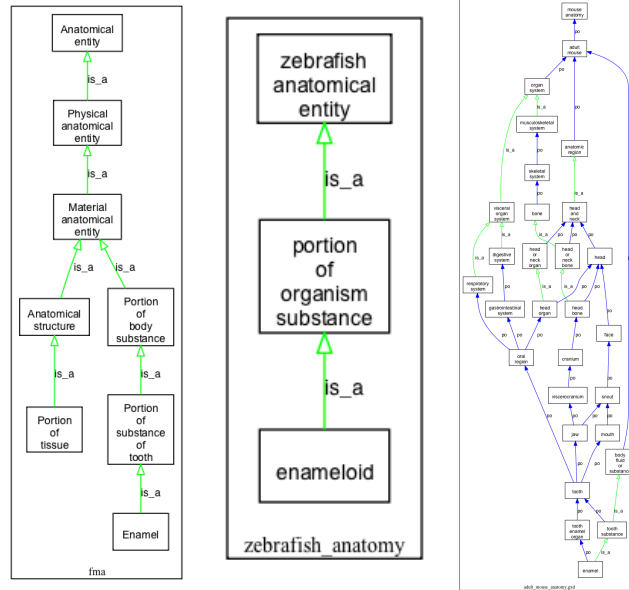
Figure 3: Enamel/enameloid in FMA (left), ZFA (middle) and MA (right). In all cases enamel is treated as a portion of organism substance. The diagrams look identical for dentine and cementum.

and dentine as tissues is fundamental.

Similar issues may exist for other classes, e.g. blood. Intuitively we might think of substances as lacking cells - but this is not what the current definitions say, and this would not be without problems. Tooth substances like dentine contain some proportion of cells, and some bones are acellular, yet it would be odd to not classify this is bone tissue.

Perhaps the existing single-inheritance upper-level structures are too restrictive here. In fact, the stuff from which organisms are made can be classified on multiple axes of characteristics that may be continuous rather than discrete in nature.

2.4 Proposed Upper Level for Skeletal Anatomy

I propose a simplified scheme that uses four upper level categories: **unit**, **stuff**, **cell** and **cell component**. The latter two are covered by CL and GO, so the gross skeletal AO would need to make distinctions only between the first two.

Intuitively, "unit" is intended to be a gross anatomical entity with some

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kind of distinct boundary. "Element" might be an equally good name. Individual units in an organism can typically be enumerated and named. Stuff is the building blocks of units. Stuff can be relatively homogeneuous, or it can be mixed.

Individual bones such as the frontal, the ethmoid, the phalanges and so on are subclasses of unit. Portions of tissue such as cartilage, bone tissue, specific bone tissues and so on are subtypes of stuff. Combinations of two or more types of stuff are still stuff. It is when stuff has an identifiable boundary that it becomes a unit.

We can equate "unit" with "organ" or sometimes "organ component" or "multi-tissue structure", but we are not so concerned with the distinction between these two here. We can equate "stuff" with "portion of tissue" and perhaps "organism substance" in some cases (we may want to keep bodily fluids off to the side, but that doesn't concern us for now).

In this document the only units we are concerned with are skeletal elements such as bones, teeth, cartilaginous elements and so on. I'm not concerned with whether these are considered organs for now, but if you want them to be, they can be. The main "stuff" we are concerned with is skeletal tissue and its subtypes, as identified by H&W - bone tissue, cartilage, tooth tissue (and intermediates). I will call these types of stuff "tissues" for now, since that sounds a bit better than "stuff", but there is no intended commitment to whether in CARO/FMA terms this is a single tissue, multi-tissue structure or substance.

3   Ontology principles

3.1   Single inheritance

Multiple inheritance and logical definitions of bone types: Multiple inheritance, in which a term has more than one isa parent, can be difficult to maintain in an ontology and could lead to errors in reasoning. Often, however, use of multiple isa parents reflects biological reality; for example, a bone may exhibit two different modes of development within the same organism (such as tripus develops by endochondral and intramembranous ossification). A logically preferable way to represent these relationships is to use cross products in which terms are defined such that their classification can be automated by a reasoner. Can bone types/bone development be defined using cross products?

There is a lot of confusion over the single inheritance issue. Sometimes this is treated as a matter of religion. I would advocate against

asserted multiple inheritance, and in favour of letting the reasoner *infer* the (poly)hierarchy. But this is just good engineering practice, rather than a matter of dogma. Sometimes it's convenient to assert two isa parents as a temporary measure whilst figuring out what the main differentiating characteristics are. This is fine, the world won't end, just don't get carried away.

3.2 Logical definitions and reasoning

I would advocate the following principles:

1. **Write genus-differentia definitions.** Definitions should constitute necessary and sufficient conditions and should be of the form "An X is a G that D", where X is the defined class, G is the generic class of entity and D is the discriminating characteristics that marks out instances of X from instances of other classes of G.

2. **Make the definitions computable where possible.** In obo-edit you can fill in the "genus" and "differentia" boxes in the cross-product tab. In Protege you would specify "Equivalent Classes". Note that the genus will be an isa parent in the hierarchy.

3. **Use the reasoner to infer the poly-hierarchy**.

If the genus is a very general term such as "skeletal element", don't worry. The important thing is listing all the characteristics of the entity being defined.

These logical definitions are sometimes known as cross-product definitions, because they are drawn from the cross-product of these different characteristics [12].

3.3 Skeletal tissue and skeletal elements properties

Some of the important characteristics for skeletal elements and skeletal tissue types:

1. **Composition.** For organs, this would be the tissues they are composed from. For tissues, this would be cells, cellular components, protein complexes and chemical entities.

2. **Qualities and morphology.** For example, shape. Also texture, histology.

3. **Location and topology.** Where is it in the body.

4. **Developmental origin and developmental process.** E.g. develops from mesenchyme.

5. **Function.** The GO biological process could be used here.

I would recommend not worrying too much if the genus is a very generic class such as "bone". Focus on enumerating the essential characteristics and let the reasoner do the work. This is very much in line with H&W.

Similar conclusions have been reached by groups classifying complex cell types such as neurons and other cell types. Rather than everyone agreeing on an optimal axis of classification, everything is asserted to be a subtype of "neuron", aand the characteristics are listed. The reasoner is then used to group things under functional classes such as "dopamine-producing neuron", structural classes such as "binculeate cell".

Table 1 shows some example classes and genus-differentia definitions. Note that there is no need for a human to assert the polyhierarchy - a reasoner could infer the subsumption relationship between *calcareous tooth of mouth* and *calcareous tooth*. We can even make grouping classes such as *dentine-based skeletal element* and have the reasoner automatically classify under here. Sometimes it's simpler to just inherit properties - for example, *cartiliginious vertebra* uses *vertebra* as genus and thus inherits its properties. In general you should not need to assert multiple isa parents / genii.

4   Skeletal tissue types

H&W classify 4 skeletal tissue types, plus intermediates. These types include dentine and enamel (which may not be classically classified here, but this makes sense from an evolutionary, cellular and developmental perspective).

4.1   Cartilage tissue

All AOs classify cartilage as a subtype of connective tissue. However, this appears not to be the case in H&W, or at least implied:

The best-recognized intermediate tissues are chondroid and chondroid bone, which are intermediate between connective tissue and cartilage and cartilage and bone, respectively

Like bone, cartilage can arise ectopically outside the skeleton in connective tissue, muscle, and the heart.

See also fig 1.1 in H&W.

Class	Genus	Develops from	Part of	Composition
dermal denticle	skeletal element	dermis	skin	dentine
calcareous tooth of mouth	skeletal element	tooth bud	mouth	dentine and enamel
calcareous tooth	skeletal element	tooth bud		dentine and enamel
dentine-based skeletal element	skeletal element			dentine
chondrocranium	skeletal element		cranium	cartilage or replacement bones
vertebra	skeletal element	somite	vertebral column	cartilage or bone tissue
cartiliginious vertebra	vertebra			cartilage
dentine	skeletal tissue	odontoblasts		...

Table 1: Matrix showing relationships between subtypes of a skeletal element which is endochondral in bony species (vertebra). Italics shows inferred relationships. We use devFrom to be consistent with TAO/ZFA but a more specific relation may be required for endochondral bones (e.g. replaces).
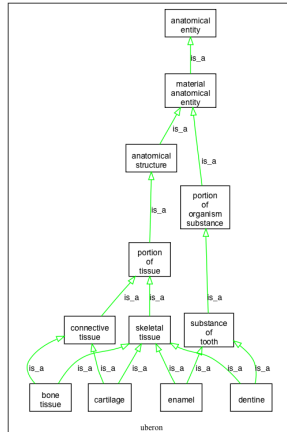
Figure 4: Skeletal tissue classification in Uberon. Only subclass relationships shown.

Figure 5: Skeletal tissue classification in Uberon, MA, ZFA and FMA. Only subclass relationships shown.

4.2   Bone tissue subtypes

Existing AOs include two (orthogonal?) classifications of bone tissue - lamellar vs woven, and replacement vs intramembrane.

4.2.1   Lamellar vs woven bone tissue

FMA includes lamellar and woven bone classes. MA only has lamellar bone (presumably because woven is only in the developing mouse, or in the pathological mouse, which would be covered by separate ontologies such as EMAP, MP and MPATH).

4.2.2   Replacement vs intermembrane bone tissue

MA includes both endochondral bone and intramembrane bone as subclasses of bone tissue. The classification is less granular than TAO which has an intermediate superclass *replacement bone*, but it is not inconsistent with it. See figure 6 for an illustration of this in Uberon.

However, individual bones such as the long bones (phalanx), the bones of the skull are *not* classified into this hierarchy (Figure 7). This is perhaps both deliberate and a good choice - perhaps some bone organs have both tissue types (TODO - check). These are developmental distinctions, and MA may deliberately leave this to the EMAP ontology (TODO - check). Nevertheless, it may be useful to include this information somewhere. From a pan-vertebrate evo-devo ontology perspective, it may make sense to record some of these as evolutionary phenotype statements, especially where the development of a bone varies across clades (see further on).

In contrast, FMA includes neither endochondral nor intramembrane bone. This would not be in keeping with it's rigid structure-based single inheritance hierarchy.

4.2.3   Unifying developmental and anatomical perspectives

Can we use terms from the Gene Ontology (Biological Process) for **intramembranous ossification** *and* **endochondral ossification**?

   The GO can be used here, but there is a danger of circularity.

   Also, the GO definitions need to be checked for accuracy:

1. **endochondral ossification** *The formation of bone by the replacement of cartilage tissue with mineralized bone.*

Figure 6: Endochondral vs intramembranous distinction in Uberon, ZFA and MA. Inter-ontology subsumption is denoted by box-containment. Only subclass hierarchy shown (thick lines are relationships in Uberon). At this time Uberon does not make fine-grained distinctions as these have not yet been applied in AOs outside teleosts.

Figure 7: MA showing endochondral vs intramembrane bone tissue, plus two specific bones - the ethmoid and the frontal.

2. **endochondral bone morphogenesis** *The process by which bones are generated and organized as a result of the conversion of initial cartilaginous anlage into bone.*

Ossification is part of morphogenesis. We need to be careful here about the meaning of terms like "replaced". Are these two GO definitions completely consistent? Do we always mean direct replacement, or is there sometimes conversion involved?

But it in general it is fine to use GO here. The *output of* relation could be used. Another approach is to use specific developmental relations. For example, a *replaces* relationship to cartilage and a *develops from* relationship to mesenchyme.

5 Cell Types and Cell Components

Accurate representation of cell types and cell components is crucial for doing justice to the different skeletal tissue types - especially where intermediate types are concerned.
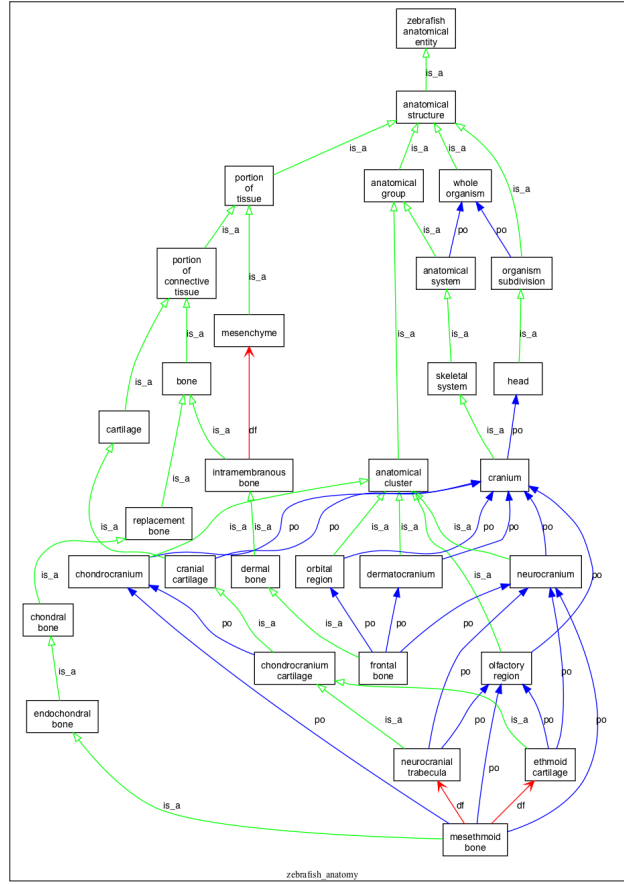
Figure 8: ZFA showing endochondral vs intramembrane bone tissue, plus two specific bones - the ethmoid and the frontal. In contrast to MA, specific bones are classified in the endo-vs-intramembranous hierarchy. Note also ZFA does not distinguish between bone tissue and bone organs
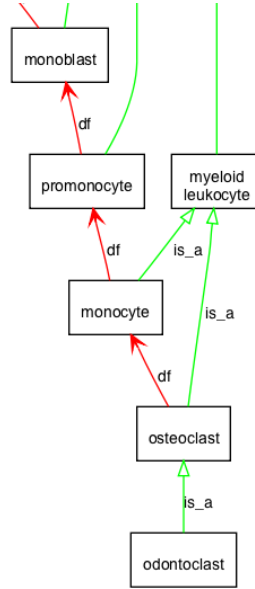
Figure 9: Osteoclast and Odontoclast in CL.

5.1   Bone resorbing cells

1. osteoclast *A specialized phagocytic cell associated with the absorption and removal of the mineralized matrix of bone tissue, which typically differentiates from monocytes.*

2. odontoclast *A specialized osteoclast associated with the absorption and removal of cementum.*

In CL, odontoclast is a subtype of osteoclast. This doesn't fit with the text definitions, unless we consider cementum a subtype of bone tissue. H&W states: *odontoclasts remove dentine, osteoclasts remove bone, chondroclasts remove calcified cartilage ... there is much evidence that all skeleton-resorbing cells belong to the same cell type, suggesting that a basic type of skeletonresorbing cells evolved together with a basic type of skeleton-forming cell.* This seems to suggest that CL should rearrange the hierarchy to make osteoclast and odontoclast siblings, under a new parent of *skeletal resorbing cell* (synonym *scleroblast*?), and to also add a new child of *chondroclast*.

The CL definition of odontoblast also mentions *cementum*, rather than *dentine*, as in H&W.

It is expected that CL will also eventually specify capabilities such as GO:0006909 *phagocytosis*, allowing cell types with this capability to be grouped together. Similarly for multi-nucleate and mon-nucleate cell types.

5.2 Collagen

6 Classifying skeletal elements

Given a classification of skeletal tissue types in terms of their underlying composition, we can move on to the main task at hand, defining and classifying the main skeletal elements of interest.

Here I use the term *skeletal element* as being some kind of unit or connected group of units composed primarily of one or more of the main skeletal tissue subtypes.

Thus the following would all be included as subclasses:

1. the neurocranium of an adult human. This is a multi-bone structure connected by sutures.

2. the cartiliginious neurocranium/chondrocranium of a human fetus.

3. a fused bony neurocranium (e.g. as in some birds)

4. the single-unit cartiliginious chondrocranium of a shark

My assumption is that it would be beneficial to have a single class that encompasses all these entities, with a fairly generic definition (e.g. *brain-enclosing skeletal element*) different subclasses to represent composition and structure-specific subtypes, with development, parthood and homology relationships between them.

This assumption can of course be questioned, it goes against the assumption of CARO in which each of the above classes would be in separate isa branches of the ontology, perhaps with developmental or homology relationships between them.

6.1 Classifying bones by tissue type

Even though we already have tissue types such as *cartilage* and *bone tissue* it is also useful to have classes such as *cartiliginous element* and *bone*. The latter could also be called *bone organ* to be consistent with the FMA.

The same holds for other tissue classifications. We may want to have *endochondral bone* and well as *endochondral bone tissue*. This may seem

like over-inflation and potentially confusing to users, but I think this may be necessary to accommodate mixed-tissue elements such as the tripus and the mixed cartilage-bone cranium of some species.

6.2 Mixing structure and development

*Representation of bone development within a structurally defined ontology: Bones in TAO are defined according to both development and position. For example, **intramembranous bone** is defined as bone that forms directly within mesenchyme; its children, however, are defined based on position (**dermal bone** forms superficially in the organism; **membrane bone** forms deep in the organism). Should development and position be represented separately in ontologies? For example, do we need separate ontological branches for development vs. position/evolutionary history (e.g., endoskeletal elements with subtypes endochondral and membrane bones, and exoskeletal elements with subtype dermal bones)?*

The current structure of TAO is fine here (assuming that dermal and membrane bone are **always** intramembranous), given that TAO mixes tissue and bones.

Another option is to assert these classes to be direct subclasses of bone (tissue), and to state either the appropriate development relationships or the relationship to the GO processes and let the reasoner do the placement. It doesn't really matter, in this case it's simplest to just assert this under intramembranous bone.

6.3 Tripus and intermediate types

TAO includes classes that are intermediate between different categories. Figure 10 shows the asserted polyhierarchy for *tripus* in TAO. What is problematic here is not the violation of single inheritance per-se, but the fact that the class is inheriting from classes that would best be declared disjoint.

(TODO - check - is tripus a bone that contains mixed tissue types, or is the composition consistent but intermediate, or does it vary across taxa, or all 3?)

To do full justice to the tripus, claustrum bone etc it may be necessary to adopt a more complex scheme. Following from our distinction of whole bones and bone tissues, we would introduce a further distinction between endochondral bone tissue and endochondral bones. We could define 3 classes:
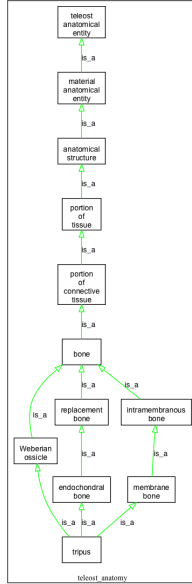
Figure 10: Tripus in TAO is both membrane and endochondral.

1. **endochondral bone** *A bone (organ) that hasPart endochondral bone tissue, and no intramembrane bone tissue*

2. **intramembrane bone** *A bone (organ) that hasPart intramembrane bone tissue, and no endochondral bone tissue*

3. **mixed endochondral/intramembrane bone** *A bone (organ) that hasPart intramembrane bone tissue and endochondral bone tissue*

We would then state that claustrum bone hasPart endochondral bone and hasPart intramembrane bone, and the reasoner would classify membership automatically.

It is also possible to explicitly name sub-regions such as "endochondral part of tripus".

6.4   Cartilaginous and non-cartiliginous skeletons

Currently TAO classifies "rib", "vertebra", "claustrum bone" etc as subtypes of bones. But what about the homologous structures in sharks etc?

One approach is to essentially duplicate all classes: we would have "cartiliginous vertebra" as well as "vertebra". It may also be beneficial to have

-	VE	CV	BV	CVA	SCV
vertebra element	=				
cartiliginous vertebra	isA	=			
bony vertebra	isA	*devFrom*	=	devFrom	homol?
cartiliginous vertebra anlage	*isA*	isA	-	=	homol?
shark cartiliginous vertebra	*isA*	isA	homol?	homol?	=

Table 2: Matrix showing relationships between subtypes of a skeletal element which is endochondral in bony species (vertebra). Italics shows inferred relationships. We use devFrom to be consistent with TAO/ZFA but a more specific relation may be required for endochondral bones (e.g. replaces).

a superclass that unifies both tissue-specific subtypes. For example: "cartiliginous vertebra" SubClassOf "vertebra", "ossified vertebra" SubClassOf "vertebra". Here the skeletal element superclass would be defined not by tissue composition, but by position, morphology, function and developmental lineage.

This brings up an interesting question regarding homology. What is the relationship between the mature bony-tissue endochondral skeletal elements of bony vertebrates and the corresponding cartiliginous elements in Chondrichthyes? Is the homology relationship from shark cartiliginous elements to the mature bony elements, the immature cartiliginous anlage, or some kind of temporal union of the two?

Table 2 shows some of the possibilities.

The story is likely more intricate and fascinating, and will differ depending on individual taxa and specific bones. See [13].

For intramembrane bones, the situation is presumably simpler.

6.4.1   Test case: chondrocranium and neurocranium

TAO has:

1. **chondrocranium** [synonym: "neurocranium" (exact)] *Anatomical cluster that is part of the cranium and composed of cartilage and cartilage replacement bones.*

2. **neurocranium** [synonym: "braincase" (exact)] *Anatomical cluster that consists of the cartilages and bones that surround the brain.*

(in ZFA the neuro/chondro synonym is related, not exact).

The definition is quite clear - the chondrocranium is the sum of skeletal elements in the cranium that are either (a) cartiliginious elements or (b) replacement bones derived from cartilage. The definitions should work for all vertebrates (M Haendel, pers. comm.). A shark a single (?) cartiliginious brain box, this would be classified under chondrocranium (note: may not technically be an anatomical cluster in a shark - ask a shark person - in any case if we use a more generic genus such as skeletal element, all is good).
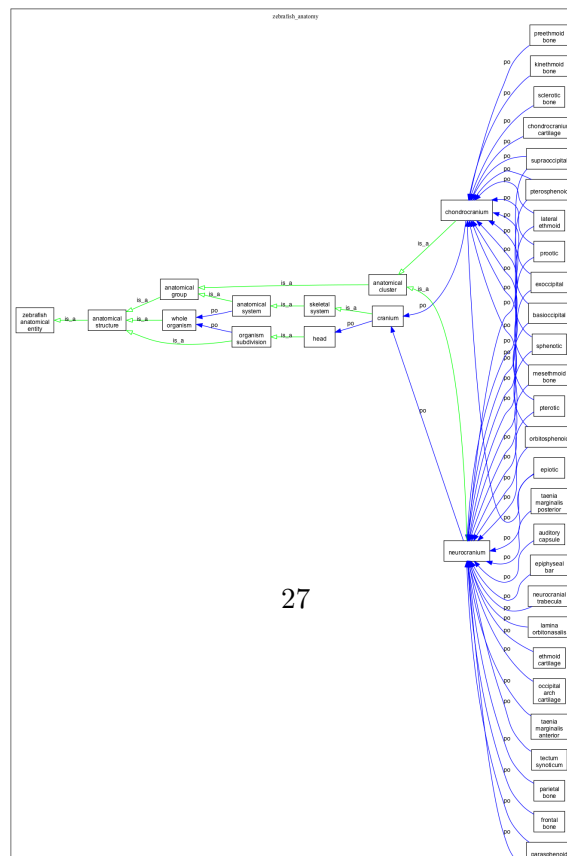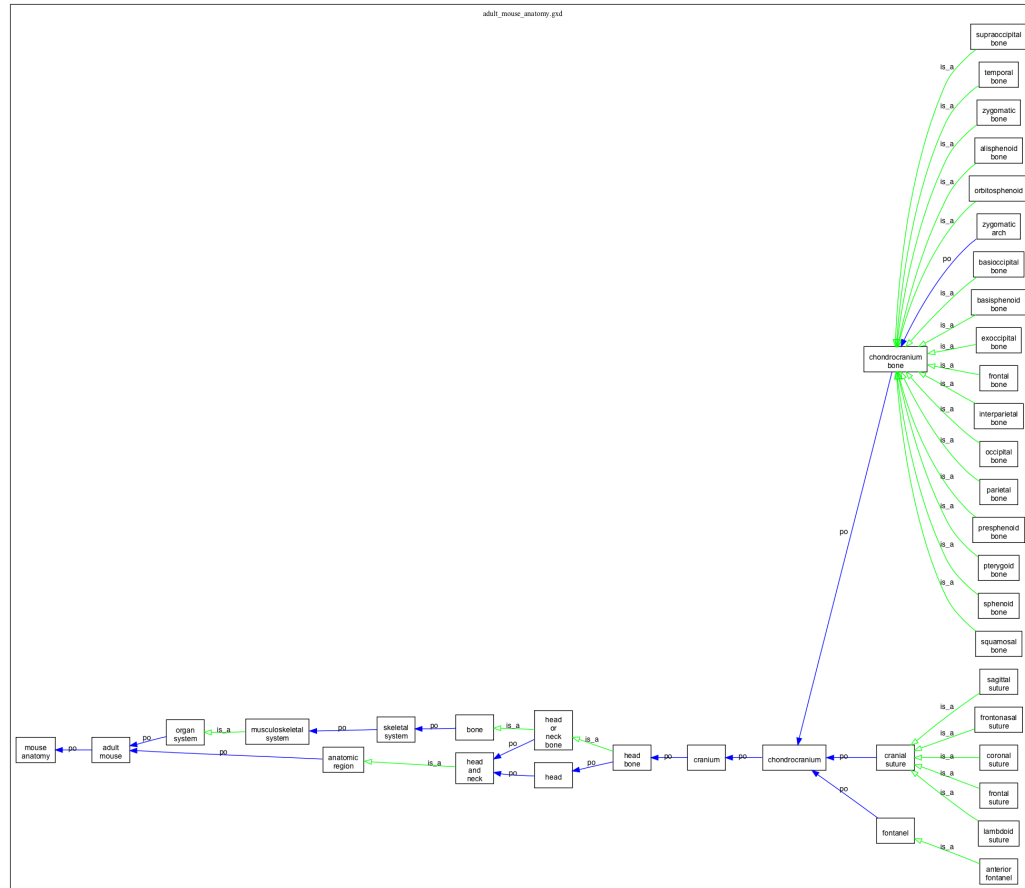
However, the terminology is complicated, further muddled by the picture in mammals, where according to Wikipedia:

The chondrocranium (or cartilaginous neurocranium) is the primitive cartilagionous skeletal structure of the fetal skull that grows to envelop the rapidly growing embyonic brain. In humans, the chondrocranium begins forming at 28 days from mesenchymal condensations and is fully formed between week 7 and 9 of fetal development. While the majority of the chondrocranium is succeeded by the bony skull in most higher vertebrates, some components do persist into adulthood.[1] In Cartilaginious fishes and Agnathans, the chondrocranium persist throughout life.[2] Embryologically, the chondrocranium represent the basal cranial structure, and lay the base for the formation of the endocranium in higher vertebrates.

In MA, there is a class MA:0000317 with primary label *chondrocranium*, with related synonyms *calvaria* and *neurocranium*. Unfortunately MA lacks definitions, but given that MA is adult structures and the parts of this class include intramembranous bone (figure 11), we can surmise that the class denotes what is more conventionally called a neurocranium, not the cartilionous embryonic structure.

Figure 12 shows the two structures in ZFA (and mirrored in TAO). Note that "neurocranium" is a related synonym of the class labeled "chondrocranium" in TAO.

Of course, the evolutionary relationships between cranial bones across vertebrates is a difficult matter and we should be wary of relying on names. Nevertheless, a pan-vertebrate skeletal anatomy will have to resolve the terminological confusion here such that meaningful homology relationships can be made.

adult_mouse_anatomy.gxd

zebrafish_anatomy

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Figure 12: Chondrocranium and neurocranium in ZFA. Note that we can infer that these two entities overlap in space due to the shared parts in common. (TAO uses an explicit overlaps relation - TODO - check is this is necessary)

7   Evolutionary phenotype statements

7.1   Taxon-specific relationships

The method for describing these has been described previously[2].

When comparing skeletal elements, location and tissue composition may vary. When comparing tissues, cellular and cell component composition may vary.

7.2   Compositional phenotypes

It may be useful to capture some of the knowledge in H&W:

1. Lamprey and hagfish cartilages lack collagen type II but have different classes of fibrous proteins (lamprins, myxin) in place of collagen (McBurney and Wright, 1996; Wright et al., 2001)

2. In shark cartilage, in addition to collagen type II, collagen type I is a principal extracellular matrix component (Witten and Huysseune, 2005)

3. Cartilages of extant "agnathans" do not mineralize in vivo (Langille and Hall, 1993)

4. The extracellular matrix of invertebrate cartilage is composed of glycosaminoglycans and a modified form of type I collagen. No invertebrate cartilages mineralize (Cole and Hall, 2004a,b)

These compositional phenotype descriptions differ from the morphological characters that have been mostly captured thus far. Capturing composition in terms of GO complexes, PRO proteins and CHEBI chemical entities could perhaps provide a good ontology linkage point for integrating with genomics databases.

Also: mono-nucleate vs multi-nucleate.

8   Homology statements

8.1   A note on hypotheses in ontologies

Typically ontology axioms correspond to uncontroversial facts, and annotations correspond to attributed statements and conclusions that can be

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[2]http://phenoscape.org/wiki/Entities_with_taxonomic_context#Taxonomically variable ontology relationships

overturned by other evidence.

Following this paradigm, homology statements should be separated from the ontology, and stored as annotations.

Like all distinctions, it's not quite as clear cut as this. In fact many developsFrom relationships may be controversial and overturned by later evidence. Parthood relationships in a multi-species ontology may also be overturned by later evidence.

From a formal logical perspective it is useful to be able to mark axioms that may be less reliable, and attribute evidence. This does not mean we must separate ontology and annotations into separate files, this is an implementation issue, albeit a common way of doing this.

In OWL2 it is possible to "annotate axioms" allowing us to place more controversial statements and hypotheses in the ontology. This can also be done in obo with trailing qualifiers on a relationship (but not oboedit).

As a practical matter it may be useful to capture mostly uncontroversial homology relationships at the time of ontology authoring.

8.2 Frontal and parietal bones

We would have 4 separate classes with for want of better names { tetrapod, teleost } × { frontal, parietal } bone. These would have the appropriate homology relationships. In addition, we can introduce an additional grouping class defined entirely positionally something like "intramembranous bone at front of skull" (perhaps need extra differentia here). These would have no implicit assumption of homology.

8.3 Fusion, mereological sums and homology

Fusions make adhering to a rigid upper level ontology difficult, if we are to retain useful grouping classes.

We may have a situation where X1 fuses with X2. Here we define a new skeletal element (let's call it "X1+X2"), which is homologous to the mereological sum of X1 and X2. In terms of CARO this might be an anatomical cluster, depending on the degree of fusion.

Here, for simplicity, we can define this as a skeletal element that has 2 parts, X1 and X2 (defining this precisely is actually quite difficult in either OE or Protege. I recommend using a consistent naming scheme and we can parse out logical definitions later).

We can do this recursively, we might have a later fusion with X3, so we can define "(X1+X2)+X3".

8.4 Serial homology and strict homology

TODO - import from homology document

9 Uberon

A seperate in-progress paper on Uberon is available.

Uberon was constructed initially to unify existing anatomical ontologies, and to provide the basic classes for defining GO organismal and developmental processes. In order to do these tasks justice it will be necessary to follow some of the recommendations laid out here.

Currently Uberon is in a state of transition so some of the recommendations are followed and others are not (e.g. distinction between bones and tissues).

Some Uberon diagrams are available in the appendix.

10 Conclusions

The message of H&W is that skeletal tissue types exist on a continuum of different characteristics. This argues against any kind of rigid single-inheritance hierarchy in a corresponding ontology. Rigid hierarchies also pose problems for multi-species ontologies, where we may want to have classes such as *cranium* which differ developmentally and taxonomically in terms of their composition and structure.

Given the enormity of the task at hand, it may be beneficial to spend a large portion of the meeting simply listing the classes of relevance and providing detailed textual definitions and comprehensive notes. Then as a second pass, possibly after the meeting, the difficult task of turning these into ontology axioms (relationships between classes and computable definitions) can begin.

For Uberon, a simplified upper level consisting of units and tissue types has proved beneficial. This leads to a natural hierarchical division, with tissue types defined according to cell types and cell components.

11   References

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12 Appendix

12.1 Mouse Bone Classification

12.2 FMA Bone Classification

12.3 ZFA and TAO Bone Classification

Both ZFA and TAO collapse the distinction between bones and bone tissue.

12.4 Endochondral bone formation in GO

Compare figure 15 (http://genesdev.cshlp.org/content/16/12/1446.full) to GO:

1. cartilage condensation *The condensation of mesenchymal cells that have been committed to differentiate into chondrocytes.*

Figure 13: Bone classification in mouse (subclass hierarchy only, 3 levels down, part-ofs are hidden). Note the distinction between tissue and the bone (organ). Is the coccygeal vertebra placement unusual?
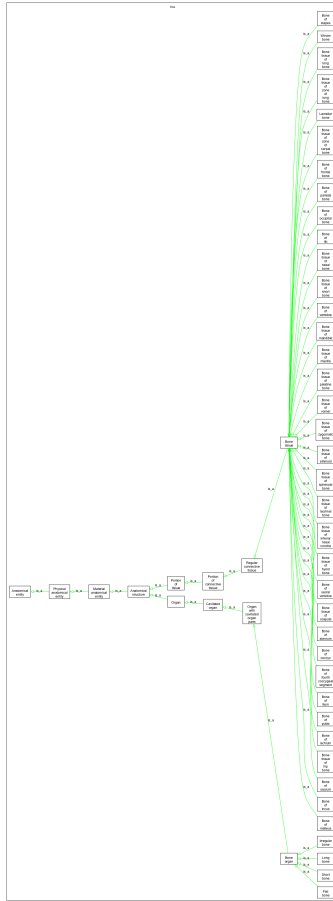
Figure 14: Bone classification in FMA (subclass hierarchy only, 1 level down, part-ofs and other relations are hidden). The distinction between Bone tissue and Bone organ doesn't appear clear to me
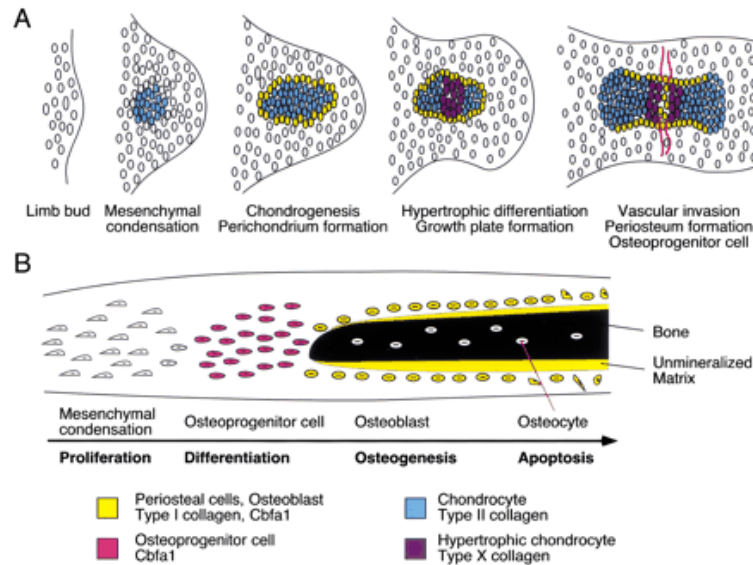
Figure 15: Endochondral bone development. From Ornitz et al.

2. growth plate cartilage chondrocyte differentiation *The process whereby a chondroblast acquires specialized structural and/or functional features of a chondrocyte that will contribute to the growth of a bone. A chondrocyte is a polymorphic cell that forms cartilage.*

3. cartilage development *The process whose specific outcome is the progression of the cartilage over time, from its formation to the mature structure. Cartilage is a connective tissue dominated by extracellular matrix containing collagen type II and large amounts of proteoglycan, particularly chondroitin sulfate.*

4. limb bud formation *The process pertaining to the initial formation of a limb bud from unspecified parts. This process begins with the formation of a local condensation of mesenchyme cells within the prospective limb field, and ends when a limb bud is recognizable.*

"chondrogenesis" is an exact synonym for "cartilage development"

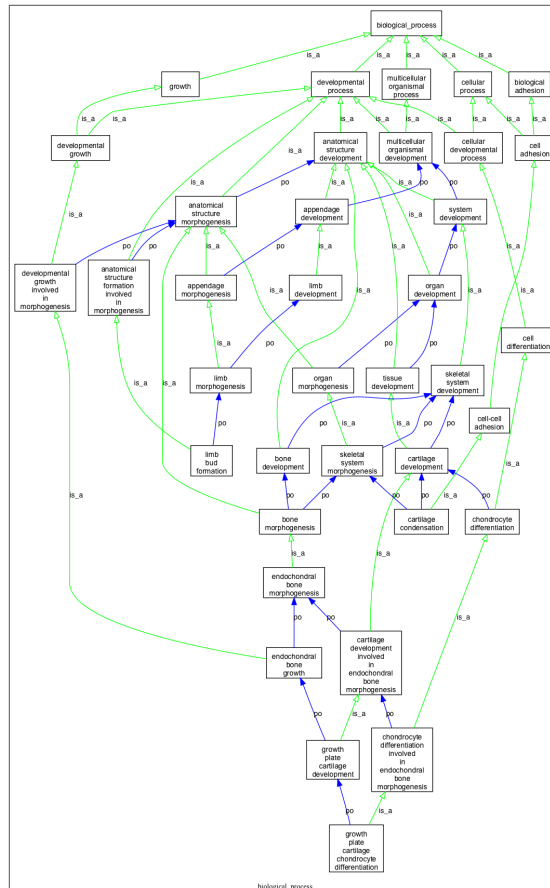No terms for perichondrium formation, periosteum formation or vascular invasion

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Figure 16: Endochondral bone development in GO.