



Anatomical Network Analysis in Evo-Devo

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Abstract

This chapter introduces the reader to anatomical network analysis (AnNA): a conceptual framework for the topological analysis of organismal form. AnNA focuses on the structural relations among anatomical parts, which allows for an evaluation of morphological organization in comparative analyses for both development and evolution. The nodes of the network represent anatomical elements, and the links that connect them represent structural relations or interactions among these elements. Network theory provides the methods to analyze these anatomical network models. The first and second sections present the historical and conceptual background of this framework. The third section explains the construction of anatomical networks and some of the basic parameters we can use to characterize the topology of these models and infer their morphological organization. The fourth section summarizes the interpretation of network parameters in terms of morphological complexity, hierarchy, integration, and modularity in the context of morphological evo-devo. The fifth section introduces the classical construction rules to build null models for networks and an example of the use of network null models in morphology. Finally, in the sixth section, we have explored some of the limits of AnNA.

Keywords

Morphology · Network theory · Quantitative methods · Topology

Introduction

The quantification of form is essential for the study of evolution (variation and diversification) and development (growth and patterning). Form is the result of the many processes acting at different levels of organization during development, from genetic control of cellular products and processes to developmental constraints on anatomy. Form is also a firsthand resource for evolutionary inquiry, since it is usually the only information preserved from extinct animals in the fossil record. The concept of organismal form covers the size and shape of the body and the structural arrangement and relations of body parts.

Anatomical network analysis (AnNA) is a conceptual framework that provides a set of tools for the analysis of the structural arrangement and relations among body parts in a morphological system. It relies on an old anatomical adagio, the *principe des connexions*, which identifies physical connections among anatomical elements (i.e., bones, muscles, cartilages) as carriers of important biological information, often more so than their size and shape. Indeed, this assumption is at the foundation of comparative anatomy itself; it was championed by the great French anatomist Étienne Geoffroy Sait-Hilaire in the nineteenth-century and it has been the focus of attention for comparative anatomy ever since. Geoffroy recognized that the shape and size of the same anatomical elements in different organisms vary greatly; so much that, to correctly identify them, it was more useful to analyze how they were

connected to their anatomical surroundings. The study of connections, although intuitively sound, lacked until very recently a suitable methodological framework to codify, manipulate, and analyze the patterns underlying these physical relationships in a meaningful way. Theoretical biologists, such as Joseph Woodger, Nicolas Rashevsky, and Rupert Riedl, proposed different ways to quantify and use the connections among anatomical parts to study the form of organisms but without a proper suite of methods. More recently, Rasskin-Gutman and Buscalioni (2001) proposed the use of graph theory to analyze anatomical connections among the skeletal elements forming the archosaurs pelvis (ischium, ilium, and pubis), setting the foundations for AnNA. In the past decade, AnNA has been developed further through its use in studies on the evolution and development (including pathology) of forms. From a methodological perspective, AnNA is based on mathematical tools from graph theory and network analysis that are available through many software packages (for a review on AnNA, see Rasskin-Gutman 2003; Rasskin-Gutman and Esteve-Altava 2014). This chapter presents the conceptual foundation of this latter approach and the methods adopted to use network analysis to study the evolution and development of form.

Connections in the Context of Organismal Form

The first step in a study of form using AnNA is to understand the biological meaning of the connectivity patterns one is about to analyze. The definition of what is a connection will determine the meaning of the results and the usefulness of the conclusions to be drawn from the study. But, why focusing on connections? Besides its classical appeal mentioned above, it is worth noting that any anatomical system can be teased apart on different levels of morphological organization. For example, Diego Rasskin-Gutman and Angela Buscalioni (2001) proposed a division into four related but semi-independent levels: (1) proportions, (2) connections, (3) orientations, and (4) articulations (Rasskin-Gutman and Buscalioni 2001; Rasskin-Gutman 2003). (A/N: in their system, the level of articulations refers to the range of motion among body elements.) Other levels could be think of, for example, that of relative positioning of parts (Woodger 1945). Each level gives insights into level-specific constraints and mechanisms that generate morphological variation and affect its evolution; hence, each level needs its level-specific type of formalisms and methods.

Of these four levels of morphological organization, the most studied one is that related to size and shape (proportions, level 1); it can be analyzed by using traditional morphometric tools with size and shape measurements or landmark-based geometric morphometrics with Cartesian coordinates (see the chapter on “► [Morphometrics and Evo-Devo](#)”). AnNA is useful to analyze the structure and topology of forms (connections, level 2), where the formalism is a codification of the physical connections among elements; this codification results on an adjacency matrix, in its simplest form, filled by 1s (representing connections) or 0s representing absence of connections; cells in an adjacency matrix can also take continuous values representing relative degrees or areas of connection among elements (see section “[The Network](#)”).

[Model and Its Analysis](#)”). The other two levels of morphological organization capture the relative positioning of parts (orientations, level 3), which can be formalized a set of angles, and the mobility of joints (articulations, level 4), which can be formalized as tables of kinetic data.

The level of connections describes the topological relations among anatomical parts, that is, their arrangement in a morphological system; in addition, topological relations often capture the presence of functional and developmental relationships (codependences) among parts (Esteve-Altava 2017). For example, connections among skull bones not only represent the topological boundaries among bones but also primary sites of bone growth and remodeling, as well as sites of stress diffusion. Function and development are also important factors acting on the origin and variation of all other levels of morphological organization, making them codependent to some extent. For example, some studies posed that the covariation of landmark positions (commonly used in geometric morphometrics to analyze shape changes) are not independent as is sometimes assumed (Chernoff and Magwene 1999); instead, landmark covariation is constrained by the connections of the parts on which they are located. At the same time, changes in shape also affect the topological arrangement of parts, which might vary during evolution. The extent to which each level of morphological organization affects each other remains an open question (Esteve-Altava 2017).

Anatomical Network Analysis

Network models are sets of interacting elements. The analysis of network models requires a specific set of tools: concepts, descriptions, and algorithms; network theory is the branch of mathematics that supplies them. In this section, we first explain the creation of anatomical network models. Then, we introduce the most popular element and network parameters that we can quantify in anatomical networks, as well as the most popular organizational features and null models used to describe the structure and properties of anatomical networks. The lists of parameters, features, and null models are thus not exhaustive but limited to those that have been used before in the context of anatomical network analysis. Specialized literature, such as reviews by Albert and Barabási (2002), Dorogovtsev and Mendes (2003), Newman et al. (2006), and Mark Newman’s book “*Networks: An Introduction*” (2010), offer comprehensive mathematical descriptions of the parameters introduced here and of others used in the analysis of various complex networks. In section “[Anatomical Network Analysis in Morphological Evo-Devo](#),” we provide the morphological interpretation of the statistics herein introduced within the framework of evo-devo.

Building an Anatomical Network: Elements and Interactions

Identifying the elements and interactions of an anatomical system is the first and most important step of the modeling process. Both, elements and interactions, must

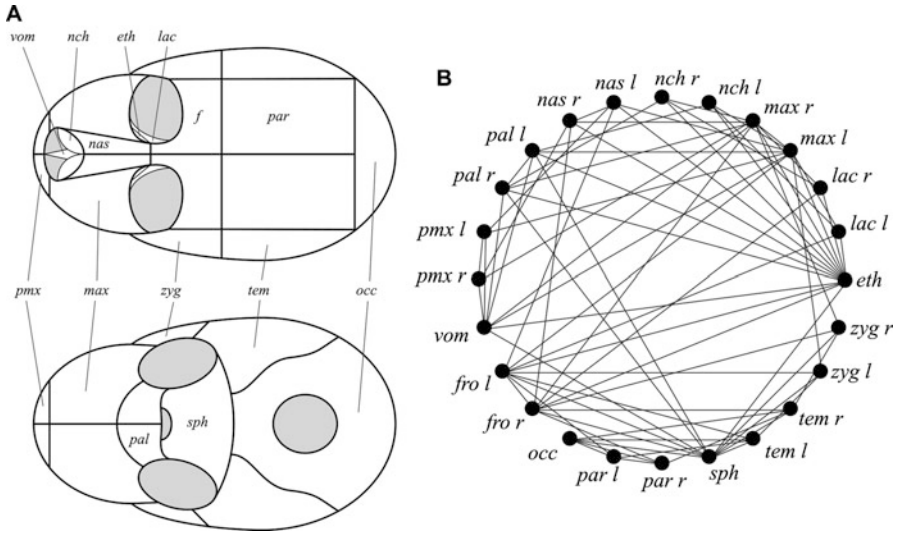


Fig. 1 Schema of a primate skull (a) and its corresponding network model (b). Labels *eth* ethmoid, *fro* frontal, *lac* lacrimal, *max* maxilla, *nas* nasal, *nch* nasal concha, *occ* occipital, *pal* palatine, *par* parietal, *pmx* premaxilla, *sph* sphenoid, *tem* temporal, *vom* vomer, *zyg* zygomatic, *l* left, *r* right. (Modified from Esteve-Altava et al. 2015a)

have a precise definition to identify them across all specimens of the study. This task implies making some compromises between the generalization required in comparative studies and the accuracy required to uncover meaningful information.

Let's consider the skeletal parts of the vertebrate's head as an anatomical system to illustrate the process of defining elements and interactions to create an anatomical network (Fig. 1). At first glance, the natural elements of the skull are its bones: a rigid body of ossified tissue. Even such a straightforward definition like this makes it difficult to identify these elements in some cases. For example, identifying bones is tricky in an ontogenetic sequence, because some adult bones are formed by many ossification centers fused sequentially, which makes it difficult to trace back their origin. A different approach would be to use ossification centers instead of bones as elements of the network. However, this would prevent a broad comparison including both living and extinct forms, because ossification centers are barely observable in fossils.

Likewise, the physical junction between two bones is the most obvious interaction between them. However, various types of joints are possible between the bones of the head, namely: cartilaginous, dental, fibrous, and synovial joints. Because different types of joints have different functions and properties, the use of a specific type constrains the range of relations modeled and interpreted. For example, craniofacial sutures (fibrous joints) act as primary sites of bone growth, while the temporomandibular joint (a synovial joint) is responsible for jaw movements too. A network model that only uses sutures as interactions captures a developmental relation among bones (i.e., growth), while a model that also uses synovial joints

captures, in addition, biomechanical relations. Furthermore, other types of junctions, such as cartilaginous joints, introduce problems in the identification of their presence, for example, in fossils. It is worth noting that anatomical networks are not restricted to the modeling of the skeletal system; for example, muscles can be modeled as nodes of a muscular network and/or as links connecting skeletal parts (Esteve-Altava et al. 2015b; Dos Santos et al. 2017).

The example above shows how the same anatomical system can be modeled in different ways, according to different definitions of its elements and interactions, each option generating a different network model. Note that any additional information other than the modeled interactions, such as physical position in the space, orientation, or shape of the elements, is not included in the anatomical network model. Such information could be added, if needed, as additional descriptors that define types of nodes and links based on biological features. Finally, we can use AnNA as part of a comparison between topological features and other biological features of the anatomical system, for example, to test the relationship between topology and shape (Esteve-Altava 2017).

The Network Model and Its Analysis

A *network model* is the combination of two sets: a set of nodes and a set of links; each link has two endpoints, that is, it represents a connection between two nodes. In this mathematical abstraction, the nodes stand for anatomical elements and the links stand for interactions among elements (in the example above, nodes are bones and links are physical joints). The most common representation of a network is a drawing of dots joined by lines: a line connecting two nodes indicates the presence of a mutual relation. Directed links indicate nonreciprocal relations, while weighted links indicate the strength of the interactions. Notice that all network representations are equivalent if the same links between nodes are kept. For simplicity, here we only provide mathematical descriptions of the algorithms used to analyze undirected (reciprocal) and unweighted networks; derived algorithms exist for the specific analysis of directed and/or weighted networks (for details on such derived algorithms, see the recommended literature at the beginning of section “[Anatomical Network Analysis](#)”).

The *adjacency matrix* ($A_{i,j}$) codifies the connections among the nodes of the network, that is, the number and the particular distribution of links between nodes. For undirected, unweighted networks, this is a symmetric binary matrix of size $N \times N$, where 1 indicates presence and 0 indicates absence of connection. Thus, the adjacency matrix defines the neighborhood, the connectivity context of each node as all the nodes to which it connects. An adjacency matrix is the main source of data in many programs used to analyze networks, but it is not the only one. For example, a list of edges (i.e., links) is also a very common source: a list in which each row indicates the origin and the destination of a link.

Network Parameters

Some important descriptors and parameters for individual nodes and the whole network are listed below. While node descriptors are very useful to study the properties of individual elements in relation to others, network descriptors are useful to compare one network to another.

Node degree: Sum of links a specific node has to other nodes in the network,

$$k_i = \sum_{j=1}^{j=n} A_{i,j}$$

Clustering coefficient: Ratio between the actual number of links among the neighbors of node i and the maximum number of links possible among them (i.e., $k_i(k_i-1)/2$),

$$C_i = \frac{2e_i}{k_i(k_i - 1)}$$

where e_i is the number of links that exists connecting the neighbors of node i .

Shortest path length between two nodes: Their shortest distance measured as number of links to go from one node to the other,

$$l_{i,j} = d(n_i, n_j)$$

where $d(n_i, n_j)$ is the minimum distance in number of links to connect nodes i and j . Note that more than one path might have the shortest length.

Density: Total number of existing links, K , divided by the maximum number of possible links for a given number of nodes, N ,

$$D = \frac{2K}{N(N - 1)}$$

Average clustering coefficient: Arithmetic mean of the clustering coefficient of all nodes in the network,

$$C = \frac{1}{N} \sum C_i$$

Average shortest path length: Arithmetic mean of the shortest path length between all pairs of nodes,

$$L = \frac{1}{N - 1} \sum l_{i,j}$$

Degree distribution: Frequency of occurrence of nodes with a given number of links,

$$P(k) = \frac{N_k}{N}$$

Clustering coefficient distribution: Clustering coefficient mean of all nodes with k links,

$$C(k) = \frac{\sum C_{i,k}}{N}$$

Types of Network Organization

In addition, the organization of the network can be informative about its properties for a given function. For example, networks are often seen as scale-free, hierarchical, and/or small-world, depending on the values of some of the parameters we just listed above compared to a null model (often a random network, see below). The presence of a community structure or modules inside the network is also very important in AnNA.

Hierarchical networks take their name from a very specific idea about hierarchy: nodes are organized as clusters within clusters – thus, C is high in these types of networks – , which promotes the formation of modules as clusters of highly interconnected nodes. Hierarchical networks are also scale free, which means that its structure is preserved at any scale of observation; in addition, these networks always host highly connected nodes or hubs. A network with a hierarchical organization shows a stratification of connections in various nested layers. Both the $P(k)$ and the $C(k)$ help assessing the presence of a hierarchical organization in a network. The functional form of these distributions (e.g., uniform, Poisson, or power law) characterizes the organization of connections among the nodes. Hierarchical networks have characteristic $P(k)$ and $C(k)$ functions that differ from those of random and scale-free networks. In random networks, $P(k)$ fits a Poisson function; in scale-free networks, it fits a power law function; in both, $C(k)$ fits a discrete uniform function. In contrast, hierarchical networks have a $P(k)$ and a $C(k)$ that fit power law functions. In general, a power law distribution in both parameters indicates that the neighborhoods of low-degree nodes are highly clustered, forming blocks, while those of high-degree nodes are sparsely connected, which suggest that high-degree nodes are acting as connectors between blocks. A hierarchical organization is commonly observed in anatomical networks with a community structure.

Small world networks have a special kind of organization between regularity and randomness; their low shortest path length (L) gives them special dynamic relationships among nodes, and their high clustering coefficient (C) provides them with distinctive structural features. Having a low L means that the communication of any kind of properties among nodes (e.g., stress forces among bones) is more efficient, thanks to shortcut links; having a high C means that there are many clusters or associations between nodes, which can be putative modules. A network with a small-world organization has a higher C and a lower or similar L to that of a random network, because of the presence of short-cut nodes. These nodes connect other

nodes that would otherwise be far apart (i.e., high shortest path length). The presence of a small-world is assessed by computing the values of C and L and then comparing them to those of random equivalent networks (i.e., networks with the same number of nodes and links but randomly rewired). A small-world organization is common in anatomical networks and is related to the identification of a community structure.

Anatomical Network Analysis in Morphological Evo-Devo

This section summarizes the current interpretation of the network parameters previously described in section “[Anatomical Network Analysis](#)” as features of the morphological organization of the body. At a connectivity level, morphological organization is the result of the overall interactions among anatomical parts. Finding the morphogenetic processes, from genetic regulatory networks to developmental constraints, that produce morphological organization is at the core of evo-devo research. To this end, we need first to describe quantitatively the morphology of organisms. Network parameters serve as proxies for features often related to the morphological organization of the body, such as complexity, hierarchy, integration, and modularity. The morphological interpretations of network parameters offered herein represent a work in progress (Rasskin-Gutman and Esteve-Altava 2014), open to discussion.

Morphological Complexity

Complexity can be defined in many ways: a state of order, unpredictability within a structured disorder, functional multitasking, structural stability, or amount of information for a minimal description to name a few (see the chapter on “[► The Evolution of Complexity](#)”). Morphological complexity has been usually defined as the number of different anatomical elements (McShea 1991). However, complex systems not only have many parts, they also achieve more structural and functional interactions. In this context, morphological complexity can be quantified explicitly by analyzing the connectivity patterns among anatomical elements using network analysis. This definition of morphological complexity, as the elements of an anatomical system and their interactions, resembles that of Herbert Simon (1962): *a large number of parts that interact in a non-simple way*.

The complexity, or simplicity, of the pattern of interactions of an anatomical network can be quantified by complementary network parameters, being the most straightforward the density of connections. The network density is the number of existing connections in the network, expressed as a fraction of the total possible connections between elements. In a network with density equal to 1, all elements are connected, that is, they are all interacting with each other. In nonanatomical systems, it is expected that more relationships would allow the system to perform more functions and show more complex behaviors. Complete connectivity is not present in anatomical structures; however, for a range of intermediate values of density, we

would also expect greater values of density to correlate with more complex behaviors. For example, a limb with more muscular connections could achieve a greater range of motion and complex behaviors (e.g., walking, climbing, manufacturing). Here, a greater number of connections would confer a greater potentiality of action; however, this does not entail that all interactions among anatomical parts are used at the same time. Analogously, the number of links of a given anatomical element can be a measure of its individual complexity, as related to the number of interactions it has, which is discussed in the literature using Rupert Riedl concept of burden (Riedl 1978; see the chapter on “► [The Concept of Burden in Evo-Devo](#)”).

Hierarchical Organization

Morphological systems are hierarchical in two ways (Mayr 1982). The first way is by aggregation, that is, each anatomical part is composed of tissues, cells, and so on downwards in the hierarchy while parts make up organs, bodies, and so on upwards in the hierarchy (see the chapter on “► [Levels of Organization](#)”). The second way is by constitution, that is, anatomical parts interact with each other as blocks within blocks, promoting hierarchical patterns of integration. A hierarchical integration of the body promotes body parts to change in form, growth, and function in a coordinate manner.

Network analysis can readily recognize constitutional hierarchy by analyzing patterns of connectivity among anatomical elements. We have already seen that, in the context of network sciences, the degree and clustering coefficient distributions help us identifying hierarchical networks as nested clusters (i.e., blocks) of anatomical elements (see section “[Anatomical Network Analysis](#)” on *hierarchical networks*).

Morphological Integration

Morphological integration means association between morphological traits, which is generally defined as the covariation among morphological traits due to common developmental and/or functional causes (Olson and Miller 1958). Depending on the definition of trait and unit of variation, the interpretation of integration varies in the context of genetics, development, and evolution, but also in morphology, depending on whether the focus is on proportions (shape and size) or connections (structure). On the one hand, when focusing on proportions, morphological integration is related to the study of correlations between body parts, that is, how much two traits (e.g., distances, landmark coordinates) change together. On the other hand, when focusing on connections, morphological integration refers to the interconnection of anatomical elements, that is, how many interactions tight them together; the statement is about the parts within the individual. For example, we would expect that two connected elements are more integrated than two disconnected elements, because a connection sets a developmental and functional dependency between them.

In a network structure, integration depends not only on direct contacts but also on indirect interactions. Two network parameters directly related to indirect interactions are the clustering coefficient and the shortest path length. Both parameters are related to information flow and correlation in networks: the clustering coefficient captures short-range information correlation due to redundancy among neighbor elements, while the shortest path length determines the speed of information transmission to distant elements depending on their effective proximity.

Morphological Modularity

Morphological systems are modular when they have differences in the degree of integration between parts, that is, the system has a heterogeneous integration of parts across different regions (see the chapter on “► [Modularity in Evo-Devo](#)”). Modularity, like integration, is a multilayered property that arises at different levels of organization: developmental, genetic, functional, and evolutionary, which converge in observable morphological modules. Traditionally, morphological modules are inferred from covariation among morphological traits, usually sets of distances or landmarks that tend to change together; thus, they are also called variational modules.

In network theory, a module is defined as a group of elements with more connections between them than to other elements outside the group (Fortunato 2010). This definition of module is also valid for any other biological system because of its generality. For instance, an anatomical module is a region of a body part with anatomical elements interacting more within the boundaries of the region than with other anatomical elements outside the region. Connectivity modules differ from the most common use today of variational modules (as related to shape covariation) in that they are inferred from the topological arrangement of anatomical units (Esteve-Altava 2017). Thus, the morphological information for variational and connectivity modules comes then from completely different sources. If, for example, the interactions modeled as links are developmental and/or functional, then connectivity modules have also a developmental and functional foundation.

Null Models in Anatomical Networks

A null model in network sciences is an idealized process-based model that generates a specific connectivity pattern of a network. A null model specifies the way nodes are added to the network and are connected by links. When this pattern is compared with that of an empirical network, it provides a comparative baseline to infer plausible mechanisms of network formation (i.e., construction rules). In an evo-devo context, null models are used, for example, to provide hypotheses about developmental mechanisms and to build generative morphospaces (as in Esteve-Altava and Rasskin-Gutman 2014b). The following are the null models that have been used

before to study the evolution and development of anatomical networks; this list is by no means exhaustive.

The Regular Model

A regular network is built so that all nodes have the same number of links. The properties of regular networks are determined by the number of nodes and the number of links per node. Biological structures conforming to a regular null model are, for example, the hexagonal prismatic wax cells of a honeycomb or the scutes of a turtle shell. These regular patterns are formed by different processes, in the case of the honeycomb, by selection of space-efficient packing while in the case of turtle shells, by stationary accretion of keratin in all directions.

The Random Model

In a random network, all pairs of nodes have the same chances to be connected. Paul Erdős and Alfréd Rényi (1959) originally proposed to build random networks by connecting nodes at random with a probability p . Some properties of random networks are size-dependent; for example, as the number of nodes increases, the degree distribution tends to a Poisson distribution, the average shortest path length increases as the logarithm of the number of nodes, and clusters tend to disappear. Likewise, the value of p affects the compactness of the network: the higher the value of p , the higher the network density and clustering coefficient, and the lower the shortest path length.

Pure random pattern of connection in biological structures are rare because of the many constraints that can prevent some links while facilitating others, thus, biasing the arrangement of connections away from randomness. For instance, the cranial cavity that hosts the human brain has a spherical shape that imposes a bias in the probability of connection between the occipital bone and the nasal bones, which is physically impossible given their range of shape (i.e., $p = 0$). However, this null model is still valid as a comparative model to establish the presence of some network features, for example, the small-world effect.

The Small-World Model

A small-world network is more clustered than a random network, but it has a similar (or slightly lower) average shortest path. Many complex systems have a small-world organization. Duncan Watts and Steven Strogatz (1998) proposed a mechanism to create small-world networks starting with a regular network that is sequentially rewired at random, with probability p (all nodes have an opportunity to change or keep their connection). By increasing the parameter p from 0 to 1, a network

switches from regular to random: the small-world organization is a transition state in this process.

A small-world organization has been found in most natural systems, including metabolic networks, brain networks, anatomical networks (e.g., skulls), and ecological networks. It is perhaps one of the default types of organization of living systems: between randomness and regularity. Mechanisms by which such systems are created are, however, very diverse, and, except perhaps for neurons, a rewiring process is not expected. An originally regular process that is later biased by emerging interactions could produce a small-world organization. In the case of skulls, for example, we could speculate about mechanisms biasing a homogeneous growth of bones from ossification centers and later interactions (fusion or boundary formation) among bones during development (see example in section “[Using Null Network Models to Study Morphological Systems: An Example](#)”).

The Scale-Free Model

A scale-free network has a degree distribution that follows a power law, which means that most nodes have a few connections and a few nodes have many (these are called *hubs*). This organization is present in many natural systems. Albert-László Barabási and Réka Albert (1999) proposed a preferential attachment mechanism to build scale-free networks. New nodes are added sequentially to a network and connected to old nodes (already present in the network) with a probability that depends on the number of connections of the old nodes. Preferential attachment creates networks with a power law degree distribution and no clusters. However, the prevalence of the preferential attachment mechanisms to produce scale-free model has been questioned (Fox-Keller 2005). Note that only a preferential attachment to the most connected nodes will produce these types of networks; other forms of preferential attachments are possible in biological systems which may not derive from the previous number of interactions.

The Gabriel Model

A Gabriel network is a type of geometric network; these are networks that are spatially constrained: two nodes only connect if they satisfy a geometric requirement. The nodes of this type of networks occupy a position in the space (e.g., in a 3D Euclidean space). Kuno Gabriel and Robert Sokal (1969) proposed a mechanism to construct spatially constrained networks based on spatial interference between nodes. Two nodes are connected if, and only if, the sphere whose diameter is the line between both nodes does not have any other node within its volume. The properties of the network depend on the number and exact position of nodes.

The Gabriel model is useful to infer developmental or structural constraints imposed by geometry. Not only because of the physical distance between, and

exact position of, ossification centers but also by the presence of insurmountable obstacles between them during development: cavities, openings, and other structures. For this reason, Gabriel models have been used before in modeling structural constraints in skull evolution and development (see example in section “[Using Null Network Models to Study Morphological Systems: An Example](#)”; Esteve-Altava and Rasskin-Gutman 2014a). When modeling the development of anatomical structures, such as skulls, the Gabriel model simulates a homogeneous growth of anatomical parts in all directions.

Using Null Network Models to Study Morphological Systems: An Example

Null models can be used to understand the mechanisms that participate in the formation of complex anatomical systems like the human head (Esteve-Altava and Rasskin-Gutman 2014a). A common hypothesis to explain the development of the human head is the functional matrix hypothesis proposed by Melvin Moss (1968). This hypothesis argues that the presence of muscle inductions and functional cavities (e.g., brain, eyeballs) guide the growth of bones and, hence, determine the shape and articulations of bones. Here is an example of the use of a network null model to study the formation of suture contacts in the human skull.

In a developing skull, bones grow until they meet with another bone and form a suture joint. Let’s assume, for simplicity, that each bone of the skull comes from only one ossification center that starts growing from its center. In the absence of any constraint on the speed and direction of growth, bones would expand as perfect spheres until they meet other bones and form a suture connection. The occupation of a region of the space by one bone will prevent the growth of other bones through this same space. Thus, an existing connection between two bones prevents other bones to connect. This is the case, for example, of the pterion region of the human skull, where the parietal, the frontal, the temporal, and the sphenoid bones meet. If the sphenoid and the parietal connect first, a fronto-temporal join is impossible; if the frontal and the temporal connect first, a spheno-parietal join is impossible. Moreover, the presence of cavities hosting soft tissues and sensory organs (i.e., functional matrices) also constrains which contacts among bones are feasible in a growing skull.

Imagine now that we model the human skull as a Gabriel network. Each bone is represented as a node, to which we give a position in a 3D space according to its center. The mechanism of linkage of the Gabriel model simulates precisely what would happen if bones had grown from its center at a uniform speed and direction (i.e., without constraints). Thus, the Gabriel model captures the impossibility of creating a suture contact between distant bones due to the presence of unavoidable obstacles between them during development, such as cavities, openings, and, in this case, other bones.

If we compare the existing connections in the human skull with those present in the Gabriel model of the human skull, we will have three types of outcomes (Fig. 2):

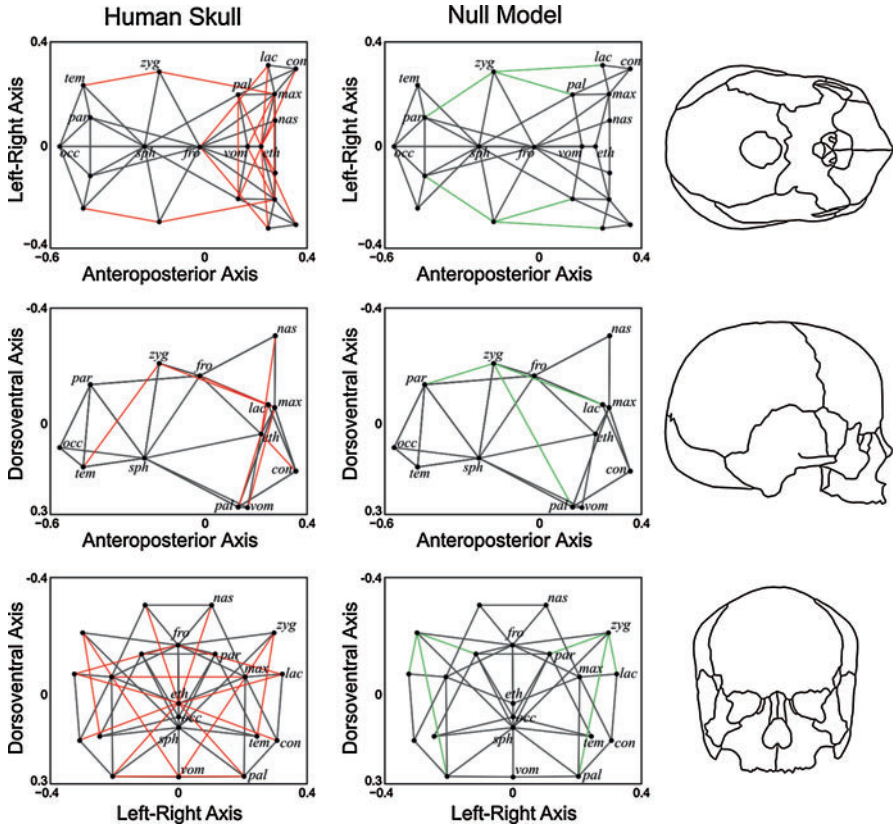


Fig. 2 Bone articulations in a human skull and in a Gabriel null model in various views. Solid gray lines show connections that are predicted by the null model as they actually occur in the human skull; dashed green lines show connections predicted by the null model but not realized; and dotted red lines show connections that are not predicted by the null model but occur in the actual human skull. Labels in Fig. 1. (Modified from Esteve-Altava and Rasskin-Gutman 2014a)

(1) a group of connections that exist in both the null model and the real skull: these are connections that can be explained by an unconstrained growth of bones. This does not mean that the formation of these connections lacks constraints. Rather, it means that there is no need to invoke constraints acting on bone growth to explain such pattern of connection. (2) Connections that exist in the null model but not in the real skull: these are connections that are prevented due to deviation from homogeneous bone growth. This can happen, for example, due to the presence of cranial cavities and openings. (3) Connections that do not exist in the null model but do exist in the real skull: these (as in case 2) are connections that require deviation from homogeneous growth of bones to exist. These connections would require a mechanism that actively pushes the bones to grow in a certain direction or at a faster rate. This difference might be important, for example, to test the validity of the functional

matrix hypothesis in the formation of sutures. In sum, by using null network models, and comparing them to real anatomical systems, we can test hypotheses about processes that participate in the formation of an anatomical system during development as well as in its evolution.

Limitations and Cautions in Anatomical Network Modeling

As it occurs in the construction of any model, the construction of anatomical networks is susceptible to some limitations that, if they are not identified (and fixed or assumed), can lead to some misunderstanding in the interpretation of results (Rasskin-Gutman and Esteve-Altava 2014). Most of these limitations arise from: (1) the definition and selection of elements and relations, (2) typological simplifications, and (3) a false sense of symmetry.

Identification of Elements and Relations

Building the appropriate network model for the system of interest and the questions asked is the first step in an anatomical network analysis. This requires a careful selection of the units of description and the relations modeled: model descriptors (nodes and links) must have precise definitions to enable their identification in all the objects of the study. However, this is not always a straightforward task neither in theory nor in practice (Butts 2009). Think, for example, of a skull network in which the units of description are bones and physical junctions. It is known that the bone unit may change in different ways during development (e.g., connections, proportions, and ossification). For instance, the frontal bone in the human skull is a single unpaired bone in the adult but two paired bones at birth. A different approach could use the ossification centers as elements of the skull network but that would exclude fossil skulls from a broader phylogenetic analysis. The same applies to the definition of physical junction as the structural relation between bones. To use suture joints is an easy way to identify most of the contacts occurring in the skull; however, it excludes from the network those bones that join the skull in a different way, such as the mandible. Moreover, a dichotomous definition of relation between bones (i.e., presence or absence) may obscure differences in the strength of junctions due to the size of their area of articulation. On the other hand, this binary definition allows one to consider all interactions even when the length of the contact is unknown, which is common in fossils.

Typological Simplifications

Even the most conserved anatomical structures show some degree of natural variation at the species or population level. For example, the pterion region of the human skull, where the temporal, parietal, sphenoid, and frontal bones converge shows

intraspecific variation in the way these bones are connected. Thus, suture contact between the sphenoid and parietal bones in this region (sphenoparietal pattern) prevents the frontal and temporal bones to contact (frontotemporal pattern); in some rare cases, the four bones meet in a single point (stellate pattern) or a wormian bone can form in their intersection (epipteric pattern). Another example will be the variable presence of some muscles or their attachments; for example, the forearm muscle, palmaris longus, is sometimes absent in humans.

Arguably, considering only the *type* form can lead to misrepresentation or, at least, oversimplification of the morphological system under study. Local variation in the connectivity pattern among anatomical parts could produce a slight variation in the network properties of a system. However, taking the *type* form (or the most common configuration) of a morphological system is a common idealization in anatomical network analysis.

Illusions of Symmetry

An example of the typological simplification in the construction of anatomical network models is the tendency to symmetrizing anatomical patterns when using *type* forms. Using the previous examples of the pterion region and the palmaris longus, variation is further increased by potential anatomical differences between the left and right side in one individual. Symmetry simplification is common in fossil descriptions, in which connectivity patterns may be obscured by conservation and taphonomic processes. The origin of this idealization lays in the well-established idea that the vertebrate body plan is bilaterally symmetric by default (although internal organs may show directional patterns of asymmetry). Small disruptions of bilateral symmetry can occur also due to errors or fluctuations during development because of alterations of the developmental program or environmental stress.

These current limitations may be overcome by new, innovative approaches that take into account – or empirically address – variability in connectivity patterns at a population or intraspecific level. Nonetheless, modeling is always an exercise of simplification, the limits of which depend on the problem at hand and the system of study.

Conclusion

Anatomical network analysis offers a conceptual framework along with the necessary tools to investigate the architecture of morphological systems at a structural (or topological) level. Thanks to its level of generality, the same tools can be applied to the study of organization and change of organismal form both at developmental and evolutionary scales. Within its limits and idealizations, an anatomical network analysis provides a systematic way of measuring structural complexity and integration, as well as exploring morphological modularity in evolution and development.

Cross-References

- ▶ [Developmental Homology](#)
- ▶ [Mechanisms of Pattern Formation, Morphogenesis, and Evolution](#)
- ▶ [Modularity in Evo-Devo](#)
- ▶ [Morphometrics and Evo-Devo](#)
- ▶ [Rupert Riedl](#)
- ▶ [The Concept of Burden in Evo-Devo](#)

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References

- Albert R, Barabási A-L (2002) Statistical mechanics of complex networks. *Rev Mod Phys* 74:47
- Barabási A-L, Albert R (1999) Emergence of scaling in random networks. *Science* 286:509–512
- Butts CT (2009) Revisiting the foundations of network analysis. *Science* 325:414–416
- Chernoff B, Magwene PM (1999) Afterword. In: Olson EC, Miller PL (eds) *Morphological integration*. University of Chicago Press, Chicago, pp 319–353
- Dorogovtsev R, Mendes JFF (2003) *Evolution of networks: from biological networks to the internet and WWW*. Oxford University Press, Oxford
- Dos Santos DA, Fratani J, Ponssa ML, Abdala V (2017) Network architecture associated with the highly specialized hindlimb of frogs. *PLoS ONE* 12(5):e0177819
- Erdős P, Rényi A (1959) On random graphs. *Publ Math Debr* 6:290–297
- Esteve-Altava B (2017) Challenges in identifying and interpreting organizational modules in morphology. *J Morphol* 278:960–974
- Esteve-Altava B, Rasskin-Gutman D (2014a) Beyond the functional matrix hypothesis: a network null model of human skull growth for the formation of bone articulations. *J Anat* 225:306–316
- Esteve-Altava B, Rasskin-Gutman D (2014b) Theoretical morphology of tetrapod skull networks. *C R Palevol* 13:41–50
- Esteve-Altava B, Boughner JC, Diogo R, Villmoare BA, Rasskin-Gutman D (2015a) Anatomical network analysis shows decoupling of modular lability and complexity in the evolution of the primate skull. *PLoS One* 10(5):e0127653
- Esteve-Altava B, Diogo R, Smith C, Boughner JC, Rasskin-Gutman D (2015b) Anatomical networks reveal the musculoskeletal modularity of the human head. *Sci Rep* 5:8298. <https://doi.org/10.1038/srep08298>
- Fortunato S (2010) Community detection in graphs. *Phys Rep* 486:75–174
- Fox-Keller E (2005) Revisiting scale-free networks. *BioEssays* 27:1060–1068
- Gabriel KR, Sokal RR (1969) A new statistical approach to geographic variation analysis. *Syst Zool* 18:259
- Mayr E (1982) *The growth of biological thought*. Harvard University Press, Cambridge
- McShea DW (1991) Complexity and evolution: what everybody knows. *Biol Philos* 6:303–324
- Moss ML (1968) A theoretical analysis of the functional matrix. *Acta Biotheor* 18:195–202
- Newman M (2010) *Networks: an introduction*. Oxford University Press, Oxford
- Newman M, Barabási A-L, Watts DJ (2006) *The structure and dynamics of networks*. Princeton University Press, Princeton
- Olson EC, Miller RL (1958) *Morphological integration*. The University of Chicago Press, Chicago
- Rasskin-Gutman D (2003) Boundary constraints for the emergence of form. In: Müller G, Newman S (eds) *Origination of organismal form*. MIT Press, Cambridge, pp 305–322

-
- Rasskin-Gutman D, Buscalioni AD (2001) Theoretical morphology of the Archosaur (Reptilia: Diapsida) pelvic girdle. *Paleobiology* 27:59–78
- Rasskin-Gutman D, Esteve-Altava B (2014) Connecting the dots: anatomical network analysis in morphological Evo-Devo. *Biol Theory* 9:178–193
- Riedl R (1978) *Order in living organisms: a systems analysis of evolution*. Wiley, New York
- Simon HA (1962) The architecture of complexity. *Proc Am Philos Soc* 106:467–482
- Watts DJ, Strogatz SH (1998) Collective dynamics of ‘small-world’ networks. *Nature* 393:440–442
- Woodger JH (1945) On biological transformations. In: Gross WEL, Medawar PB (eds) *Essays on growth and form presented to D’A. W. Thompson*. Oxford University Press, Oxford, pp 95–120