Deep homology in the age of next-generation sequencing

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Summary

The principle of homology is central to conceptualizing the comparative aspects of morphological evolution. The distinctions between homologous or non-homologous structures have become blurred, however, as modern evolutionary developmental biology (evo-devo) has shown that novel features often result from modification of pre-existing developmental modules, rather than arising completely *de novo*. With this realization in mind, the term “deep homology” was coined, in recognition of the remarkably conserved gene expression during the development of certain animal structures that would not be considered homologous by previous strict definitions. At its core, it can help to formulate an understanding of deeper layers of ontogenetic conservation for anatomical features that lack any clear phylogenetic continuity. Here, we review deep homology and related concepts in the context of a gene expression-based homology discussion. We then focus on how these conceptual frameworks have profited from the recent rise of high-throughput next-generation sequencing. These techniques have greatly expanded the range of organisms amenable to such studies. Moreover, they helped to elevate the traditional gene-by-gene comparison to a transcriptome-wide level. We will end with an outlook on the next challenges in the field and how technological advances might provide exciting new strategies to tackle these questions.

1. Introduction

Understanding the origins of the vast array of morphological features displayed by creatures in the natural world is a core problem in evolutionary biology. Much of the variety we can observe in living species arises through changes to structures present in ancestral species, a process neatly explained by Darwins' concept of descent with modification. It has been more problematic to explain the appearance of seemingly novel anatomic structures. The principle of homology was developed to distinguish between these situations, referring to the existence of shared ancestry between a pair of structures. However, how to best define homology has been contentious [1,2]. As others have previously pointed out, this has to some extent been a problem of the hierarchical level at which homology is considered [3-5]. For example, while all vertebrate forelimbs can be considered homologous as structural units, this structural entity has been modified evolutionarily to various different 'character states', facilitating distinct, yet in some cases analogous modes of locomotion, such as e.g. wings used for flying. The skeletal elements inside the wings of birds, bats or pterodactyls clearly imply a common basic pattern. This can be traced back to a common forelimb ground state that has subsequently been modified in each of the three lineages. Their inferred historical continuity therefore allows us to identify them as homologous as forelimbs. However, although all three are used for flying, they have to be considered functionally analogous as wings, since flight has evolved independently in these clades. The structures are thus homologous at the level of forelimbs but not at the level of wings. I.e., whether traits are classified as homologous or not becomes a hierarchy issue, dependent on the level at which homology is being discussed.

Conflicting semantics aside, at the core of the homology concept is the notion of "sameness" and some sort of "historical continuity." This has also led to the inclusion of the level of genes and proteins within the general realm of homology,

followed by the rise of modern molecular biology [6]. A similar case can be made at yet another level of organizational hierarchy, when considering homology amongst different cell types, rather than entire organs. Again, phylogenetic as well as functional or structural criteria have historically been used to assess homology of these entities [7].

In this context, it is perhaps best to frame a discussion of homology around its original definition. As first defined by Sir Richard Owen, homology refers to “the same organ in different animals under every variety of form and function” [8]. His pre-Darwinian definition of homology was thus based very much on underlying structural similarities that could not simply be explained by functional constraints. With the advent of Darwinism, Owen’s concept of homology became linked to the historical continuity of morphological structures (e.g. E.R. Lankester [9]), thus implying descent with modification from an “archetype” of a common ancestor. This type of homology is therefore intricately linked to phylogenetics and systematics, and how certain morphological characters are distributed over the evolutionary tree. It is often referred to as “historical homology” [5].

With the advent of comparative “evo-devo” biology, and a better understanding of the molecular mechanisms driving embryogenesis in classical model organisms, some have argued that considering ontogeny would sometimes be more informative when evaluating homology [10-12]. In particular, rather than following strict genealogical criteria, mechanistic constraints on the development of morphological features should be taken into account. This was largely inspired by the realization that distantly related species utilize a remarkably conserved gene toolkit during embryogenesis, for example for patterning their main body axes [13,14]. For the evo-devo field, this represented a unique opportunity to reframe the homology concept with a particular focus on developmental constraints. One of the prevailing criticisms of historical homology to this point was that anatomical structures, unlike genes, are not directly copied and inherited, but rather are

generated *de novo* during the embryonic development of each generation [15,16]. As individualized parts of a species' phenotype, these structures can change independently due to adaptive processes. Their development, however, is usually internally constrained by the underlying genetic blueprint as well as morphogenetic processes that can be inherently self-regulatory. There, the "biological homology" concept, as it has been referred to, is defined for anatomical structures that have a shared set of developmental constraints for their individualization [15,17]. As such, this form of homology mainly concerns phenotypes that result from complex regulatory interactions, rather than single-gene traits, such as color variants [18,19]. Moreover, it also insinuates a certain degree of modularity, within which self-contained developmental units can undergo evolutionary change, for example at the level of gene regulation.

2. Homology and gene expression - kernels, character identity networks and deep homology

Consideration of hierarchy in the regulation of genes led to the formulation of the "kernel" concept [20]. Fundamental to this approach, the genome is treated as a regulatory blueprint for embryogenesis, layered in both its functional impact on developmental patterning as well as its evolutionary age (with newer modules superimposed upon older ones). At the top of this regulatory hierarchy lie the so-called kernels, sub-units of gene regulatory networks (GRN) that are central to bodyplan patterning, exhibit deep evolutionary conservation and are refractory to regulatory rewiring. Their static behavior, and importance in defining fundamental embryonic patterns, has been argued to underlie the stability exhibited by different animal bodyplans since the Cambrian explosion [21]. At the base of this GRN hierarchy, so-called gene differentiation batteries direct terminal cell or organ differentiation. These are assemblies of effector genes that underlie functional specification, but lack regulatory information. Their deployment is controlled by so-called intermediate plug-ins, or I/O-switches, that transmit

kernel-contained patterning information down to its final differentiation output. From an evolutionary perspective, regulatory modifications are most likely to occur at this “plug-in” level, to ultimately result in structural novelty. Therefore, a hierarchy of regulatory homology can, to some extent, be inferred from the re-deployment of these switches. There are a number of examples of extraordinarily deep evolutionary GRN conservation that display “kernel”-like behavior: these include endomesoderm specification in echinoderms, hindbrain regionalization in chordates or, most spectacularly, the specification of heart development in clades as distant as arthopods and chordates (see references in [20]). Albeit structurally very distinct, a core set of regulatory interactions is equally important in directing heart development in these two distantly related phyla. Sub-circuit formations as well as downstream effector genes are remarkably conserved, implying a common regulatory blueprint that traces back to a primitive circulatory organ at the base of the Bilateria [20,22,23] (Fig. 1a). However, one of the potential shortcomings of the kernel concept in assessing homology, is its focus on transcription factors and their associated *cis*-regulatory sequences, with much less attention given to signaling pathways. Moreover, central to its definition is the deep evolutionary conservation that GRN parts have to display, in order to be considered a kernel.

A slightly more flexible approach is provided by the character identity network (ChIN) concept [24]. Again, historical continuity of character-defining gene regulatory networks is key to its definition. However, and unlike kernels, these do not have to be evolutionary ancient (i.e. phylum or sub-phylum level for kernels, down to species level for ChINs). Central to the applicability of ChINs in discussing homology is the inherent modularity of developmental systems. Different body parts and organs develop, and are patterned, in a semi-autonomous fashion, a fact known since the early days of experimental embryology [25]. The division into discrete developmental sub-systems allows for their individualized evolution, yet shared ChINs underlying their formation in

different organisms helps us to identify the resulting anatomical features as homologous. By introducing ChINs, a historical continuity is inferred by means of their repetitive re-deployment during the embryogenesis of each following generation, while modifying their output results in varying character states across species [24]. Such reasoning can help to disentangle conflicting results coming from various lines of research, such as embryology *versus* paleontology, when trying to establish homologies between morphological characters. This has been demonstrated in the assessment of digit identity in extant avian wings, where a pentadacyl ground state has been reduced to a three-digit formula. While the most anterior wing digit develops from an embryonic position usually associated with digit II, the paleontological record of theropod digit loss suggests the remaining most anterior digit to be digit I [26,27; see also Cooper & Towers, this issue]. Using comparative RNA-sequencing revealed a strong transcriptional signature uniting the most anterior digits (MAD) of the forelimbs and hindlimbs (Fig. 1b). This implies, at the ChIN level, that the most anterior digit of the avian wing shares a common developmental blueprint with its hindlimb counterpart, and hence the forelimb digit formula should be considered I, II, III, regardless of the anatomical position from which they develop [28,29]. These findings were recently corroborated by studies using gene expression patterns to identify homeotic identities of digit primordia in species that actually have lost digit I [30]. In the meantime, ChIN-based approaches of homology have also been expanded to address the evolution of novel cell types [31].

Both kernel and ChIN arguments for homology are continuous, at least in part, with the concept of “deep homology” [32], while also mechanistically refining it. The term deep homology was originally coined to describe the repeated use of highly conserved genetic circuits in the development of anatomical features that do not share homology in a strict historical or developmental sense. For example, although evolutionary separated since the Cambrian, and morphologically and developmentally highly divergent, the development of insect and vertebrate

appendages share striking similarities in specifying their embryonic axes [32,33] (Fig. 1c). Such degree of conservation in developmental patterning, it was argued, would be most parsimoniously explained by a common ancestor that possessed a primitive body-wall outgrowth program [33,34]. The genetic blueprint for this outgrowth program would then have been co-opted and re-deployed for the independent evolution of body appendages in different animal phyla, as well as being reactivated in different anatomical locations within a developing organism to give rise to serial homologs (e.g. tetrapod fore- and hindlimbs). Modification of this deeply conserved genetic program would thus represent the molecular framework within which the morphological diversifications of animal appendages would have to be considered. Their development thus shares historical continuity at the level of the gene regulatory circuits, and is said to display deep homology (Fig. 1c). Likewise, the use of similar cellular building blocks, such as the deployment of an ancestral photosensitive cell in the generation of animal eyes, led to the dependence on homologous gene regulatory interactions and thus resulted in morphological structures that display deep homology [33]. As such, the structural entity itself (in this example, the eye) is termed ‘deeply homologous’, based on the re-deployment of genetic circuitries and/or developmental mechanism that themselves display true homology, i.e. a common historical origin. Ultimately, determining whether the expression of similar genetic cassettes underlying the development of two historically non-homologous structures represents deep homology or convergent, and potentially coincidental, deployment of related genes in two independent settings, depends on assessing the number of genes used in common and, more importantly, their epistatic relationships. Accordingly, the ability to confidently identify deep homology is already enhanced through the use of more comprehensive approaches to determine gene expression similarities.

3. Deep homology goes global – from transcripts to transcriptomes

The obvious advantage of assessing deep homology at a genome-wide level is strength in numbers, each probed gene adding robustness to formulate meaningful predictions. The emergence of hybridization-based array-techniques first opened the possibility of measuring the expression of a large set of genes in a single experiment. Moreover, compared to *in situ* hybridization techniques, array-based experiments also yield information about quantitative differences in gene expression (although often at the expense of spatial information). Indeed, several pioneering evo-devo studies exploited the potential of microarrays for comparative gene expression studies across multiple species. Variation in hybridization efficiencies, due to species-inherent sequence differences in the targeted parts of mRNAs, and other technical issues made direct interpretations difficult. A number of normalization and analytics procedures, helped to bypass these problems [35,36]. However, most of these shortcomings can now easily be avoided, thanks to the development of high-throughput next-generation sequencing (NGS) techniques [37]. The advantages of NGS over array-based methods of gene expression measurements are manifold. Massively paralleled sequencing of cDNAs, known as RNA-seq, now allows for genome-wide interrogation of expression status, as well as the global description of transcript structures [38]. RNA-seq experiments also yield a better quantitative assessment of gene expression differences, thanks to a higher dynamic range as compared to hybridization-based methods [39]. Moreover, RNA-seq opens the possibility to compare a broad range of species that traditionally would have been considered “non-model organism”, including those previously excluded by a lack of an available genome sequence [40,41]. As a consequence, comparative large-scale transcriptome studies, covering different species and tissue types, have emerged in recent years as a powerful approach to address questions of morphological evolution and homology [42]. These approaches have been utilized across several taxonomic levels, from comparing transcriptomes of closely related species in a given genus, to spanning the entire metazoan kingdom [43-45]. Such studies are a powerful proof of how one can now go far beyond the

standard realm of model organisms, eliminating the need to focus on just a select few taxa [46,47]. Moreover, NGS enables for the global assessment of quantitative gene expression differences, a parameter known to influence a variety of phenotypes [48-50]. There are, however, also analytical challenges, in normalizing and comparing these data-sets, especially when working with evolutionary distant organisms [50-53]. While many of the early trans-species RNA-seq studies have mainly focused on adult tissue samples, increasingly this approach is being expanded to developmental time-series, across species and embryonic stages. Heterochrony, i.e. species-specific differences in the relative order certain morphological structures appear during embryogenesis, may potentially confound such transcriptome comparisons [53,54]. However, focusing on the temporal dynamics of transcriptome evolution holds the greatest potential to inform us about putative developmental homologies of different anatomical features [28,51]. NGS-based global and quantitative assessment of transcriptome dynamics, across a range of species and developmental time-points, is therefore likely to reveal more cases of deep homology in the near future.

Combining next-generation transcriptomic analyses with comparative embryological and functional experiments can inform us about the developmental mechanisms that lead to the appearance of deep homology. For example, such was the motivation in studying the evolution of genital bud transcriptomes in amniotes, an embryonic structure that was known to share substantial gene expression similarities with developing limbs [55,56]. Comparative lineage tracing experiments uncovered considerable variation in the embryonic origins of amniote external genitalia [51]. In the case of squamates (lizards and snakes), early limb and genital buds share a common cellular source that results in high transcriptome similarity between the two tissues (Fig. 2a,c). In its most radical interpretation, squamate external genitalia development could therefore be considered serially homologous to hindlimbs. In contrast, in mammals, the genital

bud originates from the tailbud mesenchyme. As the genitalia of squamates and amniotes form from distinct embryonic tissues, they are not historically homologous. How then did these critical, functionally analogous, structures evolve? The answer comes from the realization that this shift in tissue of origin in the two clades is accompanied by a relative repositioning of the genitalia-inducing signaling center, the embryonic cloaca. Once positioned next to a different tissue source, the cloaca elicited similar downstream transcriptional responses in a different mesenchymal cell population. This indicates that the mammalian tail bud is competent to respond in a manner reminiscent of the squamate lateral plate tissue. Given the lack of a well-documented fossil record for developing amniote external genitalia, it is difficult to argue for either squamate or mammalian situation to be the ancestral condition. However, the still recognizable similarities in gene regulatory programs underlying both amniote limb and genital development suggest an ancestral limb-like embryonic origin for amniote external genitalia as the more likely scenario [51]. In this manner, mammalian genitalia development maintained a limb-like regulatory blueprint similar to the squamates, despite its now distinct embryonic origin (Fig. 2b,c). Thanks to this regulatory co-option, due to the common inductive signals originating from the cloaca, and a shared transcriptional response to it, squamate and mammalian external genitalia display a deep homology in their development [51]. These transcriptional similarities are likely to be mechanistically linked at the *cis*-regulatory level, as suggested by comparative enhancer studies [57]. Intriguingly, a similar co-option of an ancestral gene regulatory network seems to have paved the way for the emergence of morphological novelties in the external genitalia of several members of the *Drosophila melanogaster* clade [58]. Although shared developmental trajectories seem unlikely in this case of regulatory co-option, the *Drosophila* example further underscores the power of re-utilizing pre-existing genetic cassettes, especially in rapidly evolving structures such as external genitalia [59,60].

4. Homology assessment - gene expression and regulatory strategies

A pressing question when evaluating homology based on gene expression similarities is whether the observed common patterns of gene activity are caused by the same underlying regulatory strategies, or are rather the result of convergent evolution. Even though the evolution of gene expression seems much more constrained than originally assumed [61], it still displays a considerable amount of plasticity and can rapidly diverge in response to altered selective pressures [62]. Consequently, it has been suggested that studying underlying regulatory strategies, rather than gene expression patterns alone, is more informative when evaluating potential synapomorphies of anatomical structures [63]. Likewise, a much stronger argument for deep homology can be made if the *cis*-regulatory circuitries causing the observed gene expression similarities are conserved as well. In the case of deep homology, however, pre-existing gene regulatory modules that can easily be co-opted by the gain of expression of single “gatekeeper” transcription factors also need to be assessed. Probing gene expression at a transcriptome-wide level, thanks to NGS approaches, has opened new experimental avenues to address these questions. By restricting the analysis of whole-transcriptome data to functional sub-classes of genes, GRN inputs can be approximated from the expression status of signaling molecules and/or transcription factors [28,51]. Given the propensity of the latter to bind to DNA, some similarity in GRN regulatory input can be expected in tissue types that show a high degree of correlation for their transcription factor expression profiles. Eventually, though, dedicated experimental data is required to arrive at a more molecular understanding of the regulatory mechanism causing any observed gene expression similarities. Again, several NGS-based approaches pave the way for such epigenomic annotation of regulatory elements, in a variety of tissue types and species. These include chromatin immunoprecipitation followed by sequencing (ChIP-seq) of histone modifications associated with enhancer function [64], as well as methods to assess local and/or global

chromatin structure [65]. Of particular interest are transcriptional enhancers, *cis*-regulatory elements that can activate target genes over hundreds of kilobases away. These elements can function in a highly tissue-specific and temporally resolved fashion, making them potent drivers of morphological evolution. At the same time, by changing only the regulation of a gene, rather than its coding sequence, pleiotropic effects can be avoided [66]. Hence, potential evolutionary modifications of enhancer activities have been the subject of intense investigation in the field of regulatory evolution. For example, using ChIP-seq for histone H3 Lysine 27 acetylation (H3K27Ac), an active enhancer mark, the evolutionary and developmental dynamics of enhancer activities have been mapped in various different organs and across several taxonomic ranks [67-69]. Ideally, such enhancer activity maps will be complemented by the binding profiles of transcription factors known to be important for the development or function of the tissues in question [70,71]. At the level of chromatin structure, local DNA accessibility as well as higher-order folding can inform us of potential enhancer sequences and regulatory strategies at a given locus. DNA accessibility, as defined by nucleosome-sparse regions, can be probed with a variety of NGS-based assays, such as DnaseI-, FAIRE- or ATAC-seq [72-74]. As a result, potential transcription factor binding sites can be defined bioinformatically, using motif search algorithms. Advantages of these techniques include the considerably smaller cell numbers that they require as input, compared to transcription factor ChIP-seq experiments. These methods have been successfully employed to compare global DNA accessibility maps for different species and organs, in adult tissues or across developmental time-points [75,76].

The importance of three-dimensional folding of the DNA strand itself for correct execution of gene regulatory programs, is also becoming increasingly appreciated. Such DNA looping can range from enhancer-promoter interactions over several hundred kilobases, all the way to supra-structural territories on the mega-base scale called topological associated domains, or TADs [77]. Inside of

these TADs regulatory “promiscuity” of enhancer-promoter contacts can occur to some degree. However, regulatory interactions across TAD boundaries seem inhibited, underlining their importance in maintaining proper genome organization and gene regulation [78,79]. Cross-species comparisons of TADs have revealed booth deeply conserved, as well as step-wise evolutionary dynamic assembly of such regulatory domains [80,81]. TAD conservation could thus reveal deep homology of entire regulatory landscapes, just as deep conservation of enhancer activities might inform us about evolutionary relationships of different morphological structures that they help to pattern [75]. Clearly, however, the most instructive insights at the gene regulatory level would come from the integration of different NGS technologies to first describe the regulatory architecture at loci of interest, and then test them in reciprocal, cross-species enhancer reporter experiments [82-84].

5. Concluding remarks and outlook

NGS has already significantly advanced our ability to address questions of homology, both experimentally as well as conceptually, and will likely continue to do so. Overall, it’s fair to assume that the trend of incorporating technological advances from different fields of biology, and indeed the natural sciences in general, will continue in the field of evolutionary and developmental biology, to make it a more inclusive science [85,86]. Already, the next revolution in transcriptome sequencing is on its way, with the recent ability to use single cells as input material in high-throughput experiments [87]. For the field of homology assessment, this holds special importance at several levels. Firstly, it will allow us to study the molecular mechanisms driving cell type differentiation at the relevant resolution, and learn about the evolution and putative homologous counterparts in different species, using comparative approaches [7]. Moreover, at the organ-level, it enables for the cellular deconstruction of morphological character development, which will help to resolve confounding organ cell

heterogeneity that might differ from species to species [53]. Spatial transcriptome information lost during organ dissociation can then be recovered *in silico*, down to cellular resolution, by re-mapping single-cell RNA-seq data onto grids of known marker gene *in situ* hybridization patterns [88,89]. Such high-throughput *in vivo* approaches will benefit from complementary cell culture experiments, where the controlled parameters of an *in vitro* environment can be exploited. Various cell and organ development pathways can already be re-capitulated *in vitro*, thereby helping to define their minimal differentiation requirements [90,91]. Expanding such rationale to a comparative level, between cell lines and organoids from different organisms, will allow for a reductionist approach to character identity development. Moreover, cell culture-based assays for large-scale comparative studies of epigenomic states and enhancer activities can function as invaluable proxies to delineate regulatory logic across species boundaries [92,93].

A more comprehensive inclusion of published data-sets, for example through large-scale consortia, has the potential to increase the predictive power of more targeted comparative studies on gene regulatory strategies, across, species, organs and developmental time-points [94-96]. The creation of public repositories will certainly help this endeavor (see resources in [97]), especially when created with an explicit evo-devo approach in mind [98]. Undoubtedly, though, this will present new challenges in terms of data analysis and integration. Dedicated bioinformatics efforts will thus be required, to tackle the problems inherent to cross-species comparisons, especially when considering large evolutionary distances [50,52,99]. Ideally, the future of evo-devo will gravitate toward such an integrative approach, where comparative embryology, transcriptomics and epigenomics, bioinformatics, as well as functional *in vivo* and *in vitro* work will be incorporated to study these questions at the systems level [85,86,100]. Including comparative embryological and gene regulatory data at the micro-evolutionary level, in addition to the macro-evolutionary perspective of classical evo-devo, will

allow for the integration of ecological constraints, as well as population genetics data [101].

Ultimately, a definite answer to address questions of homology amongst morphological features will only result from a highly integrative approach, taking into account a well-curated paleontological record, studies on the developmental dynamics underlying the ontogeny of these structures in extant species, as well as a molecular and genetic understanding of their underlying mechanism. At the very least, exploiting the power of NGS-technologies to investigate molecular mechanisms during development might help to disentangle conflicting results from the two former fields, i.e. paleontology and embryology (see e.g. digit identities in bird wings [29]) Moreover, building on such qualitative and quantitative molecular data during embryogenesis, modeling approaches could then move the field forward and help to consider deep homology beyond a simple comparative description of gene expression. In particular, a combination of experimental data and modeling approaches might help to delineate a 'configuration space', in which different individualized developmental and gene regulatory systems were free to evolve [86,102]. Following such synthesis, evo-devo research in general might indeed gain certain predictive powers about possible evolutionary trajectories, by defining the range of possible ontogenetic roadmaps [46,47,86]. The concept of deep homology, with its emphasis on how gene transcription can be co-opted in an evolutionary novel context, is likely to prove particularly powerful in delineating the gene regulatory dimension of such configuration spaces.

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Authors' contribution

P.Tschopp and C.J. Tabin wrote the paper.

Competing Interests

The authors declare no competing interests

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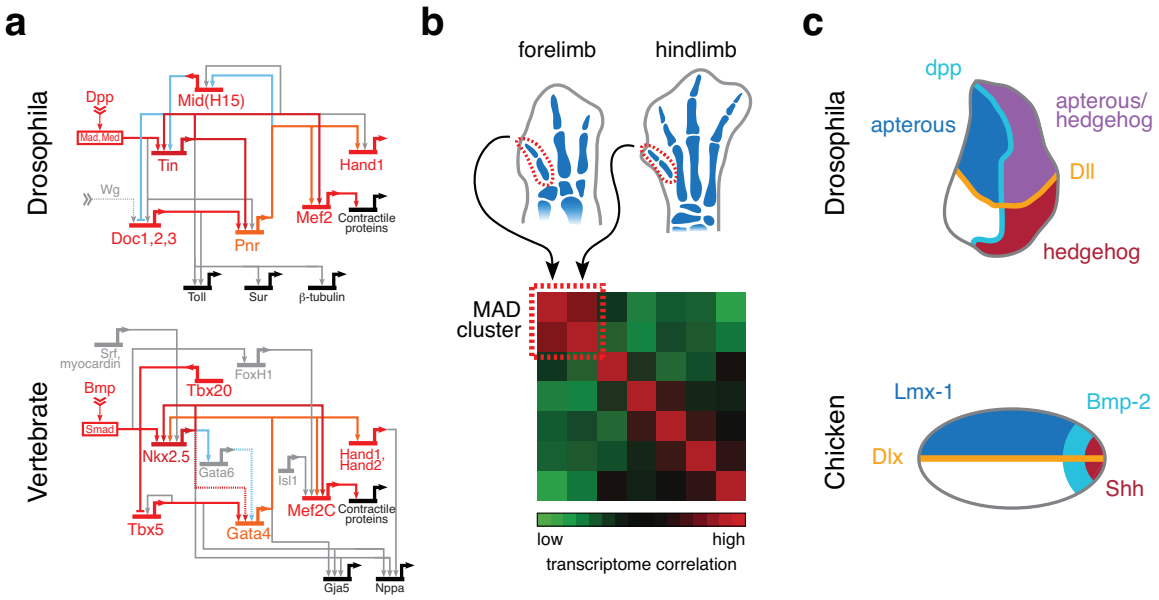
Figure captions

Figure 1

Gene expression-based homology assessment. a) The kernel concept. Deep evolutionary conservation of the core gene regulatory network underlying bilaterian heart development. Parts of the gene regulatory diagrams for *Drosophila* (top) and mouse (bottom) heart development are depicted. Genes and regulatory interactions conserved between the two distantly related species, i.e. the kernel, are highlighted in red. Interactions conserved *via* intermediate relays are highlighted in blue. Modified after [20,23]. b) The character identity network concept. During their development, the most anterior digits (MAD) of both chicken fore- and hindlimbs cluster together based on high transcriptome correlation, identifying them as homologous with regards to their underlying gene regulatory signature. Modified after [28]. c) The deep homology concept. Molecular patterning of developing bilaterian body appendages, in the *Drosophila* wing disc (top) and the chicken limb bud (bottom). Although these structures do not share any historical homology, they have their embryonic axes defined by remarkably conserved genetic circuitries, and hence display deep homology. Modified after [32].

Figure 2

Deep homology of amniote external genitalia. a) Stylized scheme of external genitalia development in a squamate, the anole lizard. For hemipenis bud initiation, the cloacal signaling center recruits cells with a hindlimb-like developmental origin (black arrow). b) In mammals, by contrast, the cloaca is positioned closer to the posterior end, and attracts cells from the tailbud for genital tubercle outgrowth (black arrow). c) Representative principal component analysis (PCA) depiction of limb bud and genitalia transcriptomes in anole lizard and mouse. While PC1 is dominated by a species signal, PC2 reveals tissue-type similarities between the two species, anoles (triangles) and mice (circles). Anole limb and genitalia transcriptomes show a higher degree of relatedness than in mouse, due to a shared embryonic origin. The development of both lizard and mouse genitalia, however, still shows high PC2 tissue-type similarity, and hence displays deep homology at the transcriptome level. lb, hindlimb; hp, hemipenis; gt, genital tubercle; cl, cloaca. Modified after [51].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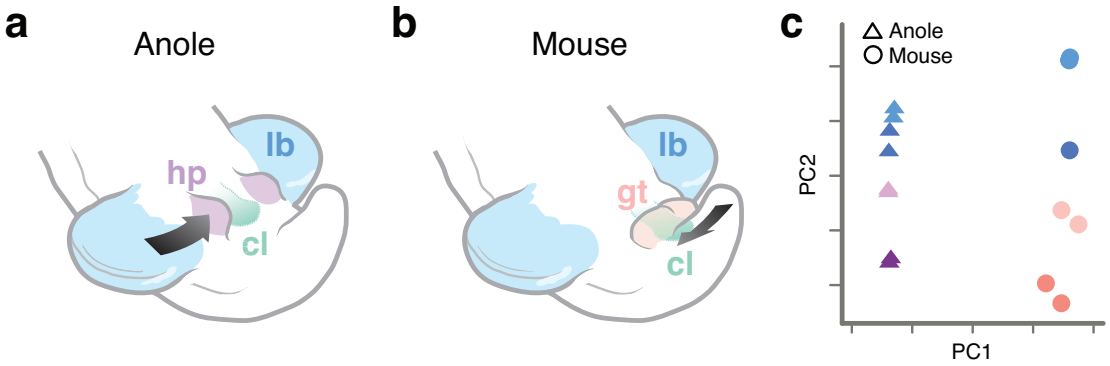
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