OPINION

The developmental genetics of homology

Günter P. Wagner

Abstract | Homology is an essential idea of biology, referring to the historical continuity of characters, but it is also conceptually highly elusive. The main difficulty is the apparently loose relationship between morphological characters and their genetic basis. Here I propose that it is the historical continuity of gene regulatory networks rather than the expression of individual homologous genes that underlies the homology of morphological characters. These networks, here referred to as 'character identity networks', enable the execution of a character-specific developmental programme.

Characters found in different species are homologous if they are derived from the same character in their most recent common ancestor (MRCA), regardless of similarity in form or function (FIG. 1). Whenever we compare two or more species, or use a model organism to learn about the molecular basis of human disease, we implicitly need to identify corresponding body parts and functional systems; that is, we make assessments about homology. Intuitively, one would expect that the historical continuity of morphological characters is underpinned by the continuity of the genes that govern the development of these characters. However, things are not that simple: one of the most important results of the past 15 years of molecular developmental genetics is the realization that homologous characters can have different genetic and developmental bases[1–3]. This seems paradoxical, because the historical continuity of morphological characters implies continuity of the (genetic) information about the characters[4]. But where else should we look for this continuity, other than in the genes? Here I review some of the conceptual issues, and the experimental results that suggest a solution to this conundrum. I argue that the continuity of morphological characters could be underwritten by homologous regulatory networks of co-adapted transcription factor genes, whereas other aspects of their development can be variable. These networks control the execution of character-specific developmental programmes, which allow for quasi-independent variation of characters[5] with respect to other parts of the body.

What does homology mean?

In the eighteenth and nineteenth centuries, it became clear that the similarities and differences among organisms are not random, but follow patterns that call for an explanation. Most intriguing are the similarities among some body parts that cannot be explained by shared functional necessity. For example, the tetrapod limb shows a highly stereotypical pattern of bony elements (FIG. 2), regardless of whether it is used for running, flying, swimming or grasping. This pattern was conceptualized by Richard Owen as homology[6], paving the way for the theory of evolutionary change. Even today, the existence of homologous body parts in different animals and plants is cited as standard evidence for this process[7].

The easy case: homology of genes. Since the time of Owen and Darwin, the idea of homology has been extended to other biological entities, such as genes, nucleotides, physiological processes and behavioural patterns[7,8]. What it means to speak of homologous genes is well understood[9], and I recapitulate these ideas here because they can guide us in a similar understanding of homology among morphological structures. Two genes are homologous as long as they are derived from the same gene in a common ancestor, regardless of whether they have the same function and regardless of the extent of similarity in their nucleotide sequences. The gene retains its identity despite evolutionary change in its function and sequence, as long as all changes result from mutations at the same genomic locus. The basis of gene identity is the historical continuity of the locus undergoing evolutionary change. Of course, things become more complicated with gene duplications and loss or fusion of parts of genes (for example, exon shuffling) to form new genes, and when extensive sequence divergence erases the evidence of shared ancestry. The mode of evolution that preserves the historical identity of a gene is the replacement of alleles at the same genomic locus.

Homology of morphological characters: what does 'sameness' mean? The homology of morphological characters is also a case of historical continuity in the face of descent with modification. In a population, a character exists in different states of size, shape or colour. Evolutionary change usually proceeds by changing the frequency of these character states in the population, eventually leading to the replacement of the ancestral character state by a derived character state (FIG. 1). Sameness, then, by the definition of homology, does not refer to similarity of structure or function as such, but to historical continuity through inheritance with modification. In other words, the homology concept can be applied to anything that forms a lineage[10–13]. Of course, things become more complicated when new characters arise (novelties) or characters duplicate like genes (for example, teeth or fins).

At the formal phenomenological level, a morphological character corresponds to a genomic locus, and a character state to an allele[14] (BOX 1). Hence, in a more technical sense, a character is a unit of evolutionary change at the phenotypic or morphological level in the same way that a gene is the unit of evolutionary change at the genetic level[15].

Developmental genetics and homology?

The semi-conservative mode by which DNA replicates ensures that genes directly give rise to copies of themselves, and is therefore the mechanistic basis for their historical continuity. In the case of morphological characters, however, the situation is more complicated, because morphological characters and even cell types do not usually directly spawn copies of themselves between generations, but are recreated in each generation from a single cell, the zygote[16]. The recreation of a character is controlled by developmental genes, so it is tempting to speculate that the continuity of morphological characters can be explained by the continuity of genetic information. Since the beginning of experimental developmental biology in the early twentieth century, the emerging picture has been disappointing and confusing[2,17–19]. For instance, there is no question that body segments in all orders of insects are homologous and derived from a single common ancestor — there is not a single lineage of organisms more closely related to an insect group than to other animals that is not segmented and, furthermore, the insects are nested in an even larger clade, the arthropods, which consists exclusively of segmented animals. Yet some genes that are essential for segmentation in *Drosophila melanogaster*[20], for example, the pair-rule genes *fushi tarazu* (*ftz*) and *even skipped* (*eve*), do not have pair-rule function in the grasshopper *Schistocerca americana*, but are instead expressed in the developing CNS[21,22]. Clearly, the way in which segments are formed in development has changed since the MRCA of crown-group insects.

A solution to this conundrum can be found in the fact that developmental variation in homologous characters is not randomly distributed, but affects some aspects of development more than others. For example, in *D. melanogaster*, segmentation proceeds through three stages that are controlled by particular genes: gap genes, which determine larger body regions, the pair-rule genes, which divide the embryo into stripes of alternating half segments, and the segment-polarity genes, which activate the actual morphogenetic process of segment formation[2]. Surprisingly, the most extensive interspecific variation has been found in the higher levels of the segmentation hierarchy, namely the gap genes and the pair-rule genes[2,23]. Examples are the pair-rule genes *ftz* and *eve*, mentioned above, and the gap gene *bicoid* (*bcd*), which exists only in the higher Diptera, not even in the dipteran mosquito *Anopheles*. By contrast, the segment-polarity gene network, which includes the interaction of *engrailed* (*en*) and *wingless* (*wg*), seems to be invariant, at least among insects[2]. This suggests that the genetic regulatory network (GRN) that controls the execution of the segment-specific morphogenetic processes is less variable than the upstream processes that activate it.

If the pattern that is suggested by the data on insect segmentation can be generalized, it seems that the most conservative parts of the developmental process are the GRNs that control the developmental programme that specifies the identity of the character; that is, the character identity network (ChIN). For example, individual cell types are determined by a characteristic set of regulatory genes over vast evolutionary distances[24–27]. Another example is the genetic network for the endomesoderm that starfish and sea urchins share[28]. By contrast, other aspects of development, from early patterning to the execution of the developmental programme, are more variable[2].

Here I review evidence that shows that these networks determine character identity rather than character state, that non-homologous morphological characters are determined by non-homologous ChINs, and that the genes participating in a ChIN are co-adapted for their task; that is, they are functionally non-equivalent to orthologues in species that do not have the character, and to paralogues that do not participate in the development of that character.

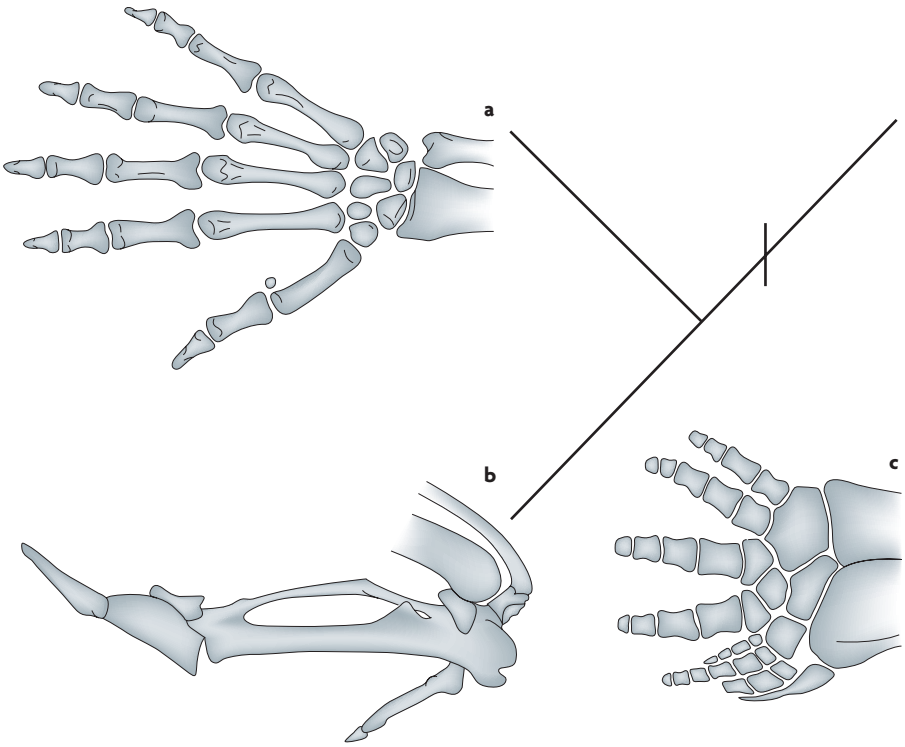

Figure 1 | **Homology of morphological characters.** The hand of a human (part **a**) differs greatly in terms of detailed structure and function from the wing of a bird (part **b**), but both are considered homologous because they arose from a corresponding character in the tetrapod common ancestor(part **c**) through descent with modification. This figure illustrates that morphological characters form lineages of descent in the same way that genes form unique lineages of descent, as long as they are not duplicated. Homology is the historical continuity of characters in multiple lineages despite variations in their character state.

ChIN genes determine character identity

The idea that the genes that control character identity are distinct from the genes that determine the special shape and state of a character has been well documented in the case of Ultrabithorax (*Ubx*) function in insect wing development. Ancestrally, pterygote (or winged) insects have two pairs of proper wings associated with T2 and T3 (the second and third segments in the thorax), as seen, for instance, in honey bees, grasshoppers and most spectacularly in butterflies (FIG. 3a). Dipterans have only one pair of wings, which is localized on T2; that is, they are forewings. A homologue of the hind wing lies on T3 but does not take

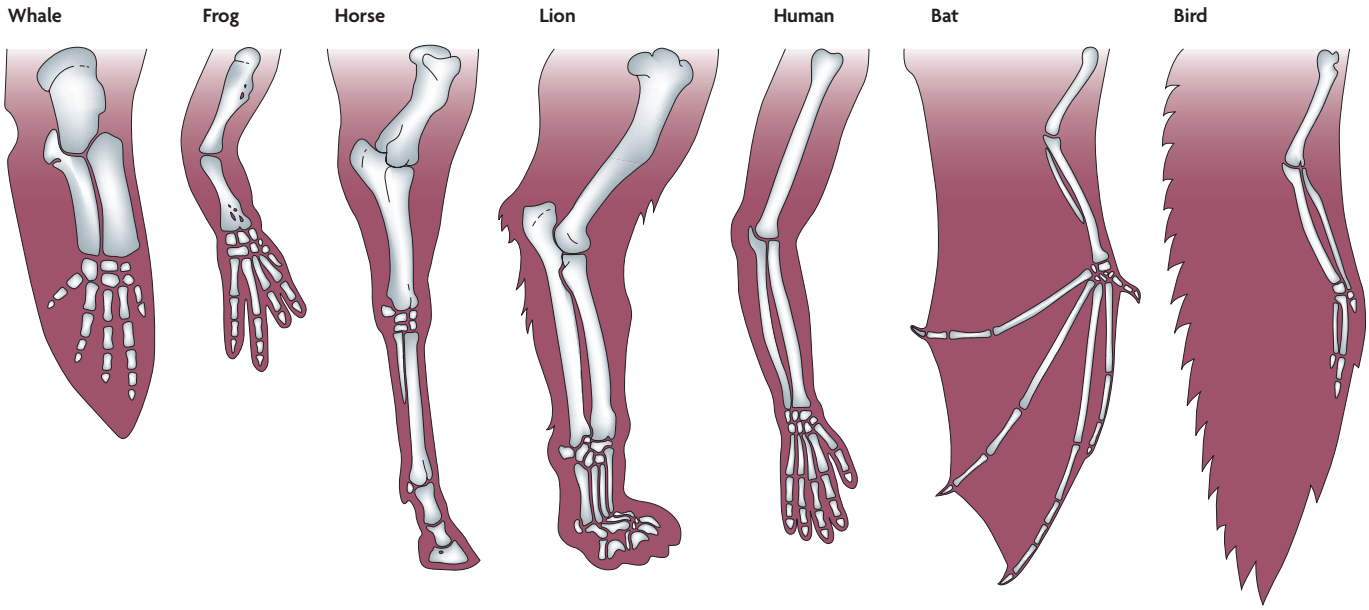

Figure 2 | **Homologous characters can have different shapes and functions.** Forelimbs of seven tetrapod species exemplify the fact that corresponding body parts have a similar design but can serve different functions, from swimming to flying. Hence, functional necessity cannot explain the similarity of the basic construction of homologous characters. This remains one of the standard arguments in favour of evolution; that is, that species derive from common ancestors by a process of descent with modification.

the shape of a wing blade. Instead, it is a small, club-like appendage called the haltere (FIG. 3b). Beetles also have only one pair of wing blades, but as they are associated with T3 they are hind wings. The forewings in beetles have been transformed into a pair of highly scleratized structures, called elytra, that function as protective covers (FIG. 3c). So, butterflies, flies and beetles all have two pairs of dorsal appendages that are homologous, because they are nested within a larger clade of winged insects, almost all of which have two pairs of wings on their T2 and T3: the forewings, which are flying organs in flies and butterflies but protective organs in beetles, and the hind wings, which form functional wing blades in butterflies and beetles but are sensory organs (halteres) in dipteran insects.

Hence, morphologically, we can distinguish between two kinds of entities. On the one hand, there are two character identities: forewings and hind wings. On the other hand, there are various character states that insect wings can assume: the forewing can be a wing blade or an elytra, and the hind wing can be a wing blade or a haltere. Distinguishing between character identities and character states also removes the confusion that is inherent in the character concept[29] between parts, such as wings and legs (character identities), and attributes of parts, such as size, shape and colour (character states).

Experimental evidence shows that this distinction between character identities and character states is the result of different genetic underpinnings. ChIN genes, like *Ubx* and abdominal-A (*abdA*), determine character identity (forewing versus hind wing) across species, regardless of their character state[30]. In *D. melanogaster*, a loss-of-function mutation of *Ubx* leads to the development of a second set of wing blades (FIG. 4a). This does not mean that *Ubx* function is to suppress wing development; Warren and collaborators demonstrated that the four-winged butterfly also expresses *Ubx* in its T3 (REF. 31). So, in general *Ubx* determines hind wing identity, regardless of whether the hind wing is shaped as a wing blade or a haltere, as in dipterans[32]. This was confirmed in the flour beetle *Tribolium castaneum*[33]. As in the butterfly, the beetle expresses *Ubx* in T3, and suppression of *Ubx* function by RNAi leads to a second set of elytra on T3 (FIG 4b). Clearly, *Ubx* does not determine the shape of a wing but determines hind-wing identity "…regardless of form and function"[6,30]. The character state can change in evolution, but it remains under the control of *Ubx*.

The experimental evidence that is cited here pertains to only one gene, *Ubx*, but it is unlikely that *Ubx* is acting alone. One would expect that *Ubx* is part of a small network that also includes *abdA* and other transcription factor genes.

ChINs of non-homologous characters

The GRN underlying eye development is a celebrated example of evolutionary conservation, as it suggests that there is homology between vertebrate and insect eyes[34]. This assertion largely comes from the common role of paired-box gene 6 (*Pax6*) in the two systems, but detailed studies show that the rest of the network is strikingly different (see below). The most parsimonious interpretation is that *Pax6* is part of the ancestral cell-differentiation pathway for photoreceptors and was then separately incorporated into the ChINs for both types of image-forming eyes[28].

Glossary

Orthologue
Two genes are orthologues if their lineages are connected through a speciation event and without a duplication event.

Paralogue
Two genes are paralogues if their lineages are connected through a gene duplication event.

Pro-orthologue
For example, when one species has two copies of a gene, say Ga and Gb, and another species has a single copy G, and the speciation event that separated the species lineages occurred earlier than the gene duplication event, G is the pro-orthologue of Ga and Gb.

Semi-orthologue
For example, if G is the pro-orthologue of Ga and Gb, then both Ga and Gb are the semi-orthologues of G.

Box 1 | **Equivalent terms for genes and morphological characters**

Morphological characters are equivalent to a genetic locus that undergoes evolutionary modification. Different instantiations of a gene are called alleles and different instantiations of a character are called character states. Genes that were inherited from a common ancestor without duplication are orthologues, whereas body parts in two species that were inherited from a common ancestor are homologues. Different instances of a gene caused by gene duplication are called paralogues, and repeated instances of a morphological character in the same organisms are called serial homologues. New genes can arise through gene duplication and divergence or from the fusion of parts of genes. For morphological characters, a new character that creates a new lineage of descent with modifications is called an evolutionary novelty[67]. These can arise in various ways, for instance, by duplication and differentiation, much like for new genes, or by *de novo* origination.

Genetic terms and their equivalents for morphological characters:

Genetic term	Morphological equivalent
Locus	Character
Allele	Character state
Orthology	Special homology
Paralogy	Serial homology
Origin of new genes	Evolutionary novelty

In *D. melanogaster*, the gene *eyeless* (*ey*; with homology to the Pax gene family in vertebrates) is necessary for eye development and is sufficient to induce eyes[35]. In fact, *ey* is part of a small network that includes another transcription factor, *sine oculis* (*so*; with homology to the Six gene family in vertebrates) and two transcriptional cofactors, *eyes absent* (*eya*) and *dachshund* (*dac*), and is activated by a paralogue of *ey* called *twin of eyeless* (*toy*) (FIG. 5a). Surprisingly, it was found that homologous genes, most notably *Pax6*, which is the pro-orthologue[36] of *ey*, are involved in eye development in all animals that have been examined[34,37–39], and can induce ectopic eyes in *D. melanogaster*[35] as well as in *Xenopus laevis*[40].

All the genes in the *D. melanogaster* eye ChIN are members of larger gene families (TABLE 1). In vertebrates, genes from these four gene families (Pax, Six, Eya and Dach) are also involved in the development of several other organs and tissues, such as muscle and ear[41,42].

However, the GRN of eye morphogenesis in vertebrates is not the same as that in *D. melanogaster*[37,41,43] (FIG. 5). For instance, *Pax6/ey* is upregulated by non-homologous genes in the two systems[43]: in insects, *ey* is regulated by *toy*, whereas in vertebrates, the transcription factor gene retinal homeobox (*Rx*; also known as *Rax*) is upstream of *Pax6*. Although a *D. melanogaster* homologue of *Rx* does exist, it is not involved in eye development. Similarly, in vertebrates, *Eya1,2,3* (homologues of *eya*) do not regulate *Dach1*, the homologue of *D. melanogaster dac*.

There are two possible reasons for the dissimilarity between the GRNs of eye development in *D. melanogaster* and vertebrates. It could be that the MRCA of flies and mammals had a GRN that involved members from all four gene families, but this network later changed. Alternatively, it could be that, just as the image-forming eye structures are independently derived, the regulatory interactions among these genes in eye development also evolved independently, and the GRN was not present in the MRCA of flies and mammals.

The gene lineages of the Six gene family suggest that the latter is the case. Although Six and Pax genes tend to be expressed together in various organs and cell types[41,42], the different Six genes that are involved in *D. melanogaster* and vertebrate eye development are not orthologues. According to a phylogenetic analysis of Six genes from animals and unicellular flagellates, *so* of *D. melanogaster* and optix from vertebrates are ancient paralogues predating the origin of multicellular animals[44,45]. Hence, it is most parsimonious to assume that *so* and the optix genes were independently recruited into eye development in the vertebrate and insect lineages just as their morphological eye structures are independently derived. This shows that ChINs differentiate as they assume control of the development of a new character, so that different characters are controlled by non-orthologous sets of genes. Furthermore, the ciliary, vertebrate-type photoreceptor and the rhabdomeric, insect-type photoreceptor coexist in a polychaete worm, *Platynereis dumerilii*, showing that vertebrate and insect eyes derive from 'paralogous' cell populations[25,46] and are therefore likely to be non-homologous.

Figure 3 | **Characters and character states.** The insects shown all have two pairs of dorsal appendages, forewings and hind wings. They can both be wing blades that function in producing lift, as in the case of butterflies (panel **a**), or only one might form a proper wing blade, as in Diptera (panel **b**) or beetles (panel **c**). In the Diptera (flies, mosquitos and so on), the proper wing blade is the forewing, whereas the hind wing is transformed into a club-shaped appendage, termed the haltere (indicated by a black arrow). In beetles, the proper wing blade is the hind wing, whereas the forewing is transformed into a protective cover called the elytra (indicated by a white arrow). Images in panels **b**,**c** courtesy of J. Tanis and A. Andrasi, respectively.

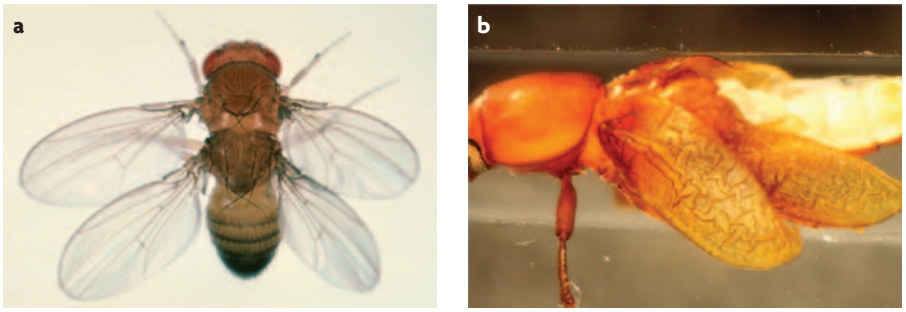

Figure 4 | ***Ubx* determines character identity, not character states.** Loss of *Ultrabithorax* (*Ubx*) function leads to development of a forewing in the position of the hind wing (in body segment T3), regardless of the character state. In *Drosophila melanogaster* (panel **a**), a loss-of-function mutation of *Ubx* leads to two forewings, whereas in *Tribolium castaneum* (panel **b**) the knockdown of *Ubx* leads to a second set of elytra[33]. So, *Ubx* is necessary to determine hind wing identity but does not determine character state[30]. Part **b** reproduced with permission from *Nature* REF. 33 © (2005) Macmillan Publishers Ltd.

Unfortunately, little is known about the genetics of cephalopod eyes[47,48], the third type of independently derived complex light-sensory organs. If the ChIN concept is correct, one would predict that the GRN of cephalopod eye development includes *Pax6* but is otherwise independently derived, and different from both the insect and the vertebrate eye GRN.

Co-adapted transcription factors

What mechanism keeps the GRNs that determine character identity more conserved than other aspects of development? A possible answer to this question derives from the fact that transcription factor proteins do not remain equivalent, but undergo functionally important changes during evolution, particularly with respect to protein–protein interactions. This is a well documented but often overlooked fact of the evolution of gene regulation. It is now well established that the functional specificity of transcription factors is not solely related to DNA binding specificity, which is low, but also involves interactions with other transcription factor proteins[49], and differences in the functional activity of a transcription factor depend on parts of the protein that are not engaged in DNA binding. These functionally important differences in transcription factor proteins might lead to co-adaptation among the transcription factor genes that partake in the same GRNs. Although several lines of evidence have created the misleading impression that transcription factors are functionally invariant[50], there is a growing amount of compelling evidence[51] that fly transcription factor genes like *Ubx*[52–55], *tinman* (*tin*)[56], and *ftz*[57], as well as vertebrate Hox genes[58,59], do not remain functionally equivalent in evolution, and that these differences are not due to changes in DNA binding, but rather to changes in protein–protein interactions among transcription factors.

A particularly well investigated case of functional non-equivalence is that of *Ubx* in *D. melanogaster* compared with its homologues in the velvet worm *Acanthokara kaputensis*[52,53] and the brine shrimp *Artemia franciscana*[55]. Grenier and Carroll[52] compared the *in vivo* activity of *A. kaputensis Ubx* (Onychophora: *O-Ubx*) with that of *D. melanogaster Ubx1a* (*D-Ubx1a*) in a misexpression approach. O-Ubx and D-UBX1A have similar homeodomains (97% identical amino-acid sequence), but their overall similarity is low. O-UBX is only 214 amino acids long, compared with 380 amino acids in D-UBX1A. Like D-UBX1A, O-Ubx expressed in flies can transform antenna towards a leg phenotype and forewings into halteres. More specifically, O-Ubx can repress *Surf wings* (*Srf*) in the wing disc and drive the expression of *decapentaplegic* (*dpp*) in the visceral mesoderm, both of which are specific targets of D-UBX1A. But other typical effects of D-Ubx cannot be reproduced by O-Ubx, such as repression of *Distal-less* (*Dll*) in the leg rudiments. These D-UBX1A-specific activities are caused by differences outside the homeodomain. The authors suggest that D-UBX1A can engage in protein–protein interactions specific for these characters that O-Ubx cannot, although the specific interaction partners have not been identified. In a later paper, the sequence that is responsible for the D-UBX1A-specific activity was identified as a repressor domain on the carboxy (C)-terminal side of the protein, with a QAQAQK$(A)_n$ motif (QA motif)[53]. Similar results were obtained in a comparison of D-UBX1A and *A. franciscana* Ubx (Af-Ubx) by McGinnis and collaborators[55]. In another study in *D. melanogaster*, in which the wild-type *Ubx* allele was replaced with an allele in which the QA motif was deleted ($Ubx^{\Delta QA}$)[54], it was shown that QA has an additive effect together with other peptide motifs[60] and ABDA protein activity. This series of studies shows that functional non-equivalence of UBX evolved not through changes in the DNA binding activity of the transcription factor, but most likely through changes in protein–protein or protein–RNA interactions.

Two important studies show that there are also specific functional differences between the homeodomains of Hox genes within the same organism (the mouse in this case). Zhao and Potter[58,59] replaced the *HoxA11* homeobox with the *HoxA13* homeobox (creating $HoxA11^{(a13Hd)}$) and found that this allele can rescue the $HoxA11^{-/-}$ phenotype in the development of the body axis, kidney and male reproductive tract. Interestingly, the $HoxA11^{(a13Hd)}$ allele antagonizes normal *HoxA11* function in limb development and causes homeotic transformation of the uterine epithelium into cervical epithelium.

This study was followed up with homeodomain swapping experiments between *HoxA11*, *HoxA10* and *HoxA4* (REF. 59). *HoxA10* is even more similar to *HoxA11* than *HoxA13*, and the $HoxA11^{(a10Hd)}$ allele was functionally quasi-equivalent even in the female reproductive tract, in addition to the body axis, kidney and male reproductive tract. By contrast, replacing the *HoxA11* homeodomain with that of *HoxA4* led to near-recessive null function in all but the axial development.

The pattern emerging from these studies is that functional non-equivalence is more likely for more derived functions, such as the mammalian female reproductive tract, than for more ancient characters, such as body axis. That is, the older the paralogues and the more derived the characters, the less likely it is that the transcription factors are still functionally equivalent. These results are interesting in light of the recent findings of adaptive evolution of the homeodomain following Hox gene duplication[61], and the fact that paralogue-specific amino-acid residues in the homeodomain are likely to function in protein–protein interactions rather than DNA binding[62].

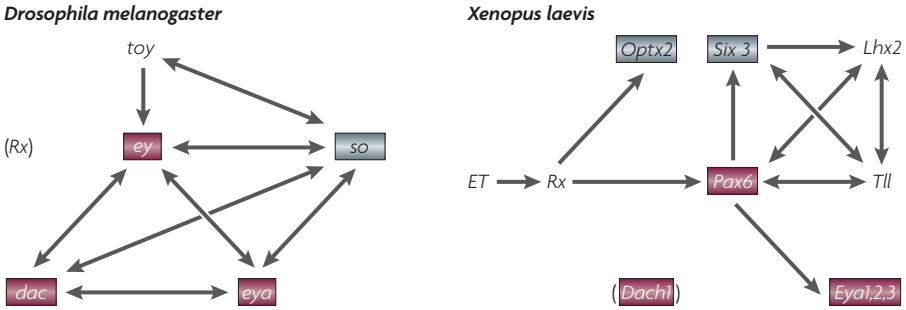

Figure 5 | **Gene regulatory networks for eye development.** The non-homologous eyes of insects and vertebrates are controlled by non-homologous genetic regulatory networks[37,39]. In *Drosophila melanogaster*, the five genes *eyeless (ey)*, *twin of eyeless (toy)*, *eyes absent (eya)*, *dachshund (dac)* and *sine oculis (so)* are jointly necessary for eye development. Four of these are also sufficient to induce eye development and can mutually induce each other's expression: *toy*, *ey*, *eya* and *dac*. The retinal homeobox (*Rx*) transcription factor gene, which is essential in vertebrate eye development, is also present in the *D. melanogaster* genome but is not involved in eye development. *dac* is essential in *D. melanogaster* eye development, but its semi-orthologue, *Dach1*, is not essential in vertebrate (for example, *Xenopus laevis*) eye development. For vertebrates, similar genes are involved in eye development but the functional relationships among them are different and involve non-orthologous genes. Orthologous genes are in red boxes and paralogous genes are in blue boxes. Instead of the vertebrate semi-orthologue of *sine oculis*, the genes sine oculis homeobox homologue 3 (*Six3*) and *Optx2* (also known as *Six6*) are involved in eye development. The gene duplication event that created the *sine oculis* and the *Six3/Optx2* paralogues occurred long before the most recent common ancestor of insects and vertebrates[44,45]. This shows that the genetic regulatory networks of insect and vertebrate eye development are most likely not homologous but derived independently. *ET*, transcription repression factor Tbx3; *Lhx2*, LIM homeobox 2; *Pax6*, paired-box 6; *Tll*, nuclear receptor subfamily 2, group E, member 1, nr2e1-A, Xtll.

The few examples reviewed above show that a transcription factor that becomes involved in a novel character has a tendency to become modified to carry out that novel function. This probably happens because of co-adaptation among transcription factors in the same GRN[63], but because there is no published example in which the evolution of both protein–protein interaction partners has been documented, we cannot be sure that this is the case. It could be that we see the result of adaptation of one transcription factor to another without both of them evolving. In either case, the transcription factors in a ChIN for a specific character are specialized for this task and remain associated with each other and with their character. They thus share historical continuity with the character they control.

ChINs and other GRNs. In a recent synthesis of the current knowledge of GRN evolution, Eric Davidson also noted that interspecific variation in GRNs is non-random[64]. He coined the term 'kernel' for the most conserved set of GRNs[28], and it is legitimate to ask whether the kernel concept is equivalent to the ChIN concept proposed here. Davidson defines a kernel as "…[a] conserved subcircuit consisting of genes which interact with one another and which are dedicated to a specific developmental function."[28] Although instances of kernels certainly overlap with examples of ChINs, the conceptual goal differs. From Davidson's examples, it is clear that kernels are only the most conserved GRNs regardless of their biological function. For instance, Hox GRNs do not constitute kernels because they are not old or invariant enough. By contrast, ChINs are defined as GRNs that subscribe the execution of a character-specific developmental programme, regardless of how old they are. Certainly, Hox-gene-based GRNs are likely candidates for ChINs, but are not kernels according to Davidson's use of the term[28]. Any new character is expected to have its ChIN, regardless of how old it is, whereas kernels are supposed to be associated with and be invariant in the oldest clades in the animal kingdom. Kernels and ChINs are both classes of GRN, but they serve different conceptual ends and are only partially overlapping.

Future directions

Consistent with modern views of homology[10,11,13,65,66], character identity is not tied to particular manifest features, like structure, composition and shape. Instead, homologues have a single historical origin, form a lineage of descent with modification, and can go extinct. From a developmental point of view, character identity and thus homology requires the ability to express an evolutionarily variable developmental programme that is different from those in other parts of the body. In this paper, I propose that this capability is underwritten by GRNs of co-adapted transcription factor genes. This concept implies a number of predictions that can be tested with the aid of standard gene knockdown and rescue experiments and functional genomic tools. For instance, the knockdown of a transcription factor gene that is part of a ChIN should affect the majority of genes that are regulated during the development of that character. In addition, because the members of a ChIN are assumed to be jointly necessary for the development of the character, the knockdown of different members is predicted to affect largely overlapping sets of downstream genes. The assertion that members of a ChIN are co-adapted can be tested by rescue experiments.

Table 1 | **Gene families in eye development: the Pax, Ey, Six, Eya and Dach families**

Cnidarians	*Drosophila melanogaster*	Vertebrates
PaxA	*Pox neuro*	
PaxB	*sparkling* (also known as *shaven*)	*Pax2*, *Pax5*, *Pax8*
PaxC	*eyeless*, *twin of eyeless*	*Pax4*, *Pax6*
PaxD	*Pox meso*, *gooseberry*, *gooseberry neuro*, *paired*	*Pax3*, *Pax7*
Six1/2	*sine oculis*	*Six1*, *Six2*
Six3/6	*Optix*	*Six3*, *Optx2*
Six4/5	*myotonix*	*Six4*, *Six5*
?	*eyes absent*	*Eya1*, *Eya2*, *Eya3*, *Eya4*
?	*dachshund*	*Dach1*, *Dach2*

See REFS 41,45,68,69. Note that the *sine oculis* and *Optix* genes duplicated before the most recent common ancestor of cnidarians and the bilaterian animals. ? indicates that family members have not been identified so far. Dach, dachshund family; Ey, eyeless family; Eya, eyes absent family; Pax, paired-box family; Six, sine oculis family.

Co-adaptation predicts that the knockdown effects can be reversed with a gene from the clade that shares this character, but not by genes from species that diverged before the origin of that character. High-throughput methods make these predictions testable at a scale that would allow assessment of the utility of the ChIN concept in explaining homology of morphological characters.

Günter P. Wagner is at the Department of Ecology and Evolutionary Biology, Yale University, POB 208106, New Haven, Connecticut 06520-8106, USA.

e-mail: gunter.wagner@yale.edu

doi:10.1038/nrg2099
Published online 8 May 2007

1. Hall, B. K. *Evolutionary Developmental Biology* (Chapman & Hall, London, 1998).
2. Wilkins, A. S. *The Evolution of Developmental Pathways* (Sinauer, Sunderland, 2002).
3. Butler, A. B. & Saidel, W. M. Defining sameness: historical, biological, and generative homology. *BioEssays* **22**, 846–853 (2000).
4. VanValen, L. Homology and causes. *J. Morphol.* **173**, 305–312 (1982).
5. Lewontin, R. C. Adaptation. *Am. Sci.* **239**, 156–169 (1978).
6. Owen, R. *On the Archetype and Homologies of the Vertebrate Skeleton* (Voorst, London, 1848).
7. Futuyma, D. J. *Evolutionary Biology* (Sinauer, Sunderland, 2005).
8. Brigant, I. Homology in comparative, molecular, and evolutionary developmental biology: the radiation of a concept. *J. Exp. Zool. B Mol. Dev. Evol.* **299**, 9–17 (2003).
9. Graur, D. & Li, W.-H. *Fundamentals of Molecular Evolution* (Sinauer, Sunderland, 2000).
10. Geeta, R. Structure trees and species trees: what they say about morphological development and evolution. *Evol. Dev.* **5**, 609–621 (2003).
11. Oakley, T. H. The eye as a replicating and diverging, modular developmental unit. *Trends Ecol. Evol.* **18**, 623–627 (2003).
12. Roth, V. L. Homology and hierarchies: problems solved and unresolved. *J. Evol. Biol.* **4**, 167–194 (1991).
13. Ghiselin, M. T. Homology as a relation of correspondence between parts of individuals. *Theor. Biosci.* **124**, 91–103 (2005).
14. Wagner, G. P. The biological role of homologues: a building block hypothesis. *N. Jb. Geol. Paläont. Abh.* **195**, 279–288 (1995).
15. Wagner, G. P. in *The Character Concept in Evolutionary Biology* (ed. Wagner, G. P.) 1–10 (Academic, San Diego, 2001).
16. Wagner, G. P. The biological homology concept. *Ann. Rev. Ecol. Syst.* **20**, 51–69 (1989).
17. Hall, B. K. in *Evolutionary Biology* Vol. 28 (eds Hecht, M. K., MacIntyre, R. J. & Clegg, M. T.) 1–30 (Plenum, New York, 1994).
18. Wray, G. A. & Abouheif, E. When is homology not homology? *Curr. Opin. Genet. Dev.* **8**, 675–680 (1998).
19. Hall, B. K. Descent with modification: the unity underlying homology and homoplasy as seen through an analysis of development and evolution. *Biol. Rev. Camb. Philos. Soc.* **78**, 409–433 (2003).
20. Pankratz, M. J. & Jackle, H. in *The Development of Drosophila melanogaster* (eds Bate, M. & Martinez-Arias, A.) 467–516 (Cold Spring Harbor Laboratory Press, Plainview, 1993).
21. Patel, N. H., Ball, E. F. & Goodman, C. S. Changing role of *even-skipped* during the evolution of insect pattern formation. *Nature* **357**, 339–342 (1992).
22. Dawes, R., Dawson, I., Falciani, F., Tear, G. & Akam, M. *Dax*, a locust Hox gene related to *fushi-tarazu* but showing no pair-rule expression. *Development* **120**, 1561–1572 (1994).
23. Peel, A. D., Chipman, A. D. & Akam, M. Arthropod segmentation: beyond the *Drosophila* paradigm. *Nature Rev. Genet.* **6**, 905–916 (2005).
24. Arendt, D. Evolution of eyes and photoreceptor cell types. *Int. J. Dev. Biol.* **47**, 563–571 (2003).
25. Arendt, D. Genes and homology in nervous system evolution: comparing gene functions, expression patterns, and cell type molecular fingerprints. *Theor. Biosci.* **124**, 185–197 (2005).
26. Allan, D. W., Park, D., Pierre, S. E. S., Taghert, P. H. & Thor, S. Regulators acting in combinatorial codes also act independently in single differentiating neurons. *Neuron* **45**, 689–700 (2005).
27. Shirasaki, R. & Pfaff, S. L. Transcriptional codes and the control of neuronal identity. *Ann. Rev. Neurosci.* **25**, 251–281 (2002).
28. Davidson, E. H. *The Regulatory Genome: Gene Regulatory Networks in Development and Evolution* 144–159, 28 (Academic, Amsterdam, 2006).
29. Ghiselin, M. 'Definition, ' 'character' and other equivocal terms. *Syst. Zool.* **33**, 104–110 (1984).
30. Deutsch, J. Hox and wings. *BioEssays* **27**, 673–675 (2005).
31. Warren, R. W., Nagy, L., Senegue, J., Gates, J. & Carroll, S. Evolution of homeotic gene regulation and function in flies and butterflies. *Nature* **372**, 458–461 (1994).
32. Wagner, G. P., Chiu, C.-H. & Laubichler, M. Developmental evolution as a mechanistic science: the inference from developmental mechanisms to evolutionary processes. *Am. Zool.* **40**, 819–831 (2000).
33. Tomoyasu, Y., Wheeler, S. R. & Denell, R. E. Ultrabithorax is required for membranous wing identity in the beetle *Tribolium castaneum*. *Nature* **375**, 58–61 (2005).
34. Gehring, W. J. & Ikeo, K. Pax6 mastering eye morphogenesis and eye evolution. *Trends Genet.* **15**, 371–377 (1999).
35. Halder, G., Callerts, P. & Gehring, W. J. Induction of ectopic eyes by targeted expression of the *eyeless* gene in *Drosophila*. *Science* **267**, 1788–1792 (1995).
36. Holland, P. W. H. in *Homology* Vol. 222 (eds Bock, G. R. & Cardew, G.) 226–236 (Wiley & Sons, Chichester, 1999).
37. Donner, A. L. & Maas, R. L. Conservation and non-conservation of genetic pathways in eye specification. *Int. J. Dev. Biol.* **48**, 743–753 (2004).
38. Kozmik, Z. Pax genes in eye development and evolution. *Curr. Opin. Genet. Dev.* **15**, 430–438 (2005).
39. Zuber, M. E., Gestri, G., Viczian, A. S., Barsacchi, G. & Harris, W. A. Specification of the vertebrate eye by a network of eye field transcription factors. *Development* **130**, 5155–5167 (2003).
40. Chow, R. L., Altmann, C. R., Lang, R. A. & Hemmati-Brivanlou, A. Pax6 induced ectopic eyes in a vertebrate. *Development* **126**, 4213–4222 (1999).
41. Kardon, G., Heanue, T. A. & Tabin, C. J. in *Modularity in Development and Evolution* (eds Schlosser, G. & Wagner, G. P.) 59–80 (Univ. Chicago Press, Chicago and London, 2004).
42. Schlosser, G. Evolutionary origins of vertebrate placods: insights from developmental studies and from comparisons with other deuterostomes. *J. Exp. Zool. B Mol. Dev. Evol.* **304**, 347–399 (2005).
43. Bailey, T. J. *et al.* Regulation of vertebrate eye development by Rx genes. *Int. J. Dev. Biol.* **48**, 761–770 (2004).
44. Bebenek, I. G., Gates, R. D., Morris, J., Hartenstein, V. & Jacobs, D. K. *sine oculis* in basal Metazoa. *Dev. Genes Evol.* **214**, 342–351 (2004).
45. Stierwald, M., Yanze, N., Bamert, R. P., Kammermeier, L. & Schmid, V. The *sine oculis*/*six* class family of homeobox genes in jellyfish with and without eyes: development and regeneration. *Dev. Biol.* **274**, 70–81 (2004).
46. Arendt, D., Tessmar-Raible, K., Snyman, K., Dorresteijn, A. W. & Wittbrodt, J. Ciliary photoreceptors with a vertebrate-type opsin in an invertebrate brain. *Science* **306**, 869–871 (2004).
47. Nilsson, D.-E. Eye evolution: a question of genetic promiscuity. *Curr. Opin. Neurobiol.* **14**, 407–414 (2004).
48. Ogura, A., Ikeo, K. & Gojobori, T. Comparative analysis of gene expression for convergent evolution of camera eye between octopus and human. *Genome Res.* **14**, 1555–1561 (2004).
49. Chan, S. K., Jaffe, L., Capovilla, M., Botas, J. & Mann, R. S. The DNA binding specificity of Ultrabithorax is modulated by cooperative interactions with extradenticle, another homeoprotein. *Cell* **78**, 603–615 (1994).
50. McGinnis, N., Kuziora, M. A. & McGinnis, W. Human Hox-4.2 and *Drosophila deformed* encode similar regulatory specificities in *Drosophila* embryos and larvae. *Cell* **63**, 969–976 (1990).
51. Hsia, C. C. & McGinnis, W. Evolution of transcription factor function. *Curr. Opin. Genet. Dev.* **13**, 199–206 (2003).
52. Grenier, J. K. & Carroll, S. B. Functional evolution of the Ultrabithorax protein. *Proc. Natl Acad. Sci. USA* **97**, 704–709 (2000).
53. Galant, R. & Carroll, S. B. Evolution of a transcriptional repression domain in an insect Hox protein. *Nature* **415**, 910–913 (2002).
54. Hittinger, C. T., Stern, D. L. & Carroll, S. B. Pleiotropic functions of a conserved insect-specific Hox peptide motif. *Development* **132**, 5261–5270 (2005).
55. Ronshaugen, M., McGinnis, N. & McGinnis, W. Hox protein mutation and macroevolution of the insect body plan. *Nature* **415**, 914–917 (2002).
56. Ranganayakulu, G., Elliott, D. A., Harvey, R. P. & Olson, E. N. Divergent roles for NK-2 class homeobox genes in cardionenesis in flies and mice. *Development* **125**, 3037–3048 (1998).
57. Lohr, U., Yussa, M. & Pick, L. *Drosophila fishi tarazu*: a gene on the border of homeotic function. *Curr. Biol.* **11**, 1403–1412 (2001).
58. Zhao, Y. & Potter, S. S. Functional specificity of the Hoxa 13 homeobox. *Development* **128**, 3197–3207 (2001).
59. Zhao, Y. & Potter, S. S. Functional comparison of the *Hoxa 4*, *Hoxa 10*, and *Hoxa 11* Homeoboxes. *Dev. Biol.* **244**, 21–36 (2002).
60. Tour, E., Hittinger, C. T. & McGinnis, W. Evolutionarily conserved domains required for activation and repression functions of the *Drosophila* Hox protein Ultrabithorax. *Development* **132**, 5271–5281 (2005).
61. Lynch, V. J., Roth, J. J. & Wagner, G. P. Adaptive evolution of Hox-gene homoedomains after cluster duuplications. *BMC Evol. Biol.* **6**, 86 (2006).
62. Sharkey, M., Graba, Y. & Scott, M. P. Hox genes in evolution: protein surfaces and paralog groups. *Trends Genet.* **13**, 145–151 (1997).
63. Wagner, G. P. & Lynch, V. J. Molecular evolution of evolutionary novelties: the vagina and uterus of therian mammals. *J. Exp. Zool. B Mol. Dev. Evol.* **304**, 580–592 (2005).
64. Davidson, E. H. & Erwin, D. H. Gene regulatory networks and the evolution of animal body plans. *Science* **311**, 796–800 (2006).
65. Wagner, G. P. & Stadler, P. F. Quasi-independence, homology and the unity of type: a topological theory of characters. *J. Theor. Biol.* **220**, 505–527 (2003).
66. Wiley, E. O. Homology, identity and transformation in Mesozoic fishes (eds Arrtia, G. & Schultze, H.-P.) (in the press).
67. Müller, G. B. & Wagner, G. P. Novelty in evolution: restructuring the concept. *Ann. Rev. Ecol. Syst.* **22**, 229–256 (1991).
68. Miller, D. J. *et al.* Pax gene diversity in the basal cnidarian *Acropora millepora* (Cnidaria, Anthozoa): implications for the evolution of the Pax gene family. *Proc. Natl Acad. Sci. USA* **97**, 4475–4480 (2000).
69. Hadrys, T., DeSalle, R., Sagasser, S., Fischer, N. & Schierwater, B. The Trichoplax PaxB gene: a putative proto-PaxA/B/C gene predating the origin of nerve and sensory cells. *Mol. Biol. Evol.* **22**, 1569–1578 (2005).

Acknowledgements
The author gratefully acknowledges the financial support for the research that led to the ideas expressed in this article: NSF IBN#0321470, OIB#0445971 and the Research Prize from the Alexander von Humboldt Foundation. I also want to thank the reviewers of this paper as well as M. Laubichler, M. Ghiselin, E. Wiley, M. Donaghue, A. Monteiro and the members of my laboratory for discussion and inspiration.

Competing interests statement
The author declares no competing financial interests.

DATABASES
The following terms in this article are linked online to:
Entrez Gene: http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=gene
abdA | *bcd* | *dac* | *Dach1* | *dpp* | *en* | *eve* | *ey* | *eya* | *ftz* | *HoxA4* | *HoxA10* | *HoxA11* | *HoxA13* | *Pax6* | *Rx* | *so* | *Srf* | *tin* | *toy* | *Ubx* | *wg*

FURTHER INFORMATION
Wagner laboratory homepage:
http://pantheon.yale.edu/%7Egpwagner/index.html
Access to this links box is available online.