REVIEW ARTICLE

Levels of Biological Organization and the Origin of Novelty

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ABSTRACT

The concept of novelty in evolutionary biology pertains to multiple tiers of biological organization from behavioral and morphological changes to changes at the molecular level. Identifying novel features requires assessments of similarity (homology and homoplasy) of relationships (phylogenetic history) and of shared developmental and genetic pathways or networks. After a brief discussion of how novelty is used in recent literature, we discuss whether the evolutionary approach to homology and homoplasy initially formulated by Lankester in the 19th century informs our understanding of novelty today. We then discuss six examples of morphological features described in the recent literature as novelties, and assess the basis upon which they are regarded as novel. The six are: origin of the turtle shell, transition from fish fins to tetrapod limbs, origination of the neural crest and neural crest cells, cement glands in frogs and casquettes in fish, whale bone-eating tubeworms, and the digestion of plant proteins by nematodes. The article concludes with a discussion of means of acquiring novel genetic information that can account for novelty recognized at higher levels. These are co-options of existing genetic circuitry, gene duplication followed by neofunctionalization, gene rearrangements through mobile genetic elements, and lateral gene transfer. We conclude that on the molecular level only the latter category provides novel genetic information, in that there is no homologous precursor. However, novel phenotypes can be generated through both neofunctionalization and gene rearrangements. Therefore, assigning phenotypic or genotypic "novelty" is contingent on the level of biological organization addressed. *J. Exp. Zool. (Mol. Dev. Evol.) 314B, 2011.* © 2011 Wiley-Liss, Inc.

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What is Novelty? The term/concept of novelty in recent literature on evolutionary developmental biology (evo-devo) is used in publications dealing with (1) similarity (homology and homoplasy), (2) relationships (evolutionary history), and/or (3) divergent developmental and genetic pathways/networks (mechanisms of morphological change) (Müller, '89; Müller and Wagner, '91; Shubin and Marshall, 2000; Stone and Hall, 2004; Moczek, 2008; Brigandt and Love, 2010; Wagner and Lynch, 2010 and references therein). We begin with a brief discussion of definitions and concepts of novelty. Then, because of its central importance to any discussion about comparisons in biology, we discuss homology, especially whether the evolutionary context of homology/homoplasy developed by Lankester (1870a,b and see also Gould, 2002; Hall, 2003) informs our understanding of novelty. Third, and using the three criteria of similarity, phylogenetic relationships, and shared developmental/genetic mechanisms, we evaluate six examples of morphological features discussed in the recent literature as novelties: the turtle shell; the transition from fish fins to tetrapod limbs; the origination of the neural crest and neural crest cells (NCCs); cement glands in frogs and casquettes in fish; whale bone-eating tubeworms, and the digestion of plant proteins by nematodes. Several of these examples reveal potential genetic origins of phenotypic novelty—gene co-option, neofunctionalization, gene rearrangements through mobile genetic elements, and lateral gene transfer—that provide genetic bases for the novelties recognized

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at higher levels. The article concludes with a discussion of these examples for our understanding of mechanisms that generate novelties at various levels of the biological hierarchy.

ASSIGNING NOVELTY

Although more could be found and discussed (Nitecki, '90; Brigandt and Love, 2010; Wagner and Lynch, 2010 and references therein), in the recent literature novelty is assigned when a feature has no homologous precursor.

Reflecting the importance making comparisons at a particular level in the biological hierarchy, novelty must be "rooted in a character concept that is adequate for the respective level of organization" (Müller and Wagner, 2003, p 221). A feature may be inappropriately regarded as novel only because we lack detailed information on their same (homologous) character in other taxa. The turtle shell discussed below may fall into this category. Further, a novelty has been defined as "A new constructional element in a body plan that neither has a homologous counterpart in the ancestral species nor in the same organism (serial homolog)" (Müller and Wagner, 2003, p 221; reiterated by Wagner and Lynch, 2010, p R. 49). Finally, a novelty need not be "a new constructional element in a body plan" but also could be a new behavior. "A novelty (whether structure or behaviour) is a new feature in a group of organisms that is not homologous to a feature in an ancestral taxon" Hall (2005, p 549). Therefore, accurately identifying novelties requires an assessment of homologous precursors in ancestral or, as a proxy, outgroup taxa.

HOMOLOGY

Homology has been discussed and debated for more than a century (Osborn, 1902; Patterson, '82; Hall, '94; Wake, '94, '99; Gilbert and Bolker, 2001; Wheeler, 2001; Scholtz, 2005, and references therein). We can begin with the first elaboration of an evolutionary concept of homology, that of the English Zoologist E. Ray Lankester (1847–1929) building on the research of the comparative anatomist Richard Owen (1804–1892) (for more detailed histories of the concept see Hall, '94; Panchen, '99; Hall, 2003).

Owen (1843) defined a homolog as: "The same organ in different animals under every variety of form and function." Owen contrasted homology with analogy ("A part or organ in one animal which has the same function as another part or organ in a different animal"). Owen never modified his definitions to accommodate Darwin's theory of evolution by natural selection. Because of its typological and Platonic connotations, Lankester (1870a,b) advocated abandoning the term homology, proposing in its place "homogeny" for similarity resulting from shared ancestry; "Structures which are genetically[1] related, in so far as they have a single representative in a common ancestor, may be called *homogenous*. We may trace an *homogeny* between them, and speak of one as the *homogen* of the other" (1870b, p 36, his emphases). The term homogeny did not catch on but biologists adopted Lankester's definition of similarity resulting from shared ancestry and applied it to the existing term, homology. Lankester introduced "homoplasy" for what he regarded as the second and only other class of similarity, which was similarity resulting from independent evolution. Importantly to Lankester, both homogeny and homoplasy were classes of homology, i.e. related to evolutionary history and so to organismal relationships.

Lankester's (1870a,b) evolutionary approach to comparisons, when combined with current knowledge of evolutionary and developmental mechanisms, leads to an expanded category of homology to include reversals, rudiments and atavism, leaving convergence as the only class of homoplasy or independent evolution (see Gould, 2002; Hall, 2003 for detailed development of this position).

Many lines of evidence lead to the conclusion that all organisms on Earth share a deep evolutionary ancestry (Theobald, 2010); all are based on cells in which DNA and RNA provide the genetic material, upon the basis of which organic molecules and phenotypes are made. Lineages of organisms share subsets of information (e.g. orthologous genes or paralogous members of gene families). Differences in shared genetic information are often used to determine the evolutionary relationships of extant groups, which helps to identify the timing of shared ancestry and set temporal boundaries on stem and crown group taxa. These are often identifiable within the fossil record on the basis of key innovations; uniquely derived character states that also define contemporary lineages. Should we consider these uniquely derived character states novelties? If yes then how do they differ from the derived character states that define taxa?

Lankester replaced Owen's dichotomy of homology and analogy with an evolutionary concept, but not a mechanistic concept of how phenotypic features arise. The definition of novelty as a uniquely derived character state with implicit nonhomology does not take us beyond autapomorphy or synapomorphy unless the concept causes us to focus on the mechanisms that produce novelties.

Homology can further be subdivided into two varieties: structural and developmental. Structural homology pertains to the presence of the same character in two lineages that share a common ancestor (a synapomorphy/symplesiomorphy), whereas developmental homology pertains to the same developmental mechanisms producing a shared character. Structural homology need not always equate with developmental homology. For instance developmental mechanisms, down to the level of gene regulation, can evolve, despite forming structurally homologous features (Ludwig et al., 2000; True and Haag, 2001).

Whether the evolution of a novel developmental process is sufficient to regard the features that arise as novel is taken up in

[1] This was pre-Mendelian, so Lankester is referring to inheritance rather than to a specific genetic mechanism.

the examples discussed below, several of which—turtle shells, tetrapod limbs, vertebrate neural crest—are classic examples of novelty discussed in the literature, others of which are newly discovered phenotypic features that immediately raise the question of homology and how new features (novelties) arise. Hallgrímsson et al. (2011) tackles these questions in relation to the origin of variation.

FEATURES PRESENTED IN THE LITERATURE AS CLASSIC EXAMPLES OF NOVELTY

Turtle Shells

Fossil evidence for the precursors of shells in turtle ancestors is lacking or was until 2008 and the discovery of the oldest turtle, the 220 million-year-old *Odontochelys semitestacea*. This turtle has the ventral portion of the shell (the plastron) but only two bony elements in the dorsal portion of the shell or carapace, which Li et al. (2008) interpret as indicating that the carapace developed without the ribs encompassing the pectoral girdle (as also seen in nonturtle amniotes). This intermediate morphology of the orientation of the ribs and scapula in *Odontochelys* corresponds with the embryology of carapace development in the Chinese soft-shelled turtle (*Pleodiscus sinensis*); however, Reisz and Head (2008) suggest that the carapace is secondarily simplified in *Odontochelys* as it is in extant leatherback and soft-shelled turtles. *Pleodiscus sinensis* developmentally recapitulates the basic amniote body plan before a unique arrest of lateral rib expansion in relation to the girdles, an expansion that accounts for the ribs surrounding the girdles in their support of the carapace (Nagashima et al., 2009). These paleontological and developmental findings revise our understanding of the "novelty" of the turtle body plan. Discoveries of intermediate fossils and intermediate ancestral developmental sequences continue to dispel such claims of novelty in vertebrate evolution. The character concept of the shell involving *Baupläne* re-construction (Gilbert et al., 2001) does not hold up in the face of the ontogenetic and phylogenetic intermediates.

Tetrapod Limbs

It has been known since the middle of the 19th century that paired fish fins are homologs of paired tetrapod limbs (Owen, 1849) and it has been proposed for almost as long that tetrapod limbs evolved from fish fins, pectoral fins transforming into forelimbs, pelvic fins into hind limbs. An enormous literature now exists on this transition (comparative anatomy, fossils, embryonic development, shared gene networks), much of it summarized and discussed in Hall (2007).

Fins are composed of basal cartilaginous elements supporting a distal and usually much more extensive set of bony fin rays (lepidotrichia). Limbs lack these fin rays but have expanded the cartilaginous elements to form digits. Simplistically, the transition at the morphological level may be expressed as "Fins minus fin rays plus digits equals limbs."

Important transitional stages in this transformation are known from the fossil record, of which the sarcopterygian fish *Tiktaalik roseae* is the most recently discovered (Daeschler et al., 2006). *Tiktaalik* bears fin rays along with homologs of all elements of the tetrapod limb skeleton, including digit-like radials but not digits. These anatomical elements in *Tiktaalik* call into question the novel origin of the autopod. Four years ago, we would have said that the wrist/ankle and digits are novel (Hall, 2007). Tetrapod ancestors were not thought to have homologs of wrist and ankle (carpal and tarsal) bones. However, discoveries of taxa such as *Tiktaalik* have changed our thinking on how much of the limb is novel to tetrapods.

But what if earlier fish with digits were found? The criteria of nonhomology to a feature in an ancestral taxon would not be met. Would this mean abandoning the concept of autopod novelty or only removing tetrapod digits as an example of a novelty? Recent CT scanning of the pectoral fin of *Panderichthys*, a sarcopterygian fish by Boisvert et al. (2008) led to the reinterpretation of what had been identified as a plate-like ulnare as distal radials; the latter also found in *Tiktaalik*. Boisvert et al. concluded that radials could be precursors to proximal digits, a conclusion that removes digits as a tetrapod novelty—the homolog of proximal digital elements is present in *Panderichthys*, which is a lobe-finned (elpistostegid) fish. Based on comparisons of zebrafish and amniotes, novel late-phase *Hoxd* gene expression was thought to coincide with the origin of the autopod (Sordino et al., '95; Wagner and Chiu, 2001). However, while late-phase *Hoxd* expression is absent from teleosts (zebrafish), it does occur in a basal actinopterygian (Davis et al., 2007) and lungfish (Johanson et al., 2007), further supporting the previous existence of autopod patterning mechanisms before the origin of the autopod itself. Therefore, neither paleontological nor developmental data any longer support the long-held belief that the autopod is a tetrapod neomorph.

The Neural Crest and NCCs

As a germ layer (Hall, 2009) the neural crest arises from the apex of the neural folds from otherwise neural ectoderm. The neural crest and NCCs are found only in vertebrates (including hagfish; Ota et al., 2007) but NC-like trunk lateral cells derived from blastomere A7.6 have been identified in several species of ascidians (Jeffery et al., 2004; Jeffery, 2006, 2007; Bishop et al., 2010). In vertebrates, NCCs form much of the skeleton of the head as well as many other cell and tissue types (Stone and Hall, 2004; Hall, 2009).

Whether the neural crest is a vertebrate, novelty depends on whether their ancestor possessed a homologous feature. Recent developmental/genetic analyses of extant tunicates (urochordates) such as *Ciona* spp. have challenged the interpretation from comparative morphological analyses that ancestors of vertebrates

were animals akin to living cephalochordates such as amphioxus (*Branchiostoma* spp). We can assess the neural crest, NCCs, or NCC derivatives as novel against these two phylogenetic relationships (Fig. 1). We use two features: the origin of NCC themselves and the origin of cartilage.

Cephalochordates possess a mesodermal branchial basket supported by gill bars composed of fibrillar collagen and chondroitin sulfate in an acellular matrix (Meulemans and Bronner-Fraser, 2007). Cephalochordates lack a neural crest or NCCs. Ascidians lack cartilage but have what Jeffery (2007) designated as neural crest-like cells (NCLCs) based on similar migratory and molecular properties. If cephalochordates are the vertebrate outgroup (Fig. 1A) and *if* amphioxus reflects the *ancestral condition*, then the neural crest and NCCs are vertebrate novelties, and cellular cartilage is a vertebrate novelty. *If* the absence of NCCs in extant cephalochordates is *derived*, then NCCs and NCLCs would be plesiomorphic for chordates. If ascidians are the vertebrate outgroup (Fig. 1B) then NCLCs (and most likely NCCs) are an ascidian+vertebrate novelty. The origination of neural-crest-derived cartilage can be examined in greater detail thanks to pioneering studies of gene networks in vertebrate NCCs, and in cephalochordate and ascidian neural ectoderm and mesoderm. Figure 2 shows the gene network associated with the formation of neural crest from the neural plate border and the expression of orthologous genes in amphioxus and *Ciona intestinalis*. As summarized by Meulemans and Bronner-Fraser (2007, p 1): "No single amphioxus tissue co-expresses all or most of these genes. However, most are variously co-expressed in mesodermal derivatives. Our results suggest that neural crest-derived cartilage evolved by serial cooption of genes which functioned primitively in mesoderm." The origin of neural crest and NCC-derived cartilage in vertebrates required co-option of mesodermal genes into the evolving neural crest or NCLCs. However, expression analysis of the gene regulatory network (GRN) in *C. intestinalis* reveals a "rewiring" of the GRN that includes not only co-option of mesoderm-associated genes into NCLCs but also loss of neural border specifier genes from the network (Jeffery et al., 2008). Experimental verification in *C. intestinalis* is required to determine whether expression of neural crest specifiers in the NCLCs is dependent on earlier border induction signals. The presence of border specifiers in cephalochordates and vertebrates, but not in *C. intestinalis*, suggests a secondary loss of this expression pattern in urochordates. Regardless of the immediate vertebrate outgroup, gene co-option, a mechanism by which genes are expressed in a novel location in descendants, is the probable basis by which these novelties arose.

Analysis of genes and gene networks (GRNs) at the border of the neural tube in amphioxus and vertebrates adds additional strong support to co-option of gene networks in the origin of the neural crest (reviewed in Yu, 2010). The GRN that specifies the border of the neural tube is conserved between amphioxus and

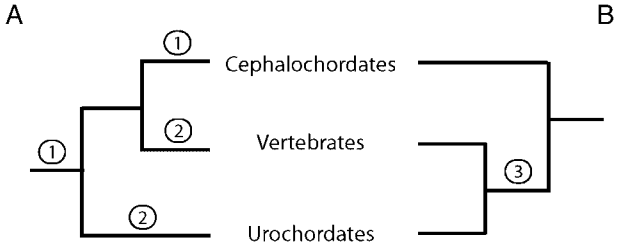

Figure 1. Chordate phylogenies with alternate sister taxon relationships between vertebrates and cephalochordates (**A**) and vertebrates and urochordates (**B**). Possible scenarios of neural crest evolution are mapped onto the chordate clade of each tree. In Figure 1A, an ancestral chordate neural crest with subsequent loss in cephalochordates (1) is equally parsimonious with independent origins of the neural crest in vertebrates and neural crest-like cells in urochordates (2). Evolution of the neural crest requires fewer changes in the second topology (B), with a single origin of NCLCs at the base of the vertebrate-urochordate clade.

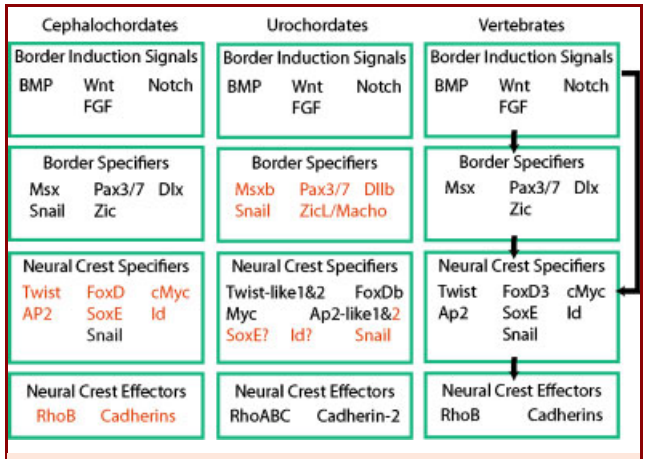

Figure 2. Gene co-option of the neural crest gene regulatory network (NC-GRN) of the neural plate border in chordates. The expression of orthologous genes has been assayed in amphioxus *(Branchiostoma floridae*; Yu et al., 2008) a tunicate *(Ciona intestinalis;* Jeffery et al., 2008) and multiple vertebrates (reviewed by Yu et al., 2008). Black colored text indicates conserved expression with the vertebrate network. Gene names in red are not conserved. Arrows indicate experimentally verified inductive or regulatory interactions. Amphioxus expresses border induction signals and border specifiers, with only transient expression of the neural crest specifier *Snail* on the neural border. *Ciona* expresses more neural crest specifiers and downstream neural crest effectors in the A7.6/trunk lateral cell lineage (NCLC), but does not express earlier neural border specifiers. It remains to be determined whether the border induction signals are capable of controlling neural crest specifiers alone in *Ciona*, without the aid of border specifiers.

vertebrates. The GRN that specifies pigment cells is conserved. The ortholog of a central gene involved in cartilage specification in vertebrates and which is the dominant collagen of cartilage, *col2a1*, is present as *colA* in the mesoderm of amphioxus (Meulemans and Bronner-Fraser, 2007), but is not expressed in the NCLC lineage of ascidians (Jeffery et al., 2008). No neural crest border genes are expressed in amphioxus, however, although these genes are present in the amphioxus genome.

Cement Glands in Frogs and Casquettes in Fish

Early tadpoles of *Xenopus* (the South African clawed toad) and most other lineages of anurans posses paired cement glands on the *ventral* surface of the head. As their name indicates, these glands secrete an adhesive enabling tadpoles to attach to vegetation.

Cement glands are innervated by the trigeminal nerve (Huang et al., 2007). Other features shared by cement glands are listed in Table 1.

A teleost fish, the Mexican tetra *Astyanax mexicanus*, exists as both sighted surface and blind cave morphs. Larvae of both surface and cave morphs have been shown to possess paired organs on the *dorsal* surface of the head. Pottin et al. (2010) named these organs casquettes (caps) and compared them with cement glands in *Xenopus*, with which they compare (are homologous) point for point *except for their dorsal location* (Table 1).

Table 1. Comparison of the features of cement glands in *Xenopus* tadpoles with casquettes in the Mexican tetra *Astyanax mexicanus*.

Xenopus	*A. mexicanus*
Paired, *ventral*, epidermal	Paired, *dorsal*, epidermal
Temporary	Temporary
Innervated by trigeminal nerve	Innervated by trigeminal nerve
Mucous secreting	Mucous secreting
Used for attachment	Used for attachment
Mechanoreceptors	Mechanoreceptors
Mediates stopping response	Mediates stopping response
Balancers in urodele larvae are homologues	Cement glands in frog larvae homologues
Ascidians have a similar structure	
High levels of *Bmp-4* and *Pitx*	High Levels of *Bmp-4* and *Pitx*
High levels of *Otx-2*	High levels of *Otx-2*
Bmp-4, Pitx, and Otx-2 required for development[1]	Requirement for these genes not known
Serotonergic 5-HT neurotransmitter	5-HT neurotransmitter

[1]The direct-developing frog *Eleutherodactylus coqui* lacks cement gland and lacks anterior *Otx-2* expression in the larval head (Fang and Elinson, '99).

Similarities between cement glands and casquettes extend to the molecular level (Table 1). Both express high levels of *Bmp-4*, *Pitx*, and *Otx-2*. (The adhesive papillae of the Urochordate *Ciona* larvae express *Bmp-4* and *Otx*.) Both use the same neurotransmitter. Although not studied in anywhere as much detail, cement glands are known from 11 distantly related chordate groups (lungfish, bichir, sturgeon, paddlefish, gar, bowfin, cichlids, *Rana,* and *Xenopus*; Pottin et al., 2010). Cement glands represent examples of parallel evolution in larvae of aquatic vertebrates (fish) or aquatic life history stages (amphibians) presumed to arise in response to selection on shared functions, which resulted in a surprising level of neurological and developmental convergence. Such deep homology would result in not regarding these organs as novel despite the occurrence of casquettes as a unique *Astyanax* autapomorphy.

As the following two examples illustrate, the designation of novelties has not been limited to morphological features of the phenotype; biochemical and/or metabolic novelties have been described.

Tubeworms Without a Digestive System That Feed on Whalebones

At least 15 species of tubeworm in the genus *Osedax* consist of populations of females feeding on decaying whalebones at depths of 3,000 m or more. Females are surrounded by a gelatinous capsule, which is colonized by hundreds of dwarf (< 1 mm long), paedomorphic, and nonfeeding males (Vrijenhoek et al., 2008; Whiteman, 2008). The females lack a digestive system but contain endosymbiotic bacteria that produce enzymes for digesting bone, a novel feeding mechanism and symbiosis not known in other taxa. The evolution of new symbiotic relationships allows novel behaviors to evolve without a homologous precursor (Margulis and Fester, '91; Hall, '99; Gilbert and Apel, 2008).

Digestion of Plant Products by the Cotton Root-Knot Nematode Meloidogyne Incognita

The round worm, *Meloidogyne incognita* has as its niche nodules within the roots of cotton plants. In a pattern that is extremely atypical for any animal, the genome of the nematode contains 60 genes in six protein families that degrade plant cell walls (Table 2). These genes code for a variety of enzymes, including cellulases, xylanases, hemicelluloses, polygalacturonases, pectate lysases, and arabinanase. This astonishing array of genes arose from multiple, independent lateral gene transfers from different bacteria, followed by many gene duplications within the nematodes to form multigene families. Phylogenetic trees of the major protein families constructed by Danchin et al. (2010) enabled them to identify the bacterial lineages that provided the genes.

Multiple, independent lateral gene transfers from different bacteria, followed by gene duplications to form multigene families is not the way variation in animal phenotypes normally

Table 2. Plant cell wall-modifying proteins in the root-knot nematode *Meloidogyne incognita*.[1]

Family of proteins	Activity	Closest relative
Glycoside hydrolase (GH28)	Polygalacturonase	*Ralstonia*: *Ralstonia solanacearum*
Polysaccharide lyase (PL3)	Pectate lyase)	Actinomycetales
Arabinanases (GH43)	Putative arabinanase	Actinomycetales
Cellulases (GH5)	Cellulase	Coleoptera
Xylanases (GH5)	Endo-1,4-β-xylanase	*Clostridium acetobutylicum*
Expansens (EXPN)	Loosening of plant cell wall	Actinomycetales

[1]From data in Danchin et al. (2010).

arises, although several billion years ago endosymbiosis provided early eukaryotic cells with mitochondria, mtDNA, chloroplasts, and nuclear membranes (Lake and Rivera, '94; Dacks and Field, 2007; Dunning Hotopp et al., 2007). Many mitochondrial and chloroplast genes were then transferred to the nucleus and some nuclear genes to these organelles. Lateral gene transfer, when followed by rounds of gene duplication, provides a powerful basis for new (nonhomologous) genes and gene functions to arise within animals and a novel genetic basis for novelty of structure see also Erwin and Valentine ('84). The next two sections compare the rationale for regarding acquisition of a new function by a gene duplication and neofunctionalization or lateral gene transfer as novel genetic mechanisms and as bases for generating phenotypic novelty.

DOES NEOFUNCTIONALIZATION GENERATE NOVEL GENES?

The diverse functions of signaling molecule and transcription factor families (*regulatory genes*) suggests that paralogs often acquire the new roles in development which may account for the evolution of morphological novelties (Shakhnovich and Koonin, 2006). In his analysis of the evolution of the neural crest, Yu (2010) provided an example of neofunctionalization following duplication of members of the Forkhead (FoxD) transcription factor gene family. Amphioxus has a single member of the *FoxD* family, *AmphiFoxD*. Vertebrates have five members of the gene family, *FoxD1-FoxD5* that arose through gene duplication in the early vertebrate lineage. *FoxD3*, which is expressed in NCCs in the neural plate border and in migrating NC cells, is downregulated as NCCs differentiate. Expression is maintained in undifferentiated NCCs, and indeed plays an important role in keeping these cells in an undifferentiated state. A novel tissue-specific *cis*-regulatory element in *FoxD3* is hypothesized to have arisen at or near the base of vertebrate origination, enabling this new function (Yu, 2010).

Recall, that novelties may occur at several levels of the biological hierarchy. Neofunctionalization of *nonregulatory genes* can result in the evolution of *new* biochemical and metabolic pathways. Whether we define these as *novel* depends on how we assign homology or nonhomology to the altered gene or its function. A duplicate of the pancreatic ribonuclease gene (RNASE1) in leaf-eating colobine monkeys (*Pygathrix nemaeus*) allows efficient breakdown of symbiotic bacterial RNA in the foregut, allowing the nitrogen recycling required for obligate herbivory (Zhang et al., 2002). Furthermore, convergent duplicates may have independently evolved in Asian and African leaf-eating monkeys, suggesting strong selective pressures at both the behavioral and genetic levels of the biological hierarchy (Zhang, 2006).

Determining the timing of paralog duplication is essential in deciphering independent gene conversions from independent duplications (Xu et al., 2009). For instance α-globin gene clusters diversified in early stem tetrapods, while ß-globins were derived independently through convergent duplication in several extant tetrapod lineages (Hoffmann et al., 2010). Other examples of paralog neofunctionalization in structural and regulatory genes that may account for evolutionary novelty have been reported in organisms/roles as diverse as Woronin bodies in *Neurospora* (Yuan et al., 2003), C-14 photosynthesis in monocots (Monson, 2003), snake venom (Lynch, 2007), and novel morphologies in plants (Flagel and Wendel, 2009; Rosin and Kramer, 2009).

Our growing understanding of genomics has substantiated early theories concerning the importance of gene and genome duplications in evolution (Ohno, '70). Gene duplication coupled with neofunctionalization involves changing functions of genes that have ancestral homologs. Under these conditions and *because it depends on duplication of a homologous precursor*, neofunctionalization would fail the criterion of requiring nonhomology at the level of the gene for the new feature/functions outlined in the preceding two paragraphs that result from neofunctionalization to be classified as novel. But at the level of the phenotype, neofunctionalization can produce novelty. An important issue for future analyses of novelty therefore is where novelty should be assigned in situations of differential (or homologous/nonhomologous) changes at different hierarchical levels.

A mechanism that provides novel rearrangements of genetic information is the acquisition of transposable elements, that is, if regulatory regions or fragments of genes are cut from one sequence and pasted into another (Blencowe, 2006; Barash et al., 2010). Neither gene is structurally homologous to its ancestral sequence. Transposable elements may alter the function of the gene in ways that generate a new product or function. Additionally, as discussed by Wagner and Lynch (2010) transposable elements can relocate transcription factor binding sites, which, if integrated into the host genome, can provide the basis for the evolution of new genetic regulation.

LATERAL GENE TRANSFER

Provision of new genetic information from lateral gene transfer, as discussed in the digestion of plant products by the cotton root-knot nematode *Meloidogyne incognita,* provides a clear and unequivocal example of biochemical/metabolic novelty. Both gene co-option and neofunctionalization require recruiting existing genetic architecture for a new task. Just as finding true morphological novelties is often confounded by the discovery of intermediate fossils (Li et al., 2008) or closer scrutiny of developmental processes (Nagashima et al., 2009), novel genetic architecture typically originates from inherited genetic material. However, this need not always be the case. Novel genetic material, which is not inherited by vertical transmission, can be acquired from other taxa through lateral gene transfer.

Recent studies have promoted lateral gene transfer as way to generate genetic, and eventually morphological novelty. The lateral transfer of DNA is common within the microbial world, and often results in difficulties in resolving deep evolutionary relationships (Lane and Archibald, 2008). Recent studies have also found gene transfers between microbial symbionts and their animal hosts (Dunning Hotopp et al., 2007 for *Wolbachia*; Rumpho et al., 2008 for *Vaucheria litorea*), and even heritable viral genomes (Arbuckle et al., 2010) and trypanosome retro-transposons (Hecht et al., 2010) in humans.

DNA transfers in metazoans rarely account for morphological change. However, the tools to identify transferred genes and their function have only recently become available. For instance, genome sequencing reveals that the unique orange color polymorphism of pea aphids is attributable to an acquisition of fungal carotenoid genes, which are not found in other animal genomes (Moran and Jarvik, 2010). Obviously aphid color polymorphisms are not as dramatic as the traditional "novelties" interpreted from the fossil record, which are typically are associated with "macroevolutionary" events (Gould and Eldredge, '77).

The *Bauplan* changes of turtles or origins of the digits may no longer be considered novel. However, periods of rapid evolutionary change, such as the evolutionary origin of new phyla during the Cambrian may still qualify, if only because we lack intermediate fossils between dramatically different body plans. Interestingly, both viral-mediated transposons (Erwin and Valentine, '84) and lateral gene transfer (Valentine, '73) have been proposed as drivers of the abrupt morphological novelties that arose during the Cambrian explosion. These fascinating yet untestable hypotheses presage recent discoveries in the importance of lateral gene transfer in genomic evolution. Of course these are still early days in our understanding of the prevalence or relevance of lateral gene transfers and their ability to generate morphological novelties.

CONCLUSIONS

As indicated in the introduction, understanding novelty requires a deep knowledge of phylogenetic relationships, of shared development/gene pathways, and the discovery of new patterns of gene co-option and gene regulation (Brigandt and Love, 2010; Wagner and Lynch, 2010 for literature).

To reiterate the concept used at the outset, "a novelty is a new feature (structure, or behavior) in a group of organisms (taxon) that is not homologous to any feature in any taxon in the ancestral lineage." To this conception, we add that *genetic novelties can arise through acquisition of nonhomologous genetic information* through mechanisms such as lateral gene transfer. Gene co-option, duplication and neofunctionalization, and mobile genetic elements, which modify existing (homologous) genetic information, also provide a basis for the appearance of novelties in which the nonhomology is at the level of the phenotype and novel gene function. Therefore, assessment within different levels of the biological hierarchy will effect whether a particular features are designated as novel.

Of the examples discussed, the origination of tetrapod limbs (or digits) and turtle shells are both presaged by homologous features in recently described fossils. Therefore, these classic examples of novel phenotypes no longer fit the nonhomology criteria for novelty. Origination of the neural crest and NCCs in vertebrates and of casquettes in cave fish are based on co-option of existing (plesiomorphic) GRNs or the component parts of existing networks and/or co-option of existing developmental mechanisms and so are novelties at the level of phenotype and gene function. The convergence in innervation and function of casquettes and cement glands is a striking example of parallel evolution and/or convergence. Even though they have evolved multiple times, the absence of these organs in ancestors would lead to them being classified as novelties, although further knowledge of the deep homologies of the processes underlying cement gland and casquette formation might change this designation on the genetic level. Novel functions associated with lateral gene transfer as in the nematode demonstrate that systems are available in which acquisition of new genetic information *and* new features of the phenotype can be examined for the links between genotype and phenotype in the acquisition of a novelty. The identification of novelty is often a matter of first setting an investigative framework within the biological hierarchy. However, occasionally novel phenotypes can be linked to novel genetic information that is acquired from extra-parental sources.

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