

windborne odors characteristic of each direction. The pigeons supposedly memorize the scents and associate them with direction; when taken to a release site, they need only sniff the air to determine along which olfactory axis they have been displaced. The odor serves as a reverse beacon [7,8].

The olfactory hypothesis has engendered an enormous number of tests [10]. Some sought to manipulate wind direction or the incoming odors, often with elaborate clear plastic baffles or vanes; still others altered the odors experienced during transport or while homing. In other tests, the olfactory nerve was cut or anesthetized before release. Often these manipulations had an effect; other times would-be replications failed, or produced quite unexpected deviations from the predictions [14]. In certain cases the changes, which should have affected a map, actually involved the compass sense. The discovery that birds use the patterns of polarized UV light at the horizon for compass calibration seems to provide a partial explanation: glass and plastic attenuate UV, rotate the polarization plane, and reflect misleading cues from other directions. Cutting the nerve, in addition to the distracting trauma involved, could damage the site of the putative magnetic-map sense: in a clear-cut case of malevolent design, this magnetite-based organ is located adjacent to the olfactory nerve. In any case, the orientation of nerve-cut pigeons is merely reduced, not abolished. Anesthetization of the nerve, by contrast, has no effect.

Adding or withholding odors during transport or after release does indeed seem to alter the strength of initial orientation in first-flight birds more consistently than any other olfactory manipulation. It is this phenomenon that drew the attention of Jorge *et al.* [9]. If odor is irrelevant to the map sense, how could manipulating it during transport cause a change? They asked whether odor might actually be a priming stimulus, a sensory input that triggers a behavioral state without actually creating or orienting a response, as odors are known to do in a variety of contexts [15,16]. The authors provided birds during transport with either bottled odorless air, ambient air, or artificially and variably scented bottled air. The first-flight pigeons were released at the

relatively unchallenging distance of 8 km. While only the ambient-air birds could have hoped to receive olfactory-map information, the scented-air birds oriented just as well. The odorless-air birds, by comparison, were largely disoriented. This looks very much like a primer effect — an olfactory wake-up call. Quite simply, the presence of natural odors seems irrelevant to successful homing. This test deserves a prize for its elegant simplicity.

After the birds had some actual flight experience at relatively short ranges, a test at 24 km showed that none of the treatments any longer had an effect. Real odors, fake odors, or no odors, the pigeons adopted accurate homeward bearings. By this time conventionally reared pigeons have shifted from relying on cues sensed during the outward journey to the cues actually present at the release site. According to the magnetic-map model, local flight experience allows the birds to measure the direction and steepness of local gradients, and use this information to extrapolate the displacement [4,5,14]. The unprimed first-flight birds, the argument goes, were not paying attention; the primed birds were busy trying to measure the gradients. The more experienced birds, primed or not, had already estimated the relevant directions and slopes, and only needed the values at the release site to place themselves on this learned grid. We should keep in mind, however, that the results of Jorge *et al.* [9] provide no direct support for this alternative model; they do, however, seriously undermine the olfactory hypothesis. Whether the odor model can recover remains to be seen.

References

- Keeton, W.T. (1974). The orientational and navigational bases of homing in birds. *Adv. Study Behav.* 5, 47–132.
- Thorup, K., Bisson, I.A., Bowlin, M.S., Holland, R.A., Wingfield, J.C., Ramenofsky, M., and Wikelski, M. (2007). Evidence for a navigational map stretching across the continental U.S. in a migratory songbird. *Proc. Natl. Acad. Sci. USA* 104, 18115–18119.
- Schmidt-Koenig, K., and Walcott, C. (1978). Tracks of pigeons homing with frosted lenses. *Anim. Behav.* 26, 480–486.
- Gould, J.L. (1980). The evidence for magnetic sensitivity in birds and bees. *Am. Sci.* 68, 256–267.
- Lohmann, K.J., Lohmann, C.M.F., and Putman, N.F. (2007). Magnetic maps in animals: nature's GPS. *J. Exp. Biol.* 210, 3697–3705.
- Gould, J.L., and Gould, C.G. (2010). *Animal Navigation*. (Princeton: Princeton University Press).
- Papi, F. (1976). The olfactory navigation system of homing pigeons. *Verh. Dtsch. Zool. Ges.* 69, 184–285.
- Wallraff, H.G. (1981). The olfactory component of pigeon navigation. *J. Comp. Physiol.* 143, 411–422.
- Jorge, P.E., Marques, A.E., and Philips, J.B. (2009). Activational rather than navigational effects of odors on pigeon homing. *Curr. Biol.* 19, 650–654.
- Wallraff, H.G. (2005). *Animal Navigation: Pigeon Homing as a Paradigm*. (Berlin: Springer Verlag).
- Wiltschko, R., and Wiltschko, W. (1985). Pigeon homing: change in navigational strategy during ontogeny. *Anim. Behav.* 33, 583–590.
- Kramer, G. (1959). Über die Heimfindeleistung uter Sichtbegrenzung aufgewachsener Brieftauben. *Verh. Dtsch. Zool. Ges. Frankfurt a. M.* 1958, 168–176.
- Wallraff, H.G. (1967). The present status of our knowledge about pigeon homing. *Proc. 14th Intl. Ornith. Cong.* 1967, 331–358.
- Gould, J.L. (1982). The map sense of pigeons. *Nature* 296, 205–211.
- Hinde, R.A. (1970). *Animal Behaviour*. (New York: McGraw Hill).
- Rouby, C., Schaal, B., Dubois, D., Gervais, R., and Holley, A. (2002). *Olfaction, Taste, and Cognition* (Cambridge: Cambridge University Press).

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Animal Evolution: Once upon a Time

The evolutionary relationships between the earliest branches of the animal kingdom – bilaterians, cnidarians, ctenophores, sponges and placozoans – are contentious. A new phylogenomic analysis suggests a return to old ideas.

Maximilian J. Telford

Understanding the emergence of the multicellular animals, and their subsequent evolution into the complex creatures we see around us, is made difficult by the passage of half a billion

years. The fossil record is one potential source of information for reconstructing these events, but fossils from the relevant period — the Ediacaran and Cambrian — are not only rare, but are particularly difficult to interpret; by definition, such ancient

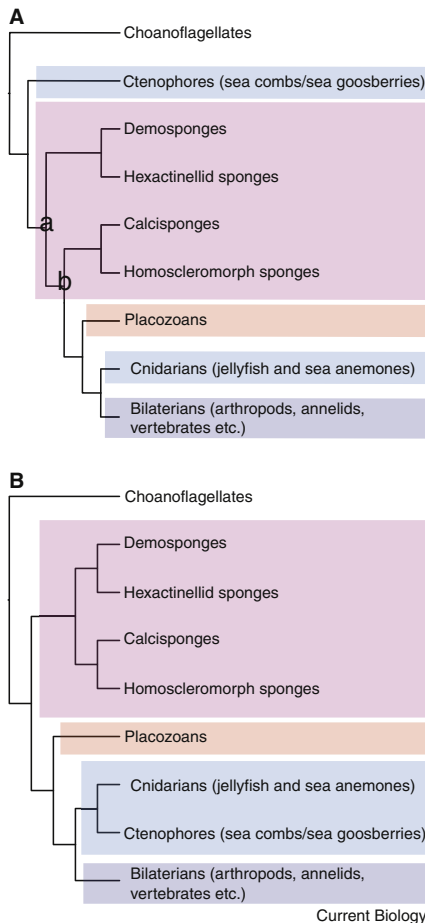


Figure 1. Old and new phylogenies of basal metazoan groups.

(A) According to some recent analyses the ctenophores are the most basal branch [6] and the sponges form two separate (paraphyletic) groups with the calcisponges and homoscleromorphs more closely related to the eumetazoans (placozoans, cnidarians and bilaterians) than to the other sponges (reviewed in [5]). This topology implies that the complexity of ctenophores and eumetazoans has been convergently evolved and that the ancestors at 'a' and 'b' were sponge like, implying that the eumetazoans are therefore derived sponges. (B) Basal metazoan relationships according to Philippe *et al.* [3]. Sponges are monophyletic and ctenophores are part of the eumetazoan clade. This phylogenetic tree closely resembles traditional morphology-based views of basal metazoan evolution.

intermediates cannot be interpreted within the framework of modern animal body plans [1]. One more readily available source of data covering some of these early transitions is derived from the few lineages that have survived. A correct evolutionary interpretation of the characteristics of such groups depends, however, on knowledge of their phylogenetic

relationships [2]. The application of molecular systematics to the relationships of the basal metazoan groups has resulted in contradictory and confusing results, but a study in this issue of *Current Biology* suggests that progress is being made [3].

The Traditional View

Most of the animals with which we are familiar fall into a single group called the 'bilaterians'. Four further distinct groups of animals are commonly recognised: the cnidarians (jellyfish and sea anemones), ctenophores (sea gooseberries/sea combs), porifera (sponges) and the placozoan *Trichoplax adhaerens*, which is so unfamiliar it has no common name. Knowing the relationships between these four very different groups and their positions relative to the bilaterians is important because mapping their characteristics onto a correct phylogenetic tree may enable us to infer the series of evolutionary events that led to the emergence of the bilaterians.

The traditional view of the relationships of these animal groups is based on their morphology and embryology. The cnidarians and ctenophores have been considered as lying closest to the bilaterians because they possess a nervous system and muscles, gastrulation, which results in ectoderm and endoderm, as well as true epithelia; the cnidarian–ctenophoran–bilaterian clade is referred to as the 'Eumetazoa'. Sponges lack these characters and have generally been considered the most basal metazoan group; fossil evidence for sponges also long predates the earliest clear ctenophore, cnidarian or bilaterian remains. The position of the placozoans has been the most difficult to understand, but the presence of epithelial tissue has suggested they are closer to the eumetazoans than to the sponges [4].

The nested series of increasingly complex characters — multicellularity, epithelial organisation, gastrulation and finally bilaterality and triploblasty — in the clades discussed above are naturally seen as a phylogenetic series indicating the route taken by evolution to arrive at the bilaterians. This morphology-based view and the evolutionary scenario it supports has, however, experienced repeated challenges from molecular systematic studies over the past few years.

Stirrings at the Bottom

The two main points of contention (ignoring for now the ever problematic placozoans) concern the question of whether sponges form a unique group (monophyly) or if some sponge groups are closer to the eumetazoans than others (paraphyly) as well as, most recently, the phylogenetic position of the ctenophores. There are four main lineages of sponge, the calcisponges, homoscleromorphs, demosponges and hexactinellids. All four groups have simply organised, sessile adults, very unlike the other metazoans, and share unique features — most notably a water canal system. Several molecular analyses in the past few years have concluded, however, that the sponges do not form a monophyletic group (Figure 1), suggesting instead that the calcisponges and/or homoscleromorphs are more closely related to the eumetazoans than they are to the other sponge groups (see references in [5]). If correct, this paraphyletic arrangement of sponges would have enormous consequences for our understanding of the evolution of the animals, because it suggests that the common ancestor of the eumetazoans and calcisponges was itself a sponge: any alternative scenario requires unparsimonious repeated evolution of 'sponge-ness'. This phylogenetic arrangement of sponge groups ultimately implies that all eumetazoans are very derived sponges [5].

We have little idea of what the precursors to the eumetazoans may have looked like and so, while unconventional, the idea of a sponge ancestor isn't entirely far fetched. A second recent result concerning the phylogenetic position of the ctenophores, however, is more difficult to explain — especially if the sponges really are paraphyletic. In an influential phylogenomic analysis of metazoan relationships, Dunn *et al.* [6] found that the two ctenophore representatives in their sample were placed right at the base of the animal tree, more basal than even their sponge exemplars (both demosponges) which were grouped, with low statistical support, alongside the cnidarians [6].

Taken at face value, this topology implies that the morphologically simple sponges are derived from an animal that must have been fairly complex, something that has given rise to

ctenophores, cnidarians and bilaterians. If we were to accept both the basal ctenophore idea and the paraphyly of sponges, our picture of animal evolution becomes very convoluted, as this topology suggests that the advanced characteristics of ctenophores, such as muscles and nerves, must have evolved independently of the same characteristics found in eumetazoans.

Plus Ça Change, Plus C'Est la Môme Chose

In this issue of *Current Biology*, Hervé Philippe and colleagues [3] present a new analysis that focuses on each of the above questions, providing new data from several members of all of these groups, including sequences from all four sponge clades. Their phylogenomic analysis of 129 concatenated protein-coding genes assembled from whole genome sequences and EST sequence databases results in a very large data set (30,257 aligned amino acids) that covers the different groups well.

One important benefit of including more taxa is to divide long branches, thus reducing the effects of long branch attraction. Long branch attraction is an infamous source of systematic error in phylogenetic tree reconstruction; it results from unrelated rapidly evolving lineages ('long branches') evolving away from their short-branched neighbours yet occasionally undergoing identical nucleotide substitutions; these occasional convergent changes in unrelated long-branched taxa result in their being erroneously grouped together. In addition to the increased breadth and depth of data sampling compared to previous studies, Philippe *et al.* [3] have made two further important improvements, both intended to counteract long branch attraction. The first is the use of the CAT model of sequence evolution used to reconstruct the tree as this has been shown to be less susceptible to long branch attraction errors than are other models. The CAT model recognises that different portions of a protein have different substitution patterns; a transmembrane domain, for example, will have frequent exchanges between hydrophobic amino acids but very rarely will a hydrophilic amino acid appear. Their second contribution is to root their trees using subsets of

outgroup taxa (assorted unicellular eukaryotes) with different branch lengths. The expectation is that as error-causing long-branched outgroups are removed, correct nodes of the tree should gain in statistical support.

The result of their efforts is to return us to the traditional *status quo*: the four sponge groups form a monophyletic unit and the ctenophores are not found at the base of the tree but are grouped with the cnidarians. As their experimental approach was specifically designed to counteract long branch attraction, the implication is that previous findings of paraphyletic poriferans and basal ctenophores [5,6] were the result of long branch attraction. This inference is borne out by the experiments altering the outgroup: as they removed long-branched outgroups they observed an increase in statistical support both for poriferans and for 'coelenterates' (the old name given to the group comprising cnidarians and ctenophores). The placozoans, meanwhile, are found as the sister group to the eumetazoans although this position has only weak statistical support.

The results from Philippe *et al.* [3] are credible as their experiments were designed to tackle long branch attraction and they successfully demonstrate its effects. Their phylogenetic conclusions also seem reasonable in terms of what we know of the morphology of these groups. The idea of the independent evolution of muscles, nerves etc. in ctenophores and other eumetazoans was particularly hard to explain. The revised tree returns us to a scheme in which there are just three branches of living animals on the path to the bilaterians (poriferans, placozoans, and coelenterates) where, according to recent molecular phylogenies, there might have been five: (ctenophora, calcisponges and homoscleromorphs, demosponges and hexactinellids, placozoans as well as cnidarians). One consequence of this reduction in the number of basal metazoan branches is the loss of a degree of resolution in our picture of the stages of evolution leading to the Bilateria.

This new tree will undoubtedly be tested by other researchers; if correct, we can expect confirmation of the

proposed clades from other phylogenetically informative traits, such as rare genomic changes discovered within the genomes of these taxa [7]. Once the dust eventually settles around the phylogeny of the basal metazoans (the question of the position of the placozoans seems still to be wide open), further progress in understanding bilaterian origins will come from two sources. The first area for progress will use comparative developmental data to extend our understanding of deep homology of embryological processes and morphological characters. This approach is exemplified by work showing that both of the supposedly diploblastic phyla cnidarians and ctenophores have probable homologs of mesodermal tissue [8,9]. The second source of progress must come from the palaeontologists, who have a unique window into this ancient period of evolution.

References

1. Donoghue, P.C., and Purnell, M.A. (2009). Distinguishing heat from light in debate over controversial fossils. *BioEssays* 31, 178–189.
2. Telford, M.J., and Budd, G.E. (2003). The place of phylogeny and cladistics in Evo-Devo research. *Int. J. Dev. Biol.* 47, 479–490.
3. Philippe, H., Derelle, R., Lopez, P., Pick, K., Borchiellini, C., Boury-Esnault, N., Vacelet, J., Renard, E., Houliston, E., Quéinnec, E., *et al.* (2009). Phylogenomics revives traditional views on deep animal relationships. *Curr. Biol.* 19, 706–712.
4. Srivastava, M., Begovic, E., Chapman, J., Putnam, N.H., Hellsten, U., Kawashima, T., Kuo, A., *et al.* (2008). The *Trichoplax* genome and the nature of placozoans. *Nature* 454, 955–960.
5. Nielsen, C. (2008). Six major steps in animal evolution: are we derived sponge larvae? *Evol. Dev.* 10, 241–257.
6. Dunn, C.W., Hejnol, A., Matus, D.Q., Pang, K., Browne, W.E., Smith, S.A., Seaver, E., Rouse, G.W., Obst, M., Edgecombe, G.D., *et al.* (2008). Broad phylogenomic sampling improves resolution of the animal tree of life. *Nature* 452, 745–749.
7. Rokas, A., and Holland, P.W.H. (2000). Rare genomic changes as a tool for phylogenetics. *Trends Ecol. Evol.* 15, 454–459.
8. Martindale, M.Q., Pang, K., and Finnerty, J.R. (2004). Investigating the origins of triploblasty: 'mesodermal' gene expression in a diploblastic animal, the sea anemone *Nematostella vectensis* (phylum, Cnidaria; class, Anthozoa). *Development* 131, 2463–2474.
9. Martindale, M.Q. (2005). The evolution of metazoan axial properties. *Nat. Rev. Genet.* 6, 917–927.

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