Only time — and pressure — will tell.

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Evolutionary biology

Even-toed fingerprints on whale ancestry

Michel C. Milinkovitch and J. G. M. Thewissen

Both morphological and molecular studies indicate that cetaceans (whales, dolphins and porpoises) and artiodactyls (even-toed ungulates, which include pigs, hippos, camels and ruminants) form a clade or monophyletic group — that is, they have a common ancestor that is not shared by any other group of mammals. This is counter-intuitive, because it implies that a cow is more closely related to a dolphin or a whale than to a pig or a camel (Fig. 1).

Although it is compatible with earlier molecular analyses (for example, refs 4, 5), the idea that cetaceans are highly derived artiodactyls was first suggested in 1994 on the basis of mitochondrial and nuclear DNA and amino-acid sequences. The idea was corroborated by other phylogenetic analyses of DNA sequences. But the issue is still controversial, because the exact means by which molecular sequence data should be analysed remains debated — although analytical settings that are particularly meaningful with respect to phylogenetic inferences can probably be identified in specific instances. But, basically, many morphologists consider that molecular data are necessarily more noisy than morphological data.

The analyses by Shimamura and colleagues now provide a remarkable example of molecular markers, which should lead...
morphologists to re-examine what might have misled them for more than a century. The authors report phylogenetic interpretations of nine retropositional events that led to the insertion of so-called ‘short interspersed elements’ at particular loci in the nuclear genome of various artiodactyls and cetacean ancestors. Three of these events unambiguously support the grouping of cetaceans, hipppos and ruminants in a clade, and the other six provide a partial resolution of the relationships within that clade. Because the likelihood of these elements being independently inserted at the same locus in different lineages (or precisely excised) seems virtually nil, these markers can reasonably be considered to be essentially noise-free.

So, these molecular results may prompt a serious revision of how we view morphological transformations in whales and artiodactyls. Three salient features of artiodactyls are: first, the axis of symmetry of hand and foot runs between the third and fourth digit (paraxony); second, the heel has developed an extremely mobile joint at a place where most mammals have a barely mobile joint; and third, the last lower milk molar consists of three rows of cusps (three-lobe deciduous lower premolar 4, DP/4). Although paraxony is a striking feature, it also occurs in primitive whales2,12 and it is uninformative for the issue at hand. But the remodelled heel is a different matter — because it is present in all artiodactyls, and in no other mammal, this character is classically interpreted as derived and, therefore, as supporting artiodactyl monophyly.

The presence of the mobile joint in artiodactyls can be inferred from bones: there is a pulley (or trochlea) on the distal part of the astragalus (one of the heel bones) — a so-called trochleated astragalar head. This led to efficient and fast locomotion in the earliest artiodactyls. Although some features of the artiodactyl heel are present in other mammals such as rabbits, the astragalar head is never trochleated. In modern cetaceans, the hindlimb is so greatly reduced that the heel cannot be recognized, and no complete functional astragalus is known for a fossil whale. However, the mesonychians — a group of land mammals that is considered to be the closest extinct relative of cetaceans — also lack a trochleated astragalus. So if Shimamura and colleagues’ hypothesis is correct, then either mesonychians are not closely related to cetaceans (and many dental characters are convergent), or the specialized heel morphology is not the exclusive character that many morphologists take it to be. It may have evolved several times independently in artiodactyls, or have been lost in the mesonychian/cetacean clade. The complete astragalus of an early cetacean would probably shed light on this issue.

The hypothesis put forward by Shimamura et al. also clashes with the DP/4 character — the tooth has three lobes in all artiodactyls, but not in early cetaceans or mesonychians. If the new molecular data are correct, the morphology of this tooth has, like the trochleated astragalus, a complicated phylogenetic history that includes reversals, convergences, or both. Dental differences could reflect dietary differences, because both early cetaceans and mesonychians were probably carrion feeders or carnivores, whereas all early artiodactyls were omnivores or herbivores. But many morphological systematists are reluctant to let functional arguments influence their evaluation of characters.

What characters, besides the molecular ones described by Shimamura et al. and by Gatesy, do hipposs, ruminants and cetaceans have in common that makes them different from pigs, peccaries and tylopods (camels and llamas)? Morphological studies have usually upheld close genealogical ties among pigs, peccaries and hipposs (and the larger group that includes hippos, anthracotheroids), but the similarities are usually uninformative primitive characters that are also present in the ancestral artiodactyl, or features that are subject to rampant convergences (such as certain dental characters, see Fig. 1). Shimamura et al. also suggest that artiodactyls and cetaceans diverged in the Cretaceous — about 15 million years before they are found in the fossil record. However, if hippos are closely related to cetaceans, and if mesonychians are not, then the discrepancy in the times of origin is considerably less: it amounts to the difference between the origin of cetaceans (approximately 50 million years ago) and that of anthracotheroids (around 49 million years ago). But because of the rapid and unique specialization of cetacean morphology, few characters can be recruited to bolster the grouping of ruminants, hipposs and cetaceans into a clade. Recovery and study of the earliest cetaceans would probably help to resolve this problem.

Few molecular studies rule out previously accepted morphological trees as convincingly as that of Shimamura et al. However, the face of phylogenetic science itself is changing rapidly — it is becoming more objective and less inductive. For example, phylogeneticists no longer need to ask whether molecular data are superior or inferior to morphological data, because the signal-to-noise ratio in morphological, molecular and combined data sets can now be measured directly, even before examining phylogenetic trees11. In any case, the new analyses indicate that the use of retropositional events as molecular markers may define a new power of resolution in estimating phylogenies. This method could even bring to a close some of the most intensive controversies12 in the field, such as whether the toothed whales12 are monophyletic or paraphyletic, and likewise for the rodents13.
Over the past two decades, putative neurotransmitters have proliferated, from biogenic amines to amino acids, neuropeptides and, most recently, gases such as nitric oxide and carbon monoxide. Amidst the ferment over recently discovered messengers, scant attention has been paid to an atypical neurotransmitter whose candidacy dates back almost 30 years—adenosine. But the field may now be rejuvenated, thanks to a backer almost 30 years—adenosine. But the field may now be rejuvenated, thanks to a backer almost 30 years—an adenosine receptor subtype, A2a.

Neurotransmission by adenosine and ATP—a designated ‘purgineur’ by Burnstock1—is difficult to study because both molecules are involved in many pathways of general cellular metabolism. Nonetheless, the evidence for adenosine as a neurotransmitter is as persuasive as for any other substance; immunohistochemical studies have shown that adenosine is found in discrete neuronal populations in which those cells that use it as a neurotransmitter have much higher localized densities; an efficient, energy-requiring uptake system exists which could account for synaptic inactivation of adenosine; and adenosine has prominent functions outside the brain. Studies by Berne4 have shown that oxygen starvation leads to a massive accumulation of adenosine, which causes vasodilation and increased blood flow.

The most compelling evidence for the specific actions of adenosine has come from the characterization of its receptors. The influences of adenosine on levels of cyclic AMP in brain slices provided the first molecular evidence for the function of receptors and differentiation of subtypes. At nanomolar concentrations, adenosine lowers the activity of adenylyl cyclase (which converts ATP to cAMP) through adenosine A1 receptors, whereas micromolar levels augment adenylyl-cyclase activity at A2 receptors.

Xanthines and caffeine both block adenosine receptors, and this ability could account for their effects as behavioural stimulants. Xanthine derivatives can block adenosine receptors in direct proportion to their potencies as behavioural stimulants, and at concentrations similar to blood levels of caffeine after a few cups of coffee. The bronchodilating, antiasmatic effects of theophylline and other xanthines may involve aortic-arterial-pressor blockade, although the evidence is controversial.

Molecular-biological techniques have further discriminated receptor subtypes, specifically A1, A2a, A2b, and A3 (ref. 9). The A2a receptor (which stimulates adenylyl cyclase) is particularly abundant in the basal ganglia, blood vessels and platelets, so it was selected by Ledent et al.10 for targeted deletion.

The A2a-receptor knockout mice seem to be normal on a gross level, and they breed successfully. But whereas the A2a-agonist drug CGS-21680 reduces locomotor activity in wild-type mice, it has no behavioural effect in the knockouts. Strikingly, caffeine, which normally stimulates locomotor activity, substantially depresses activity in the knockout mice. These findings cement the conclusion that the stimulant effects of caffeine are derived from adenosine-receptor blockade. Why should caffeine depress behaviour in the knockouts? Adenosine analogues and caffeine can either stimulate or depress locomotor activity depending on the dose. So, in the knockouts, a stimulatory action of adenosine—perhaps through A1 receptors—may be masked.

Ledent et al. also found that the knockout mice seem to be more anxious and aggressive than wild-type animals. This fits with anecdotal clinical evidence that caffeine increases anxiety, and that stimulation of the adenosine receptor can relieve anxiety. Adenosine modulates pain pathways in complex ways. There is evidence that stimulation of A2a receptors on sensory pain fibres increases pain perception. The knockout mice show reduced pain responses, suggesting that—at least at A1 receptors—adenosine normally augments pain perception. This also fits with observations that caffeine can be anxiolytic.

Adenosine has many effects in the cardiovascular system; it inhibits platelet aggregation and dilates several vascular beds. The knockout mice show more efficient platelet aggregation than wild-type animals, and they are resistant to inhibition of platelet aggregation by adenosine analogues. Ledent et al. also found that the knockout mice have increased blood pressure, supporting a tonic vasodilatory role for adenosine.

Pinning down the diverse biological functions of A2a receptors should provide a route for the pharmaceutical industry to develop related therapeutic agents (Table 1). One possibility might be new cognitive stimulants. Although the relative merits of caffeine’s stimulant effects are a source for debate, its cognition-enhancing actions are well documented. Agents that elicit these beneficial influences, without side-effects such as tachycardia, increased urination and agitation, may treat cognitive impairment of the elderly. At present, despite the wealth of available analgesics, none is entirely satisfactory. Moreover, the complex effects of adenosine on pain perception have thwarted efforts to develop adenosine-based analgesics. The findings by Ledent et al.10 from the knockout mice favour selective A2a antagonists as non-addictive analgesics. Finally, antagonists to the A2a receptor might improve defects in blood clotting and vascular shock, whereas A2a agonists should decrease clotting and guard against low blood pressure.

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Table 1: Potential adenosine-receptor drug development based on the A2a knockout phenotype

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<tr>
<th>Phenotype</th>
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<tr>
<td>Altered locomotor response to caffeine</td>
<td>A₂ antagonists as cognition enhancers</td>
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<td>Apparent anxiety</td>
<td>A₁ agonists as anxiolytics</td>
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<tr>
<td>Lesser pain responses</td>
<td>A₂ antagonists as analgesics</td>
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<tr>
<td>Increased platelet aggregation</td>
<td>A₁ agonists as anticoagulants</td>
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<td>Hypertension</td>
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<td>A₁ agonists as shock</td>
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