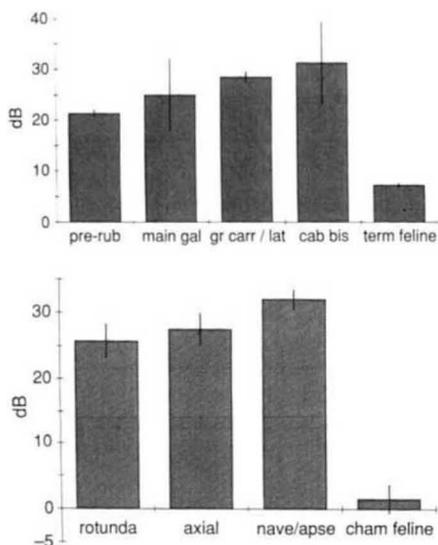


Sound and rock art

SIR — Neither the location nor subject matter of prehistoric rock art has yet been satisfactorily explained¹. Quantitative data from sites sampled in France, together with experience on two other continents, provide a possible explanation for the location and subject matter of Upper Palaeolithic parietal art.

Open-air art sites as well as deep decorated caves from the Upper Palaeolithic give reflected sound levels significantly above ambient. For example, at the open-air site of l'Oreille d'Enfer, echoes were measured at 53 ± 3 dB against a background of 45 ± 2 dB ($P < 0.0003$). Further, in the deep caves of Font-de-Gaume and Lascaux, the images of horses, bulls, bison and deer are found in regions with high levels of sound reflection, whereas feline art is found in regions of the caves with poor acoustics (see figure). The difference between reflected sound levels at ungulate versus feline art locations is statistically significant at $P < 0.0004$ within Font-de-Gaume and $P < 0.0001$ within



Comparison of reflected sound levels in different regions of Palaeolithic caves. Experiments with a sound-burst device were recorded using a PMD420 Marantz cassette recorder with a Dynamic LOZ Shure SM57 omnidirectional microphone, and analysed using a Brüel and Kjær model 2232 precision sound-level meter. The intensity of reflected sound is expressed on the value axis as decibels above background noise levels. Error bars represent one standard deviation of two to four replicate tests. Abbreviations are: pre-rub, pre-rubicon (entrance tunnel); main gal, main galleries; gr carr/lat, grand carrefour (intersection) and lateral gallery; cab bis, cabinet de bisons; term feline, terminal fissure with feline; rotunda (hall of bulls); axial, axial gallery; nave/apse, nave/apse area; and cham feline, chamber of felines. Top, Font-de-Gaume; bottom, Lascaux.

Lascaux. These results suggest an acoustic influence on both the placement and content of the art.

It has been previously observed that the shape of a cave exerted some general influence on the placement of species². Indeed, shape is one major determinant of cave acoustics. However, the highly sound-reflecting axial gallery decorated with ungulates and the acoustically dead chamber of felines in the same cave of Lascaux are both narrow dead-end tunnels, suggesting that the cave shape was influential only to the extent that it does affect the acoustics.

Echoes have been mentioned as a phenomenal attribute of certain rock art sites³, and a correspondence has been suggested between deep cave painting placement and locations that resonate at particular musical notes⁴. But no objective quantitative acoustical data have been reported, nor is there a comprehensive theory that understandably relates acoustics to the rock art content.

Statistical tabulations of the content of European Palaeolithic parietal art² can be reinterpreted as showing that more than 90% of the subjects depicted fall into the biological classes Artiodactyla, Perissodactyla and Proboscida,

Relations of fish and tetrapods

SIR — The evolutionary transition from fishes to tetrapods, which occurred about 400 million years ago, has come under intense scrutiny in the light of new molecular data and reinterpretation of the morphological evidence¹⁻⁴. There is little disagreement that tetrapods are most closely related to the lobe-finned fishes (sarcopterygians), whose members possess fin supports resembling tetrapod limb bones, and have, in at least some taxa, functional lungs. But there is debate over the relationship of the tetrapods to the two extant sarcopterygian lineages, the lungfishes and the coelacanth.

Although the traditional view^{5,6} supports the coelacanth as closest relative, some recent morphological analyses^{7,8} have favoured the lungfishes, or lungfishes + coelacanth, as sister group of the tetrapods. Previous molecular data^{1,3,9} have supported either the coelacanth or the lungfishes, and this question is generally considered to be unresolved². We have sequenced approximately three kilobases of mitochondrial DNA, including the entire 12S ribosomal (r) RNA and 16S rRNA genes, from the coelacanth and all three lineages of lungfishes. Phylogenetic analyses including all available vertebrate sequences for

collectively grouped as ungulates. Why might prehistoric humans have chosen to make images of hoofed mammals in sound-reflecting environments?

It is known that some ancient cultures considered echoing a supernatural phenomenon. Experimentation with the sound reflection at rock art sites reveals that percussion noises (from clapping or producing stone tools) can yield echoes that sound similar to the galloping of a horse, and that reverberation of percussion noises can sound like the thundering of a buffalo stampede. This phenomenon of hoofbeat-like echoes thus relates to the ungulate images as well as to the sound-reflecting canyons and caves where the art is typically located.

These measurements and observations suggest that Palaeolithic ungulate art was produced in response to percussive sound reflections perceived as hoofbeats. The production of hoofbeats via sound reflection could have been part of a ritual intended to summon up game.

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these genes support the lungfishes as the closest living relatives of the tetrapods.

We sequenced the genes for 12S rRNA, tRNA^{Val}, and 16S rRNA in *Latimeria chalumnae* (coelacanth, tissue courtesy of J. A. Musick), *Lepidosiren paradoxa* (South American lungfish), *Protopterus sp.* (African lungfish) and *Neoceratodus forsteri* (Australian lungfish, tissue courtesy of W. Bemis). We also sequenced a portion of the mitochondrial cytochrome *b* gene in *L. paradoxa* and *N. forsteri* for comparison with published data for that region in the other sarcopterygian fishes. The sequenced regions correspond to sites 648–3,229 (RNA genes) and 14,842–15,124 (cytochrome *b*) in the published human sequence. For phylogenetic analyses, we included all complete vertebrate sequences for these genes available in databases. Carp, a ray-finned fish (*Cyprinus carpio*), was used as the outgroup. The available tetrapods are frog (*Xenopus laevis*), chicken (*Gallus gallus*), human (*Homo sapiens*), seal (*Phoca vitulina*), cow (*Bos taurus*), whale (*Balaenoptera physalus*), mouse (*Mus musculus*) and rat (*Rattus norvegicus*).

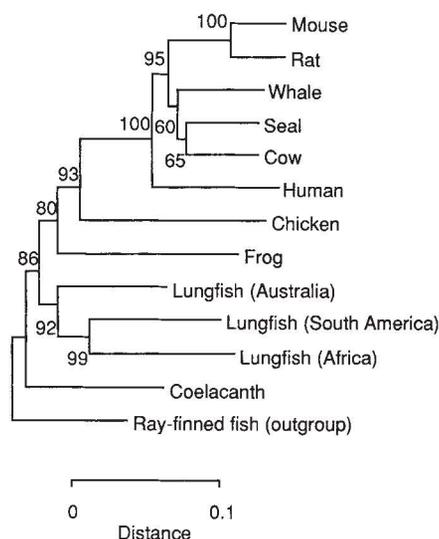
Two different methods of phylogenetic analysis, neighbour-joining and maximum parsimony, resulted in trees that

Phylogenetic tree of tetrapods and lobe-finned fishes based on mitochondrial DNA sequences of an approximately 3-kb region of the mitochondrial genome, including the entire genes for 12S rRNA, tRNA^{Val} and 16S rRNA, and a 283-bp portion of the cytochrome *b* gene. The neighbour-joining method was used with the Kimura distance to correct for different rates of substitution in transitions versus transversions, and for multiple hits. A total of 2,073 sites, of which 830 are variable, could be aligned (sites with deletions not used). Bootstrap P-values (10), from 0–100%, are indicated on the tree. A maximum parsimony analysis (not shown) resulted in a single most-parsimonious tree which also supported a lungfish–tetrapod relationship. DNA was extracted from liver and muscle samples stored at -70°C , amplified by PCR, and both complementary strands sequenced^{14,15} with the use of 23-oligonucleotide primers. For cytochrome *b*, we used two pairs of primers, each spanning the same region in *Lepidosiren* and *Neoceratodus*. The programs NJBOOT2 and Treeview (K. Tamura, Pennsylvania State University) were used to construct the neighbour-joining trees and perform the bootstrap analysis (2,000 replications). Our cytochrome *b* sequence for *Lepidosiren* differs slightly from that obtained elsewhere⁹ but both sequences, used separately, resulted in identical topologies. The programs Metree and Treeshow (A. Rzhetsky, Pennsylvania State University) were used to test the significance (from

support a lungfish–tetrapod relationship (see figure). Three statistical tests^{10,11} of this node on the phylogenetic tree gave similar results: bootstrap P-value (86%), branch-length significance (82%) and rejection of alternative topologies (89%). The same neighbour-joining topology was obtained using either a simple p-distance (proportion), a Jukes–Cantor distance to correct for multiple hits, or a Kimura distance to correct for transition bias and multiple hits.

The three lungfishes from different continents form a monophyletic group, with the South American and African species as sister taxa. Three already well-established groups, Tetrapoda, Amniota and Mammalia, are each monophyletic. This is the largest molecular dataset to address the question of the closest living relative of tetrapods, and these results support the findings of previous mitochondrial DNA studies that included fewer sites and taxa^{3,9}. A lungfish–tetrapod relationship is further supported by enzyme usage in the synthesis of urea¹². The phylogeny derived from the biochemical and molecular evidence provides an independent framework for interpreting the morphological changes involved in the transition from fishes to tetrapods.

Identification of the specific lineage of living fishes that is our closest relative is also important for understanding the evolution of non-fossilizable tetrapod traits and the genetics of tetrapod limb



zero) of internal branches in the neighbour-joining tree, and to compare the total sum of branch lengths of that tree with the length of alternative topologies¹¹. The new sequences reported here have been deposited in EMBL (Z21921-3, 21926-8). (A copy of the complete alignment has been deposited as supplementary information in the London editorial office of *Nature*; that, and other technical information, is available from S. B. H.)

development¹³. Although most molecular evidence strongly favours a lungfish–tetrapod relationship, DNA sequence data from additional slow-evolving genes should provide an increasingly robust phylogenetic framework for understanding vertebrate evolution.

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Weak queen or social contract?

SIR — There may be a simpler explanation for the experiment that Reeve and Nonacs reported as evidence for social contracts in wasps¹. Reeve and Nonacs observed increased aggression towards dominant alpha queens by beta queens when eggs were experimentally removed from the nest, and they interpret this as evidence for retaliation against alpha queens for eating eggs laid by the beta queen. Another interpretation consistent with these results is that the beta queen infers from the empty brood cells that the alpha queen is weak and might be vulnerable to a challenge. This would result in the observed escalated aggression by the beta queen and interference with the alpha queen's attempts to lay eggs, particularly by beta queens who are nearly as big as the alpha queen. This hypothesis also fits with West-Eberhard's finding² that alpha queens limit the reproduction of beta queens by restricting access to empty cells, as only the alpha queen begins new cells.

The weak-queen hypothesis is simpler than the social contract hypothesis because the former assumes only that the beta queen perceives that the cells are empty, and therefore that there is a problem with the alpha queen. The social contract hypothesis assumes that the beta queen perceives that the cells are empty, recognizes that some of those cells contained her eggs, and that she further assumes that the alpha female ate the eggs. Furthermore, she fails to recognize that most of the eggs the alpha queen ate she also laid. The weak-queen hypothesis also accords better with what we know about egg recognition. Eggs are thought to be recognized for only the brief time during which they are guarded^{2,3}. In this species eaten eggs were on average 11.4 minutes old, and females guarded their eggs for up to 20 minutes².

Another advantage of the weak-queen hypothesis is that it provides a more natural explanation of why the beta and not the alpha queen increases aggression. The alpha queen would not increase aggression as she is already the dominant queen. Under the social contract hypothesis, the alpha queen encountering empty cells formerly containing eggs could also think that the social contract had been broken by the beta queen. It would then follow that the alpha queen should also increase aggression when eggs are experimentally removed.

The beta queen was observed by Reeve and Nonacs to increase aggression towards the alpha queen in July and not