concerned. The strikingly derived nature of the Neanderthal labyrinthine features, already present before birth, and the apparent lack of continuity with modern human morphology could be seen as an argument in support of distinguishing between Neanderthals and modern humans at the species level.

The evidence from Arcy indicates that advanced Châtelperronian industries were used by late Neanderthals, suggesting a high degree of acculturation. The association of the Arcy Neanderthal with personal ornaments so similar to those found in contemporary and nearby Aurignacian layers[14,29] questions the nature of the cultural interactions with modern humans. At least in the case of these specific objects, we may be facing evidence of a trading process rather than the result of technical imitation of modern human technology by Neanderthals. ☐

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Continental breakup and the ordinal diversification of birds and mammals

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THE classical hypothesis for the diversification of birds and mammals proposes that most of the orders diverged rapidly in adaptive radiations after the Cretaceous/Tertiary (K/T) extinction event 65 million years ago [1-3]. Evidence is provided by the near-absence of fossils representing modern orders before the K/T boundary[4,5]. However, fossil-based estimates of divergence time are known to be conservative because of sampling biases[6], and some molecular/time estimates point to earlier divergences among orders[7-10]. In an attempt to resolve this controversy, we have estimated times of divergence among avian and mammalian orders with a comprehensive set of genes that exhibit a constant rate of substitution. Here we report molecular estimates of divergence times that average about 50–90% earlier than those predicted by the classical hypothesis, and show that the timing of these divergences coincides with the Mesozoic fragmentation of emergent land areas. This suggests that continental breakup may have been an important mechanism in the ordinal diversification of birds and mammals.

Molecular time estimation of evolutionary divergence requires genes that are evolving at a relatively constant rate, which often limits the number of sequences available for analysis[8,9]. Fortunately, the widespread use of model organisms for genetic

FIG. 1 Relationship between the evolutionary histories of mammals and birds and events in earth history during the Mesozoic and Cenozoic. *a*, Fossil record of the orders of eutherian mammals and birds[4] (solid line, certain; broken line, uncertain); avian taxonomy from ref. 29. *b*, Molecular estimates of divergence times based on constant-rate genes (from Table 1); the number of genes (constant rate/total) is in parentheses; filled circles, mean; horizontal bar, ±s.e.; thin vertical line, median. *c*, Land breakup (fission) and fusion events determined from reconstructions of emergent land areas during the last 250 Myr (ref. 30); only emergent land areas $> 3 \times 10^6$ km^2 (about the size of India) are considered here, although the same pattern is evident if smaller areas (say, the area of Madagascar) are considered. *d*, Total emergent land area exposed at different times during the last 250 Myr (ref. 30).

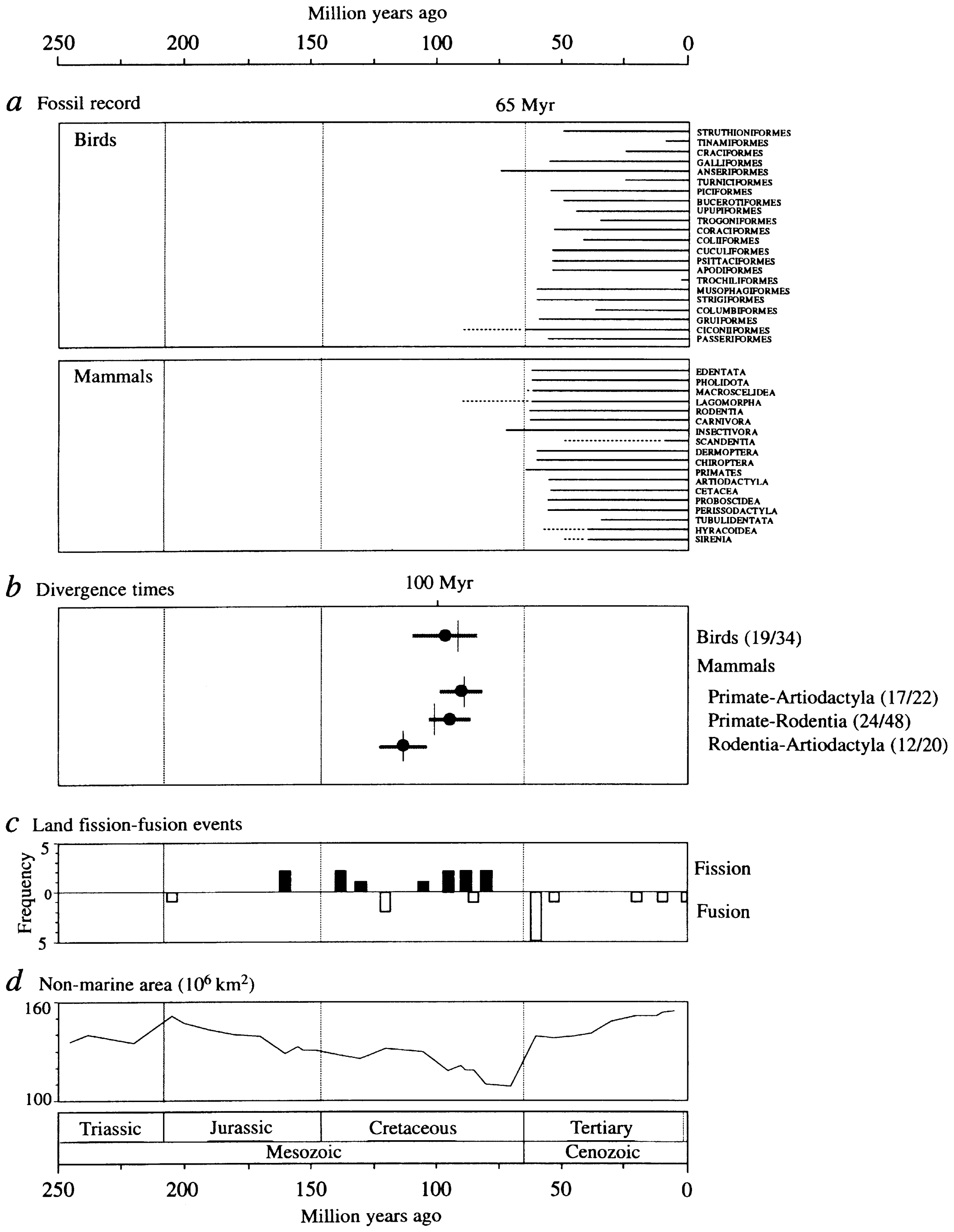
▶

METHODS. For avian comparisons (in *b*), the nuclear genes used and taxa are (g, galliform; a, anseriform; c, columbiform; s, struthioniform): acylphosphatase–muscle$_{ga}$, alcohol dehydrogenase$_{gs}$, α-crystallin$_{gacs}$, α-globin$_{gacs}$, argininosuccinate lyase$_{ac}$, β2 thyroid hormone receptor$_{hm}$, β-globin$_{gacs}$, cytochrome *c*$_{gacs}$, embryonic α-globin$_{ga}$, histone 1$_{ga}$, lactate dehydrogenase B$_{ga}$, lysozyme c$_{ga}$, malic enzyme$_{ac}$, MX protein$_{ga}$, prolactin receptor$_{gc}$, and somatotropin$_{ga}$ (for all genes, sequences of a mammal and an outgroup (or paralogous sequence) also were used). For mammalian comparisons, the nuclear genes used and taxa are (h, *H. sapiens*; m, *M. musculus*; b, *B. taurus*): acetylcholine receptor α_{hmb}, acetylcholine receptor δ_{hmb}, acetylcholine receptor γ_{hm}, α-globin$_{hmb}$, annexin II$_{hm}$, Bc12–Ig fusion gene$_{hm}$, B-myb$_{hm}$, casein kinase II α_{hb}, CD18-integrin β-2$_{mb}$, CDC2$_{hmb}$, c-kit proto-oncogene$_{hm}$, C-myb$_{hmb}$, connexin 43$_{hmb}$, cyclin A$_{hmb}$, cyclophilin B$_{hb}$, c-yes protein$_{hm}$, E-cadherin$_{hm}$, erythroblast virus oncogene homologue 2$_{hm}$, fatty-acid-binding protein$_{hmb}$, focal adhesion kinase$_{hm}$, follistatin$_{hmb}$, GATA-3$_{hm}$, histone H2A.X$_{hm}$, lactate dehydrogenase A$_{hmb}$, lamin B1$_{hm}$, midkine protein$_{hm}$, MOS proto-oncogene$_{hm}$, muscle pyruvate kinase$_{hm}$, myelin proteolipid protein$_{hmb}$, Myf-5$_{hm}$, Na$^+$/K$^+$ ATPase β-2$_{hm}$, NAD$^+$ ADP-ribosyltransferase$_{hmb}$, N-cadherin$_{hm}$, neural cell adhesion molecule$_{hmb}$, neurotrophin-3$_{hm}$, NF-κ-B$_{hm}$, N-myc$_{hm}$, nucleolar protein N038$_{hm}$, nucleolin$_{hm}$, ornithine decarboxylase$_{hmb}$, p53 cellular tumour antigen$_{hmb}$, S6 II kinase$_{hm}$, SPARC$_{hmb}$, transcription factor Eryf-1$_{hm}$, TGF-β2$_{hm}$, TGF-β3$_{hm}$, transglutaminase$_{hmb}$, tyrosine phosphatase$_{hm}$, vimentin$_{hmb}$ (for all genes, sequences of *G. gallus* and an outgroup, usually *X. laevis* or a paralogous sequence, also were used).

research has generated a large number of gene sequences for representatives of several orders of birds and mammals. This now allows us to derive a single average estimate of divergence time from many independent (single gene) estimates, thus reducing potential biases that may exist with individual genes, and increasing the accuracy of the time estimate.

An accurate calibration point is also required for a reliable estimate of divergence time from molecular data. Typically, the first occurrence of two taxa in the fossil record is used as a minimum estimate of their time of divergence. However, that may be a considerable underestimate (> 50%) because of sampling biases[6]. The fossil record of birds and mammals is particularly prone to such biases because of the long time span between the earliest fossils (150 million years (Myr) for birds, 220 Myr for mammals) and the first appearances of the modern orders (mostly 55–65 Myr)[4]. Therefore, an internal calibration based on the fossil record of the modern orders themselves may lead to a large bias in the divergence times estimated. To avoid this, we have chosen the relatively well-constrained fossil divergence time between the ancestors of birds (diapsid reptiles) and mammals (synapsid reptiles), 310 Myr, as an external calibration point[4]. The first appearance of amniotes at 335 Myr (ref. 11) and of tetrapods at 370 Myr (ref. 12) suggests that a divergence time of birds and mammals much earlier than 310 Myr is unlikely.

All of our molecular estimates of divergence times for avian and mammalian orders are Mesozoic rather than Cenozoic, and are considerably older than divergence times suggested by fossil evidence (Table 1; Fig. 1). Some previous estimates of divergence



times based on immunological distances and sequence data also pre-date the K/T boundary[7-10]. However, unlike those studies, we have tested for rate constancy both among orders and between the lineages used for calibration, used an external (bird–mammal) rather than an internal calibration, and based our divergence times exclusively on a large number of constant-rate genes.

We suggest that the fragmentation of emergent land areas during the Cretaceous, not the sudden availability of ecological niches following the K/T extinction event, was the mechanism responsible for the diversification of avian and mammalian orders. When plate tectonics and sea level are considered together, all of the major continental breakup events during the last 250 Myr occurred in the Mesozoic, and mainly during the Cretaceous (Figs 1*c* and 2). The timing of these events corresponds closely to the molecular time estimates for divergences of the orders (Fig. 1*b*). The tectonic breakup of Pangaea has been cited as a factor in the historical biogeography of some Mesozoic organisms, including birds and mammals[9,13]. However, few data on times of divergence from constant-rate genes have been available to test such a hypothesis. Moreover, comparisons have not been made between ordinal divergence times of multiple groups such as birds and mammals. The continental breakup hypothesis (Fig. 2) is compatible with the extensive speciation that occurred within orders following the sudden availability of niches in the early Tertiary period.

These early molecular dates for divergences among the orders of birds and mammals indicate an unusually strong bias in the fossil record against Middle to Late Cretaceous fossils. Other groups of terrestrial vertebrates, including dinosaurs, also show a mid-Cretaceous decline in diversity[14], but not as pronounced. Besides the sampling bias already noted[6], the highest sea levels in the Mesozoic and Cenozoic occurred in the Middle to Late Cretaceous, resulting in about 25% less emergent land area than exists at present (Fig. 1*d*). This would have proportionately reduced the area available for the deposition of terrestrial fossils.

TABLE 1 Molecular time estimates for the divergence of avian and mammalian orders*

Comparisons	No. of genes Total	Constant rate	Total length†	Time‡ (Myr)	Evolutionary rate ($\times 10^{-4}$ per site per Myr)
Birds					
Nuclear genes§	34	19	2,829	97 ± 12	1.9–13.1
Mitochondrial genes					
rRNA‖	2	2	1,267	68–131	2.8
Protein	3	0			
Mammals					
Nuclear genes					
Primate–Rodent	48	24	10,051	95 ± 7	0.8–8.5
Primate–Artiodactyl	22	17	6,573	90 ± 8	0.4–10.0
Rodent–Artiodactyl	20	12	4,497	113 ± 9	1.0–10.2
Mitochondrial genes					
rRNA‖	2	2	2,171	99–184	4.0
Protein	13	0			

Nuclear genes. Complete amino-acid sequences ($\geqslant$100 residues) of nuclear genes were obtained (HOVERGEN[18], release 10; ENTREZ, release 18). Avian taxa included a struthioniform (*Struthio camelus*), galliform (*Gallus gallus*), anseriform (*Anas platyrhynchos* or *Cairina moschata*) and columbiform (*Columba livia*); corresponding sequences of a mammal (for calibration) and a more distantly related taxon (usually *Xenopus laevis* or a paralogous sequence, for root) were included. Mammalian taxa included a primate (*Homo sapiens*), rodent (*Mus musculus*) and artiodactyl (*Bos taurus*); relationships among these orders generally have not been well established[19], although molecular evidence has favoured a basal position for rodents[10]. For mammalian analyses, each comparison included data from a bird (*G. gallus*) for calibration and an outgroup (usually *X. laevis* or a paralogous sequence) for root. Sequences of individual genes (or gene families) were aligned[20] and orthology determined by phylogenetic analysis using MEGA[21]. Sites with alignment gaps were removed and constancy of rate between mammalian orders and between *G. gallus* and the mammals was tested (5% level)[22]. Pairwise distances were estimated by use of a gamma correction[23] with shape parameter (a) of 2. For each gene, average pairwise distance between *G. gallus* and the two mammalian sequences was divided by 620 (2 × 310 Myr) to obtain evolutionary rate, and the pairwise distance between mammalian orders was converted to time using this estimate of rate. Ordinal divergences were estimated with the Poisson correction distance and a different gamma distance ($a = 1$); resulting estimates were within ±5% of those in the table. Assuming a basal position of rodents, the average time estimate (nuclear genes) of the divergence of the rodent lineage from the primate–artiodactyl lineage is 104 Myr. The timing of avian ordinal divergences was accomplished similarly, although fewer sequences ($\geqslant$approximately 100 residues) were available, so all pairwise comparisons for nuclear genes were pooled to estimate means. S.e.m. was computed by dividing the standard deviation by the square root of the number of different genes to account for non-independence in multiple comparisons. **Mitochondrial genes.** For avian comparisons, portions of the two rRNA genes were analysed by the linearized tree method using a gamma correction distance ($a = 1.7$, transversional distance under Kimura's model[21]) for representatives of 7 avian orders: galliform (*G. gallus*), anseriform (*Anas platyrhynchos*), struthioniform (*Rhea americana*), psittaciform (*Melopsittacus undulatus*), columbiform (*Columbia livia*), cuculiform (*Cuculus pallidus*), and ciconiiform (*Ciconia nigra*). The amino-acid sequences of ATP6, ATP8 and COXII from *G. gallus* and *A. platyrhynchos*[24] also were analysed using a gamma correction distance ($a = 1.4$, Poisson model[21]). In both cases, an artiodactyl (*B. taurus*) and carnivore (*Phoca vitulina*) were used for calibration, and a frog (*X. laevis*) was included for root. For mammalian comparisons, the taxa[25] are: an artiodactyl (*B. taurus*), carnivore (*P. vitulina*), cetacean (*Balaenoptera physalus*), perissodactyl (*Equus caballus*), primate (*H. sapiens*), rodent (*M. musculus*), a galliform bird (*G. gallus*, for calibration), and a frog (*X. laevis*, for root). The linearized tree method[26] was used with the gamma distance ($a = 1.4$ for protein genes, $a = 1.7$ for rRNA genes)[21,27]. In both avian and mammalian analyses using linearized tree methods, rate constancy was rejected for protein genes but not for rRNA genes. However, the likelihood ratio tests[28] (using Kimura's model with $R = 2$) rejected rate constancy in rRNA genes as well. Therefore dates obtained by the linearized tree method are not shown in Fig. 1.

* Nuclear genes were analysed individually, and mitochondrial genes were analysed tandemly as two datasets (protein and rRNA genes). Total sequence lengths, times of divergence, and evolutionary rates are shown only for genes in which constancy of evolutionary rate was not rejected.

† Number of aligned amino-acid residues (proteins) or nucleotides (rRNA) for constant-rate genes.

‡ Mean time of divergence (±1 s.e.) as computed from the time estimates for comparisons of nuclear genes. For mitochondrial genes, the range of time is given for all ordinal divergences estimated in the linearized trees.

§ Based on pairwise comparisons of galliform, anseriform, columbiform and struthioniform birds.

‖ Constant rate was rejected in likelihood ratio tests but not in linearized tree tests.

The general concordance between ordinal diversification and Earth history for birds and mammals predicts that similar patterns may be seen in other terrestrial organisms. Although the relationships and times of divergence of turtle, lizard and snake families are not yet well established[4,15], Mesozoic continental fragmentation has been implicated in dinosaur biogeography[16]. Similarly, recent DNA sequence evidence about frog relationships[17] identified two major groups of families associated with Laurasia (Archaeobatrachia) and Gondwana (Neobatrachia); the two major neobatrachian groups are associated with South America (Bufonoidea) and Africa (Ranoidea). These observations conform to the hypothesis of continental breakup and diversification. Major radiations of marine invertebrates in the mid-Cretaceous also have been causally linked to the subdivision of continents by epicontinental seas[13].

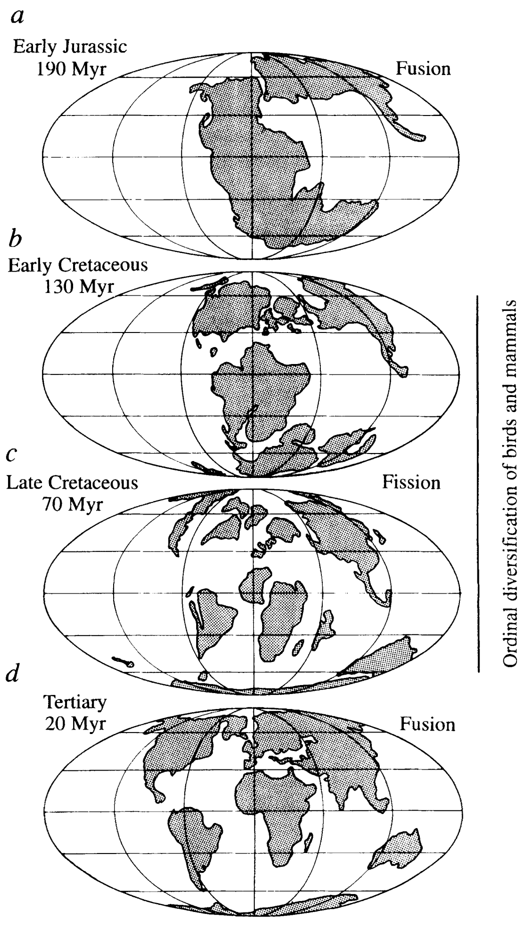


FIG. 2 Breakup (fission) and fusion of emergent land areas in the Mesozoic and Cenozoic as determined by plate-tectonic reconstructions and paleocoastline data (modified from ref. 30). *a*, Early Jurassic (190 Myr), with 2 large continents. *b*, Early Cretaceous (130 Myr), with 7 continents ($> 3 \times 10^6$ km^2). *c*, Late Cretaceous (70 Myr), with 11 continents. *d*, Mid-Tertiary (20 Myr), with 5 continents; subsequent fusion of North and South America, and Africa with Eurasia, led to the present configuration of three isolated emergent land masses. The orders of mammals and birds diverged during the Cretaceous (for example, *b* and *c*) at the time when emergent land areas were undergoing breakup.

If continental breakup were an important mechanism for ordinal diversification in birds and mammals, then it might be expected that distributional data of living fossil taxa should help in biogeographic reconstruction. Although some orders can be associated with specific palaeolandmasses[9], most now occur on multiple continents and their original distributions have not been established. This is not surprising considering that a large amount of continental fusion, allowing migration, already had occurred by the Early Tertiary when the fossils of most modern orders first appeared (Fig. 1*a*, *c*). As larger numbers of constant-rate genes for a diversity of taxa become available, it should be possible more accurately to associate ordinal divergences with specific continental breakup events. □

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Correlation between male song repertoire, extra-pair paternity and offspring survival in the great reed warbler

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In many birds, females copulate with males other than their social mate, resulting in extra-pair fertilizations (EPFs)[1–7]. It is still unknown, however, why females seek EPFs[7,8]. In one study, males that accounted for most EPFs had higher survival[6], but neither the characteristics revealing male quality nor the benefits accruing to females selecting attractive males were identified. Great reed warblers, *Acrocephalus arundinaceus*, are socially polygynous, and females base their mate choice on territory quality[9] and song-repertoire size[10], both of which predict harem size and reproductive success[11,12]. By DNA fingerprinting[13], we demonstrate that female great reed warblers obtain EPFs from neighbouring males with larger song repertoires than their social mate. In addition, the relative post-fledging survival of offspring was positively correlated with their genetical fathers' song repertoire size. These data support the hypothesis that females, by engaging in extra-pair fertilizations, seek genetic benefits for their offspring[7,8].

We studied a population of individually colour-ringed great reed warblers, a long-distance migrant with sexually monomorphic cryptic plumage, at lake Kvismaren, Sweden[9,12,14–18] from 1987 to 1993. All territories were visited daily throughout the breeding season (May–July). In this population, a substantial proportion of the returning migrants settle in their natal area[18]. Polygynous males (40% of territorial males[12,14]) attract females sequentially about every 8 days[16]. Males have a loud mate-attraction

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