APS

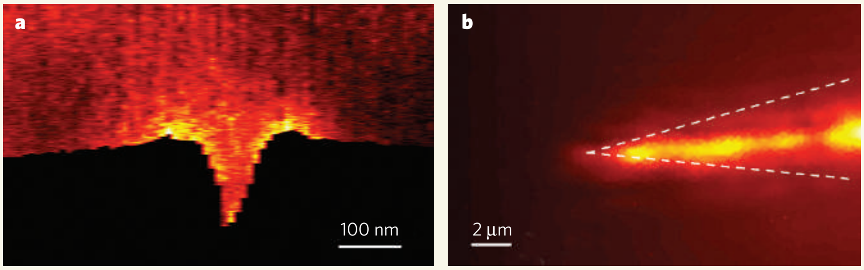

Figure 2 | **Into the groove. a**, Ropers and colleagues' 'tip-enhanced electron emission microscopy'[1] provides a picture of a nanoscale groove in a gold surface. **b**, The laser-illuminated probe tip.

'map' of the surface can be made. What STM cannot easily give us, however, is information on what happens in the third dimension above the surface. Step forward Ropers *et al.*[1], with their 'tip-enhanced electron emission microscopy'.

Like STM, the authors' technique involves measuring the effect of a sample surface on the electrical current flowing through a probe tip. So far, so conventional. But the real beauty of the technique is how that current is generated: it is stimulated by a pulsed laser beam focused on the tip. Because this laser field is affected by any kind of sample that is introduced near to the probe, the device can act in three dimensions. Furthermore, the current flow scales highly nonlinearly with changes in the laser field, making the imaging extremely sensitive to whatever is put near the probe tip.

So what sorts of things could the technique be used to look at? Ropers *et al.* use it to image a nanometre-scale groove on a gold surface (Fig. 2). But anything down to a single metal atom is theoretically possible: the spatial resolution of the authors' technique is given by the size of the metal tip, 20 nanometres, which is certainly scalable to atomic size. Questions such as the distance from which atomic-scale objects would be visible, and whether the technique could also be used for non-conducting nanostructures, will no doubt be addressed soon.

A further dimension is added to Roper and colleagues' technique through its time resolution. The laser pulses that dictate the electron emission are exceedingly short (7 femtoseconds, or 7×10^{-15} s) and have a frequency of 80 megahertz. This admits the exciting prospect of tracking atomic-scale dynamics in real time. For example, the authors suggest[1] that the dynamics of surface polaritons — discrete packets of energy that result from the interaction of an electric field and the vibrations of a material — could be studied using pairs of time-delayed pulses. Surface polaritons have been credited with wide-ranging potential for 'optical' devices that do not suffer the wavelength limitations associated with devices using light propagation.

Another recent study[3] has achieved an electron pulse resolution of below 100 femtoseconds using an identical source, and there is promise for entering the attosecond (10^{-18} s) domain[3]. Ahmed Zewail, who won the 1999 Nobel Prize in Chemistry for his studies of reaction dynamics using femtosecond spectroscopy, recently observed[4] that "Ultrafast electron microscopy should have an impact on all areas of microscopy, including biological imaging". Following Zewail's vision, Ropers *et al.* have made exciting progress in an area that might be called ultrafast near-field microscopy. ■

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EVOLUTIONARY BIOLOGY

Mass survivals

David Penny and Matthew J. Phillips

The conclusion that the primary divergences of the modern groups of mammals occurred in the mid-Cretaceous requires fresh thinking about this facet of evolutionary history — especially in ecological terms.

On page 507 of this issue, Bininda-Emonds and co-authors[1] present an evolutionary tree of more than 4,500 mammals, and conclude that more than 40 lineages of modern mammals have survived from the Cretaceous, some 100 million to 85 million years (Myr) ago, to the present. This is paralleled by Brown and colleagues' analyses for birds, just published in *Biology Letters*[2]: they claim that more than 40 avian lineages have likewise survived from before the extinctions at the Cretaceous/Tertiary (K/T) boundary 65 Myr ago. These numbers of surviving lineages push back the evolutionary history of many mammals and birds much further than earlier estimates based on smaller data sets[3,4]. But strong claims need strong evidence to support them.

However, first things first, concentrating on mammals. Bininda-Emonds *et al.* present an evolutionary tree that includes 99% of living mammal species (4,510 out of 4,554), a major achievement in itself. They used a supertree[5] approach, in which some 2,500 previously inferred subtrees were integrated into a large supertree (see Fig. 1 of the paper[1] on page 508). To date the supertree, they constructed an alignment that included 66 genes consisting of more than 51,000 nucleotides. These, plus 30 fossil calibration points, were used to estimate the times of the divergences and the rates of net speciation against time (Fig. 1, overleaf). Improvements will undoubtedly be made to the tree and its calibration points. However, inferring a good tree of such scale is groundbreaking, and the methods will be used as a model for tree-of-life studies — whether of birds, flowering plants, invertebrate groups or other organisms.

An evolutionary tree is just the initial, descriptive, part of a study; from an evolutionary viewpoint, the real interest is using trees to learn about the processes of evolution. For context, the main divisions of existing mammals are the placental mammals (eutherians), the marsupials and the monotremes (such as the platypus). Each has its further subdivisions into order, then family, and so on.

The authors[1] report a period of radiation of placental mammals around 100–85 Myr ago; all modern orders are inferred to have diverged by 75 Myr ago. In contrast, they do not detect any radiation involving current placental lineages near the end of the Cretaceous. However, they do identify various radiations of modern families from the Eocene through to the Miocene (about 55–10 Myr ago). Modern families seem to radiate at slightly different times for the main lineages (such as for marsupials, Afrotheria, Supraprimates; Fig. 1), making it unlikely that a common physical cause was responsible. But the most challenging aspect of the phylogeny is the inference that more than 40 lineages of living mammals (and of birds, as described by Brown *et al.*[2]) survived from the Cretaceous to the present.

For mammals, there are three important areas of agreement (or at least non-disagreement) between the fossil record and the supertree results[1]. The first is the initial radiation of modern eutherian lineages (from around

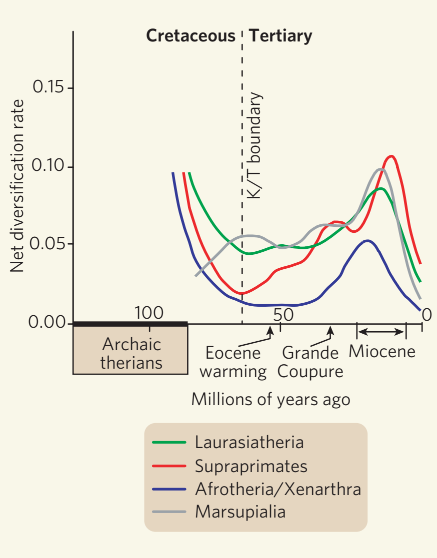

Figure 1 | Mammalian diversification over evolutionary time. The lineages shown are the Laurasiatheria (which include ungulates, whales, carnivores, core insectivores, bats), Supraprimates (primates, rodents, rabbits), and Afrotheria (elephants, sea cows, hyraxes, tenrecs, elephant shrews) and Xenarthra (armadillos, sloths, anteaters). The Marsupialia are non-placental mammals such as kangaroos. Note the overall lack of dramatic change at the K/T boundary[1]. The 'Grande Coupure' is the rapid turnover of many mammal groups, at least in Europe, Asia and North America, around the end of the Oligocene (34 Myr ago). Major diversifications of many modern families date from around this time. Archaic therians are extinct relatives of later placental and marsupial mammals. (Net diversification rate = net speciation events, per lineage, per million years. Figure based on Supplementary Information, ref. 1.)

100–85 Myr ago)[6]. This is roughly coincident with the decline in frequency of earlier fossil mammalian groups[7] (such as triconodonts, symmetrodonts and 'archaic' therians) and their replacement in several regions by placental and marsupial lineages of uncertain affinities, for example in Uzbekistan[8]. This is a key period for future work, although it may be hindered by a relatively poor fossil record from 95 to 80 Myr ago[9].

The second area of agreement is that the radiation of eutherian mammals in the Early Tertiary (65–60 Myr ago), following the end-Cretaceous impact, was not primarily of existing placental lineages, but rather of now extinct groups including 'archaic' ungulates (omnivores), plesiadapiformes (primate relatives) and multituberculates (which might have filled a rodent-like niche). It had earlier been considered that the decline and eventual extinction of dinosaurs 'allowed' modern mammals to diversify, but it is clear that there were intermediate lineages, now extinct. The first two areas of agreement change the focus for the primary radiation of placental mammals (and for birds[2,10]) back into the mid-Cretaceous. The third area of agreement between molecules and fossils is that the modern families do not radiate until the Late Eocene through to the Miocene (Fig. 1). These agreements are a major achievement of the whole supertree approach. The results eliminate any direct suppression of modern orders of mammals by dinosaurs during the Late Cretaceous.

Taken together, these three areas of agreement mean that the pivotal macroevolutionary events in the evolution of existing placental mammals occurred either well before, or well after, the K/T boundary. The former events are the divergence between orders; the latter the radiation of families. It is notable that both the mammal[1] and avian[11] data sets were generated by teams that included both palaeontologists and specialists in molecular evolution. That combination allows questions to be addressed that neither group can do well independently. Each endeavour still has its own areas of uncertainty and issues that are independent of the other, but attempts at tackling many questions benefit from these interactions. In the future, integrative studies will need to go further in incorporating both biogeography and macroecology, including long-term niche stability. A view of the past[12] in which the main taxonomic divisions of mammals occur in the Cretaceous has many such consequences that will require more thought.

For example, the results reopen the intriguing question of the ecological roles of the early mammals and birds. Here, the present is the key to the past. For example, in the present, juvenile reptiles have different ecological niches from adults[12,13], and interactions between adult mammals and juvenile reptiles can be instructive. Thus, a small rat (*Rattus exulans*), whether by direct or indirect competition with the young of tuatara (*Sphenodon*), eliminates this New Zealand reptile, which has 20 times the rat's adult body weight[14] — even though adult tuatara are not directly affected. For longer-term effects, diversification of new groups can be studied in cases such as the past invasion of South America by placental mammals from the north, or the arrival of rodents in Australia. Bininda-Emonds and colleagues' study[1] illustrates how major improvements in evolutionary trees open up new research programmes, including studies of the present to explain the past. ■

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BEHAVIOURAL GENETICS

Sex, flies and acetate

Charalambos P. Kyriacou

A receptor molecule in the fruitfly *Drosophila melanogaster* responds to a male pheromone in both sexes. But the effect of this response on sexual behaviour is not the same in males and females.

Courtship in the fruitfly *Drosophila* involves visual, gustatory, olfactory and acoustic sensations that mediate male advances and, until she mates, poorly understood female rejections[1]. Cuticular pheromones have been implicated in sexual behaviour both within and between *Drosophila* species[2,3], but the only known volatile pheromone is 11-*cis*-vaccenyl acetate (cVA), a male-specific lipid that is transferred in the ejaculate to females during copulation[4]. When deposited on eggs or food, cVA causes flies to aggregate[5] but, somewhat controversially[6], cVA has also been reported to act as an 'anti-aphrodisiac', inhibiting the courtship of males with previously mated females[7]. Perhaps as a consequence, interest in cVA in *Drosophila* waned rather. It has made a comeback in recent years[8], however, and with three new papers — one by Kurtovic and colleagues on page 542 of this issue[9], and a brace in *Current Biology*[10,11] — the role of cVA and its receptors has been clarified.

To study the role of cVA, Ejima and collaborators[10] exploited the principle that when a male fly is trained by courting an unreceptive, mated female, he remembers his experience, and subsequently shows less interest in courting a virgin female. They found that when