have also found a water depletion layer at a hydrophobic surface using neutron scattering, extending over a thickness of about 1.8 nm. They are confident that their surface is free of nanobubbles, and fairly sure that protonated impurities are not responsible for the result.

These studies are starting to produce a consistent picture of hydration at hydrophobic interfaces: a thin layer of low-density, somewhat gas-like water that precedes complete capillary evaporation between two such surfaces, and helps to nucleate nanobubbles that create an apparently long-range attraction. But that may not be the end of the story. For example, Yaminsky and Ohnishi[4] argue that experimental imperfections (protrusions from the surfaces), rather than nanobubbles, create the long-range hydrophobic force, and claim that their surface-force measurements show nothing inconsistent with the standard theory of colloidal interactions — that is, nothing unique to water — down to separations of 3 nm or so (at which point the attraction between the surfaces is so great that they spring into contact).

Moreover, would we necessarily expect 'depleted' water to be really more gas-like, or instead more ice-like, with enhanced ordering, as originally supposed[5]? Evidence of ice-like water inside a (hydrophobic) carbon nanotube at room temperature has recently been seen in simulations[10]. And what are the consequences for the hydration environment of proteins, where, for example, a local change in water density might be expected to affect important quantities such as pH and salt concentration? In other words, what does the physics of confined water have to say about cell biology? ■

Philip Ball is a consultant editor for Nature.

1. Steitz, R. *et al. Langmuir* **19,** 2409–2418 (2003).
2. Jensen, T. R. *et al. Phys. Rev. Lett.* **90,** 086101 (2003).
3. Schwendel, D. *et al. Langmuir* **19,** 2284–2293 (2003).
4. Yaminsky, V. & Ohnishi, S. *Langmuir* **19,** 1970–1976 (2003).
5. Frank, H. S. & Evans, M. W. *J. Chem. Phys.* **13,** 507–532 (1945).
6. Blokzijl, W. & Engberts, J. B. F. N. *Angew. Chem. Int. Edn* **32,** 1545–1579 (1993).
7. Lum, K., Chandler, D. & Weeks, J. D. *J. Phys. Chem. B* **103,** 4570–4577 (1999).
8. Parker, J. L., Claesson, P. M. & Attard, P. *J. Phys. Chem.* **98,** 8468 (1994).
9. Tyrrell, J. W. G. & Attard, P. *Phys. Rev. Lett.* **87,** 176104 (2001).
10. Mashl, R. J. *et al. Nano Lett.* doi:10.1021/nl0340226 (2003).

Molecular biology

Complicity of gene and pseudogene

Jeannie T. Lee

'Pseudogenes' are produced from functional genes during evolution, and are thought to be simply molecular fossils. The unexpected discovery of a biological function for one pseudogene challenges that popular belief.

Pseudogenes are defective copies of functional genes that have accumulated to an impressive number during mammalian evolution[1]. Dysfunctional in the sense that they cannot be used as a template for producing a protein, pseudogenes are in fact nearly as abundant as functional genes[2,3]. Why have mammals allowed their accumulation on so large a scale? One proposed answer is that, although pseudogenes are often cast as evolutionary relics and a nuisance to genomic analysis, the processes by which they arise are needed to create whole gene families[4], such as those involved in immunity and smell. But are pseudogenes themselves merely by-products of this process? Or do the apparent evolutionary pressures to retain them hint at some hidden biological function? For one particular pseudogene, the latter seems to be true: elsewhere in this issue (page 91), Hirotsune and colleagues[5] report the unprecedented finding that the *Makorin1-p1* pseudogene performs a specific biological task.

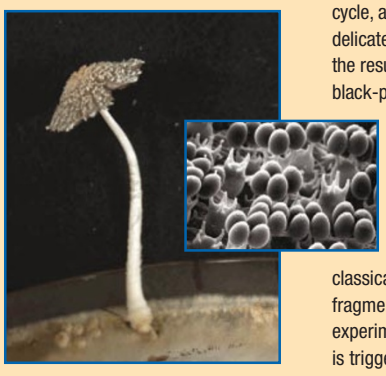
Hirotsune *et al.*[5] had been analysing mice in which copies of a fruitfly gene called *Sex-lethal* were randomly inserted in the mouse genome. In the course of their studies, they encountered one mouse line that died shortly after birth from multi-organ failure. As this occurred in only one mouse line out of many, the results could not be explained by aberrant *Sex-lethal* expression. Instead, the authors attributed their finding

Genetics

Suicidal mushroom cells

Programmed cell death — apoptosis — is a universal phenomenon among multicellular organisms, and is especially important during development. Genetically orchestrated mechanisms of cell death have also been found in single-celled protists and yeast. Writing in *Fungal Genetics and Biology* (**39,** 82–93; 2003. doi: 10.1016/S1087-1845(03)00024-0), Benjamin Lu and colleagues describe a remarkably simple version of apoptosis in the ink-cap mushroom *Coprinus cinereus* (pictured).

Lu *et al.* studied apoptosis in mutant strains of the fungus that have defects in spore formation. Spores are formed by meiosis, which is the same type of cell division that halves the number of chromosomes in the egg and sperm cells of animals. In the mushroom, meiosis occurs in a synchronous fashion,



URSEL KÜES; INSET, DAVID MCLAUGHLIN

sweeping across the gill surfaces underneath the cap, reconfiguring and sorting the chromosomes within 10 million spore-producing cells called basidia (inset). Mutants of *C. cinereus* called 'white-caps' are infertile: their basidia show defects at various points in the cell cycle, and the ashen hue of their delicate umbrellas is caused by the resulting failure to form black-pigmented spores.

It turns out that, in the mutants, basidia that experience problems at the beginning of meiosis (prophase I) undergo mass apoptosis, showing the classical apoptotic hallmark of DNA fragmentation. Lu and colleagues' experiments suggest that apoptosis is triggered at a single checkpoint in the mushroom cell cycle. This contrasts with the situation in the mouse, where the switch that activates apoptosis can be tripped at many steps throughout meiosis, implying that there is an almost continuous molecular assessment of the viability of the gamete-forming cells. Not surprisingly, it seems that mushrooms lack some of the developmental sophistication of mammals.

But why should apoptosis occur in mushrooms at all? Resource conservation is a likely explanation. Mushrooms disperse astonishing numbers of airborne spores, but there is an infinitesimal chance that any individual spore will reach a suitable patch of ground, survive, out-compete the resident microbes, grow and one day find a mate. So by aborting any basidia that have mishaps early in meiosis, the fungus conserves resources for healthy cells that will succeed in producing viable spores. **Nicholas P. Money**

Nicholas P. Money is in the Department of Botany, Miami University, Oxford, Ohio 45056, USA.
e-mail: moneynp@muohio.edu

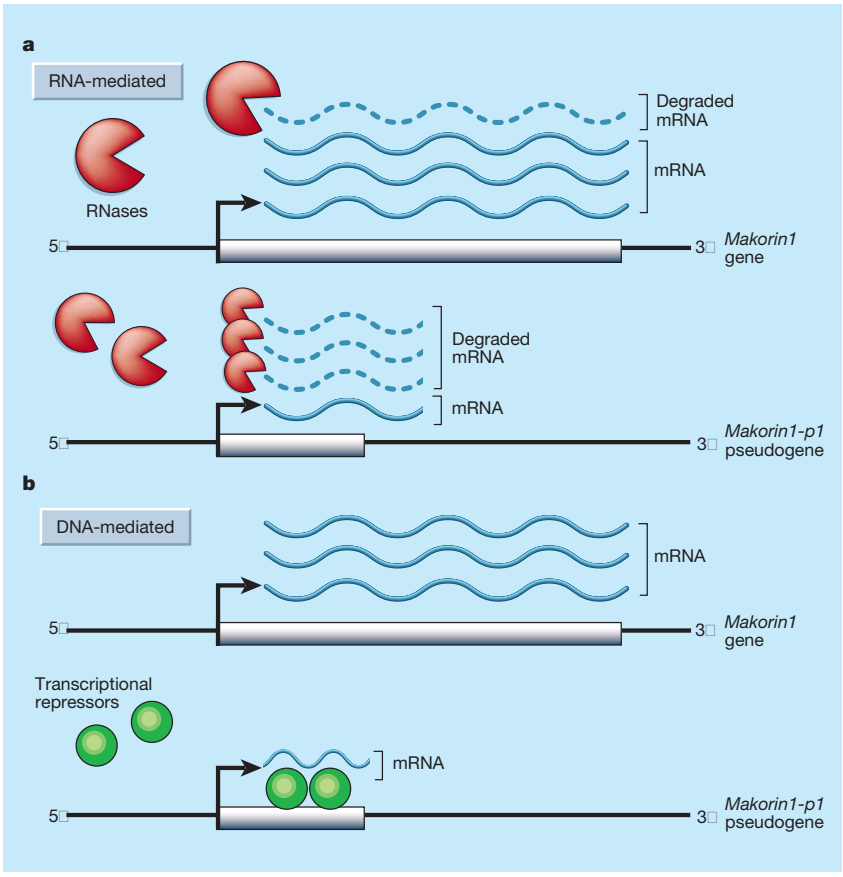


Figure 1 **Gene regulation by a pseudogene. Hirotsune *et al.*[5] have found that the expression of the *Makorin1* gene is controlled by one of its pseudogene copies, *Makorin1-p1*. The figure shows two ways in which this might happen.** a, **An RNA-mediated mechanism. Here, messenger RNA copies of the pseudogene and gene compete for a destabilizing protein that binds a crucial 700-nucleotide region near the beginning of the mRNAs. This destabilizing protein might be an RNA-digesting enzyme (RNase).** b, **A DNA-mediated mechanism. Here, regulatory elements in the 700-nucleotide region of the pseudogene and gene compete for transcriptional repressors.**

to a disruption of the particular stretch of genomic information into which *Sex-lethal* had inserted in this case. Whereas some might have dismissed the line as an aberration and unworthy of the effort required to characterize it, Hirotsune and colleagues delved deeper and were rewarded with the surprising finding that a pseudogene can regulate the expression of the functional gene from which it arose.

The authors first found that, in the mouse line in question, the inserted *Sex-lethal* gene disrupted *Makorin1-p1* — a pseudogene copy of the functional *Makorin1* gene. Only recently identified[6], *Makorin1* is an ancient gene that has been evolutionarily conserved from nematode worms to fruitflies and mammals, and encodes a putative RNA-binding protein. It is the prototype of a large family of *Makorin* genes and pseudogenes, and is located on mouse chromosome 6.

By contrast, Hirotsune *et al.* found that the pseudogene *Makorin1-p1* lies on chromosome 5. Like the original gene, this pseudogene can be 'transcribed' into a messenger RNA copy. But it has incurred mutations during evolution, so the mRNA cannot, as is usual, be used to produce a protein. Further differences between the gene and pseudogene include the fact that the *Makorin1-p1* mRNA contains only the first (that is, 5′) 700 nucleotides of the *Makorin1* mRNA. Moreover, whereas both copies (one from the mother and one from the father) of the *Makorin1* gene can be transcribed, the *Makorin1-p1* pseudogene is paternally 'imprinted', so that only the paternal copy is expressed.

Normally, *Makorin1* mRNA is expressed throughout the animal[6]. But Hirotsune *et al.* found that when the paternal *Makorin1-p1* pseudogene was disrupted, the expression of *Makorin1* was markedly reduced in embryos and throughout birth and weaning. This implies that the pseudogene is normally required for the high-level expression of *Makorin1*. Interestingly, of the two forms of *Makorin1* mRNA, only the smaller 1.7-kilobase transcript was downregulated — the larger 2.9-kilobase copy was unaffected. The long and short forms are identical except in a region at the so-called 3′ end that is not translated into protein. So, it seems that this region in the long form functions independently to keep expression levels high.

The authors also wondered whether the imprinting of *Makorin1-p1* is mechanistically central to *Makorin1* expression. However, its disruption had equal effects on both maternal and paternal *Makorin1* genes. So it seems that the imprinting of *Makorin1-p1* is an odd happenstance that has little or nothing to do with its function. Rather, it seems likely that, when *Makorin1-p1* arose, it fortuitously integrated into a chromosomal region that was already imprinted.

This study[5] generates many new and exciting questions. For instance, is *Makorin1-p1* the only *Makorin* pseudogene that regulates *Makorin1*? Considering that disruption of *Makorin1-p1* causes only a partial loss of expression of the functional gene, one might speculate that there are indeed other pseudogenes whose functions partly overlap, and that the deployment of an entire pseudogene battalion might be a feasible strategy of gene regulation.

Furthermore, how does *Makorin1-p1* regulate *Makorin1*? The authors found that the 700-nucleotide 5′ region of *Makorin1-p1* not only was required but was also sufficient for regulation in experiments *in vitro*. These experiments also suggested that the pseudogene acts sequence-specifically, affecting only those genes that show some sequence similarity to itself.

Non-protein-coding RNAs have recently been shown to perform a variety of tasks, such as gene silencing, catalysis and the regulation of development[7]. So *Makorin1-p1*'s mechanism of action might involve its non-coding RNA product, rather than the pseudogene itself. Hirotsune *et al.* propose that this product works to stabilize the *Makorin1* mRNA (Fig. 1a). They favour a model in which the first 700 nucleotides of the *Makorin1* mRNA contain a recognition site for a destabilization factor. Because this 700-nucleotide domain is shared by the *Makorin1-p1* mRNA, the expression of the pseudogene would provide a means of titrating out the destabilizing factor through direct competition. In this model, the longer *Makorin1* mRNA is unaffected because its 3′ untranslated region protects it from degradation. An extension of this idea is that *Makorin1* self-regulates: it has been suggested that it encodes an RNA-binding protein[6], which might be the destabilizing factor that downregulates the short form of its own mRNA.

Given the available data, however, another mechanism could be at work (Fig. 1b). This is suggested by the fact that mRNA stability is usually controlled by elements in the 3′ untranslated region[8] — rather than at the 5′ end, where the key 700-nucleotide region of *Makorin1* is found. The alternative

mechanism would involve the pseudogene DNA locus directly. For example, perhaps the 700-nucleotide region in the gene and pseudogene contains elements that, on binding certain proteins, repress transcription. In this model the repressor proteins would be limited in availability, so that *Makorin1-p1* would compete for repressor binding. These two models — RNA-mediated versus DNA-mediated — have mechanistic differences and could be tested.

Whatever the underlying mechanism, the work of Hirotsune *et al.*[5] is provocative for revealing the first biological function of any pseudogene. It challenges the popular belief that pseudogenes are simply molecular fossils — the evidence of Mother Nature's experiments gone awry. Indeed, it suggests that evolutionary forces can work in both directions. The forward direction is driven by pressures to create new genes from existing ones, an imperfect process that often generates defective copies of the original. But these defective copies need not be evolutionary dead ends, because pressures in the reverse direction could modify them for specific tasks. In the case of *Makorin1* and *Makorin1-p1*, the result of bidirectional selection is that one gene cannot exist without the other — an example of functional complicity between a perfected product of evolution and its derivative castaway. Might the pseudogene copies of other functional genes be similarly useful? ■

Jeannie T. Lee is at the Howard Hughes Medical Institute, Department of Molecular Biology, Massachusetts General Hospital, and the Department of Genetics, Harvard Medical School, Boston, Massachusetts 02114, USA.
e-mail: lee@molbio.mgh.harvard.edu

1. Harrison, P. M. *et al. Genome Res.* **12,** 272–280 (2002).
2. International Human Genome Sequencing Consortium *Nature* **409,** 860–921 (2001).
3. Mouse Genome Sequencing Consortium *Nature* **420,** 520–562 (2002).
4. Harrison, P. M. & Gerstein, M. *J. Mol. Biol.* **318,** 1155–1174 (2002).
5. Hirotsune, S. *et al. Nature* **423,** 91–96 (2003).
6. Gray, T. A. *et al. Genomics* **66,** 76–86 (2000).
7. Eddy, S. R. *Nature Rev. Genet.* **2,** 919–929 (2001).
8. Mignone, F., Gissi, C., Liuni, S. & Pesole, G. *Genome Biol.* **3,** 4.1–4.10 (2002).

Atmospheric chemistry

Burning domestic issues

Joel S. Levine

In the developing world much of the energy for heating, lighting and cooking comes from burning 'biomass', mainly wood. A first attempt has been made to quantify the resulting emissions to the atmosphere.

Almost a quarter-century ago, Paul Crutzen and colleagues[1] published pioneering work showing how the burning of biomass produces emission of a whole variety of trace gases. Since then there has been a growing realization of the environmental significance of this source of gases, and of associated particulate material, and the effects range from the local through the regional to the global. Biomass burning also influences the biogeochemical cycling of carbon and nitrogen compounds from the soil to the atmosphere.

Although most such burning occurs during human-initiated land clearance and changes in land use, a large component is due to the use of biomass fuels for domestic activities. That component has not previously been quantified. As they describe in the *Journal of Atmospheric Chemistry*, however, Ludwig and colleagues[2] have now provided some estimates, and the figures concerned are substantial.

Trace gases produced during biomass burning and released into the atmosphere include carbon dioxide (CO_2), carbon monoxide (CO), methane (CH_4) and non-methane hydrocarbons, hydrogen (H_2), nitric oxide (NO), ammonia (NH_3), methyl chloride (CH_3Cl) and sulphur species[3]. Carbon dioxide and methane are greenhouse gases that lead to global warming. Nitric oxide and sulphur species lead to the photochemical production of nitric acid and sulphuric acid, two of the main components of acid precipitation. Methane, carbon monoxide and nitric oxide bring about the photochemical production of ozone in the troposphere. Ozone is a pollutant and irritant, as well as a greenhouse gas.

EARL & NAZIMA KOWALL/CORBIS

Figure 1 **Domestic duty — an Ethiopian villager with firewood collected for cooking.**

In studies of this topic, it is usual to divide burning into the following components: forests (tropical, temperate and boreal); savannas; agricultural waste left after harvesting (cereals' stubble, for instance); and fuel wood for domestic heating and cooking. For the first three components, the geographical distribution and area burned are key parameters in calculating the amount of gases and particulates released into the atmosphere. Although there has never been a dedicated satellite to monitor and quantify burning, satellite instrumentation designed for other purposes has allowed identification of the location of active fires and burned scar areas in forests, savannas and agricultural regions[4]. Space-based measurements can provide little information on the fourth component. But Ludwig *et al.*[2] point out that the use of fuel wood, charcoal and non-woody biofuels for cooking, heating and lighting is a daily event for about half of the world's population, mostly in the developing world (Fig. 1), and they have produced some detailed estimates of the resulting emissions.

That task required two types of information: data on the consumption of biomass fuels, and data on the emission of gases and particulates per unit quantity of those fuels (the emission factors). Ludwig *et al.* collected the limited published data on the first topic, and in assessing the second they performed a series of laboratory measurements of the emission factors of domestic biomass burning. To estimate the contribution to the global inventory of trace-gas emissions, they assumed that 80% of domestic biomass burned is wood, 15% is agricultural residue, 2.5% is dung and 2.5% is charcoal. Finally, they assumed that about 85% of the domestic emissions are taking place in the developing countries of Asia, Africa and Latin America.

As Ludwig *et al.* themselves say, the calculations have a high degree of uncertainty. Nonetheless, the figures — given here in teragrams (1 Tg is 10^{12} grams, or 10^6 tonnes) — are illuminating. The estimated annual global release through domestic biomass burning is 1,495 Tg of carbon in the form of CO_2, 141 Tg of carbon in the form of CO, and 2.54 Tg of nitrogen in the form of NO. These are significant proportions of the annual global production of these environmentally important gases: 17% of total CO_2, 13% of total CO and 6% of total NO. But as the authors point out, the estimated CO_2 release is not necessarily a net emission, as it depends on the sustainability of wood fuels. The use of agricultural residues and dung can be assumed to be 100% sustainable and

Cell biology

Enlightened messages

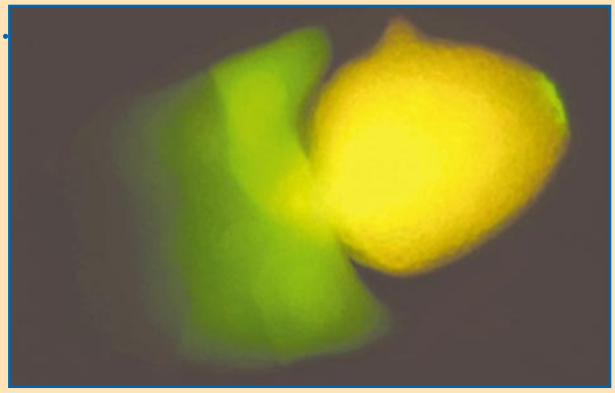
Writing in *Proceedings of the National Academy of Sciences*, Diana Bratu and colleagues describe a way to trace the movement of messenger RNA (mRNA) in living cells (doi:10.1073/pnas.2233244100).

Every moment inside cells, thousands of proteins and RNA molecules shuttle from one location to another. By fusing cellular proteins to other, fluorescent proteins, their movements can be followed visually. But it is not possible to tag RNA in the same way. Bratu *et al.* have devised a different sort of tracking system to monitor the transport of specific RNA messages. They created fluorescent 'molecular beacons' — short stretches of nucleic acids that seek out and bind to complementary mRNA sequences.

Attached to one end of the beacon is a fluorophore; a fluorescence quencher is fixed to the other end. The single-stranded beacon normally folds back on itself, forming a double-stranded hairpin structure in which the quencher and fluorophore are held in close proximity. But when the beacon binds to its complementary sequence in the RNA message, it unfolds — the fluorophore is separated from the quencher and the mRNA lights up.

To test their tracking system, the authors designed beacons for the *oskar* mRNA of fruitflies. The *oskar* message encodes the Oskar protein, which is involved in patterning the developing fruitfly egg. It is produced in 'nurse cells', which nurture the developing eggs. After entering and traversing the egg, *oskar* mRNA accumulates at the posterior end.

Bratu *et al.* injected *oskar*-specific molecular beacons into a living egg to see whether the pattern of fluorescence would unfold as expected. As the picture shows, it did — the authors detected the *oskar*-specific signal (green) in the neighbouring nurse cells and at the posterior end of the egg, whereas the signal from a control beacon was located throughout the egg (yellow). **Clare Thomas**



DIANA BRATU & SANJAY TYAGI

forest is that of Janzen[4]. In this, recruitment of individuals to a community is regarded as a consequence of dispersal from the parent plant, combined with predation by seed-eating organisms such as bruchid beetles, which are particularly adept at dealing with the many secondary plant compounds that deter most seed predators.

Because seed predators tend to concentrate around the parent tree, recruitment there is low, despite a high input of new seeds. There is a critical distance from the parent — within the range of frequent seed arrival, but with few predators — in which recruitment is most abundant. Beyond this, seed arrival is limited by dispersal ability. This model accounts for why many species of tree tend to be widely separated in tropical forests. But it does not explain why others may occur in clumps.

Working on Maracá Island in the Amazon basin of Brazil, Fragoso *et al.*[1] studied the effects of tapir feeding on the fruits of the palm *Attalea maripa* (formerly known as *Maximiliana maripa*), which is unusual in that it tends to grow in clusters. The fruits are 5–8 cm in length and are produced in large numbers. They consist of a fibrous husk, a layer of yellow pulp and a further woody layer — the endocarp — that encloses the seeds. Many small mammals feed upon the fruits, first removing the husk and then eating the pulp. The inner part is then abandoned and is usually attacked by bruchid beetles, which lay their eggs on the damaged fruits: their larvae penetrate to the seeds and consume them. Infestation close to the parent tree can approach 100%.

Tapir fruit-eating behaviour is different from that of the smaller mammals. These are large animals (about 250 kg; Fig. 1), with home ranges of several thousand hectares. They ingest the palm fruits intact, digest the pulp, and finally pass the endocarps and husk fragments in their faeces. They habitually defecate in specific latrine sites, both in the upland, drier areas and in the wetland swamps of Maracá Island. The endocarps at these sites are 98% viable, and the establishment of young palms is far more successful there than around the parent trees. Aerial surveys confirmed the existence of aggregations of palms, possibly reflecting former patterns of tapir latrines. But to find out more about the factors enhancing seed survival, experiments were required.

Fragoso *et al.* tackled matters as follows. They collected palm endocarps, placing them at different distances from the parent trees, and enclosing them in wire frames to exclude all large predators but allow access by beetles. Some plots contained clean endocarps. In others, the endocarps were covered with tapir faeces (from which any ingested endocarps had been removed) to simulate the burial conditions at latrine sites.

The results showed that, up to a point, survival was significantly enhanced by distance from the parent site (a consequence of the scarcity of bruchid beetles away from those sites). Survival was also greater when endocarps were buried in faecal material (making it more difficult for the beetles to locate the endocarps and lay their eggs on them). The effect of burial, however, became insignificant in very distant sites, presumably also because of the scarcity of the seed predator. Modelling of palm population dynamics under these conditions will evidently prove complex[5].

This work[1] illustrates the complexity of interactions between palm, tapir and beetle. But it also has a bearing on conservation. Restriction of tapir movement, by habitat fragmentation for example, could severely affect palm population dynamics. The patches of palm in the forest and sometimes also in the savanna, created at tapir latrines, constitute a mosaic of habitats in which biodiversity thrives. Such interactions between plants and animals might be a central determinant of the rates of adjustment of vegetation to changing conditions — the future of the forest could in part lie within the intestines of a tapir. ■

Peter D. Moore is in the Division of Life Sciences, King's College, Franklin–Wilkins Building, 150 Stamford Street, London SE1 9NN, UK.
e-mail: peter.moore@kcl.ac.uk

1. Fragoso, J. M., Silvius, K. M. & Correa, J. A. *Ecology* **84,** 1998–2006 (2003).
2. Cain, M. L., Milligan, B. G. & Strand, L. E. *Am. J. Bot.* **87,** 1217–1227 (2000).
3. Rousset, F. in *Dispersal* (eds Clobert, J., Danchin, E., Dhondt, A. A. & Nichols, J. D.) 18–28 (Oxford Univ. Press, 2001).
4. Janzen, D. H. *Am. Nat.* **104,** 501–528 (1970).
5. Turchin, P. *Complex Population Dynamics: A Theoretical/Empirical Synthesis* (Princeton Univ. Press, 2003).

Editorial note

The News and Views article "Molecular biology: Complicity of gene and pseudogene" (*Nature* **423,** 26–28; 2003) discussed the discovery of pseudogene function in the mouse, as described by S. Hirotsune *et al.* on pages 91–96 of the same issue. There is an earlier report of pseudogene function, in a mollusc and with a different mechanism (S. A. Korneev, J.-H. Park & M. O'Shea *J. Neurosci.* **19,** 7711–7720; 1999).