in consumer products (International Center for Technology Assessment, 2006). The FDA, which declined to comment both on the petition and on the issue of additional regulation, has until November to reply. Kimbrell, who expects the issue to end up in federal court, said the FDA stance "flies in the face of the universal scientific assessment of engineered nanoparticles, which is that adverse effects of nanoparticles cannot be reliably predicted or derived from the known toxicity of the bulk material."

The first half of the petition urges the FDA to issue a formal opinion on nanoparticles and to enact regulation such as specific nanomaterial toxicity testing and mandatory labelling. The second half raises questions about whether nanoparticles in sunscreens might be inhaled or might penetrate the skin to enter the bloodstream. Kimbrell cites a number of crucial studies on the safety of nanoparticles, showing that they can produce DNA-damaging free radicals when exposed to ultraviolet light. The petition asks the FDA to declare that these products are "an imminent hazard to public health" and order manufacturers to cease production until "broader FDA nanotechnology regulations are developed and implemented" (International Center for Technology Assessment, 2006).

Ken Donaldson, a cardiovascular and lung toxicologist at the University of Edinburgh, UK, and an advisor to a wide range of groups on the toxicology of particles, said that policymakers and the public should keep in mind that people are routinely exposed to nano-sized particles with little damage. Although he is concerned about potential dangers from nanomaterials, he pointed out that "every time you go on a busy street, you're exposed in every breath to billions of nanoparticles," he said. "Most people go about their daily business and are not much affected by it."

Donaldson suspects that, eventually, some engineered nanoparticles might prove to be harmful—such as carbon nanotubes, which in some ways resemble asbestos. "The question is whether new nanoparticles are much more harmful than the ones that we know about. We need some toxicology to benchmark these [engineered nanoparticles] against existing particles," Donaldson said. "[I]t would be madness to try and call a halt [to nanotechnology]. [...]"

There is nothing that it has thrown up so far that would cause anyone to stop nanotechnology as an endeavour."

Murdock commented that government must not ignore the fact that there are potential risks and hazards with all materials—nano or not—and should not create overly stringent regulations. "There have been calls from activist groups for moratoriums on nanotech research and commercialization and production activity, but not from the government themselves," he said. "If we create incredibly tight regulations for any nanomaterials, we run the very real risk of people saying it's going to be cost prohibitive to commercialize those technologies and no longer trying to innovate in those areas. We have to be careful with the unintended consequences of being too restrictive or requiring too much burden of proof up front."

REFERENCES

Davies JC (2006) Managing the Effects of Nanotechnology. Washington, DC, USA: Project on Emerging Nanotechnologies

ETC Group (2003) *The Big Down: Atomtech—Technologies Converging at the Nano-scale*. Ottawa, ON, Canada: ETC Group. www.etcgroup.org

International Center for Technology Assessment (2006) Citizen Petition to the United States Food and Drug Administration: Petition requesting FDA amend its regulations for products composed of engineered nanoparticles generally and sunscreen drug products composed of engineered nanoparticles specifically. Washington, DC, USA: International Center for Technology Assessment. www.icta.org

The Royal Society, The Royal Academy of Engineering (2004) *Nanoscience and Nanotechnologies: Opportunities and Uncertainties*. Summary document. London, UK: The Royal Society

SCENIHR (2006) *Modified opinion (after publish consultation) on the appropriateness of existing technologies to assess the potential risks associated with engineered and adventitious products of nanotechnologies*. SCENIHR/002/05. Brussels, Belgium: Scientific Committee on Emerging and Newly Identified Health Risks

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Entente cordiale

Multiple symbiosis illustrates the intricate interconnectivity of nature

Life is war. Ever since living organisms arose on Earth, they have competed for resources such as space and food, as well as preying on each other. But amid all this feeding and being fed upon, many organisms peacefully cooperate to derive mutual benefit. Flowering plants feed pollinating insects with nectar and, in turn, depend on them for proliferation. Other plants harbour a microbial 'zoo', which provides them with nutrients from the soil or nitrogen from the atmosphere. Similarly, bacteria in the guts of plant-eating animals break down otherwise non-digestible compounds, such as fibre, as an additional source of nutrients for the host.

There are many well-studied symbioses of two organisms in which both participants benefit. But it is only recently that scientists have discovered more complex relationships involving three or more organisms from different taxonomic groups. Such multipartite symbioses are undoubtedly more common than the few examples discovered so far, and

are likely to embrace a huge range of organisms, according to Andreas Brune, Professor of Biogeochemistry at the Max Planck Institute for Terrestrial Microbiology in Marburg, Germany. "Multiple symbiosis is probably more widespread than we think, especially in view of the symbiotic systems involving different organizational levels, for example between bacterium, nematode and insect, or bacterium, protozoan and insect," he said.

The term symbiosis is defined as a close interaction between two or more species, which includes parasitic relationships in which the host organism derives no benefit at all. By contrast, a mutualistic symbiosis—sometimes called mutualism—is a long-term arrangement in which all partners derive a benefit. These might have evolved from parasitic relationships as the participants adapted to form a stable and mutually beneficial long-term relationship. When the partners are totally dependent on each other for survival, the relationship is

obligate. So far, there are only a few known cases of obligate multiple symbiosis that involve three different organizational levels. The main reason for this has been the complexity of the relationships involved, according to Ulrich Melcher, Professor of Agricultural Biochemistry at Oklahoma State University (Stillwater, USA). "You don't read about them much because most life scientists are not ecologists and focus on simpler problems," said Melcher. "How two organisms interact with one another is a complicated enough problem for them."

Most of the known cases of multiple mutualism involve relationships in which two of the partners are at the same organizational level—usually two bacteria that might be interacting directly—but are both essential for the survival of the host. One of the most studied examples is the mutualistic relationship between the glassy-winged sharpshooter (*Homalodisca coagulata*) and two bacteria: *Baumannia cicadellinicola* and *Sulcia muelleri* (Wu *et al.*, 2006). The sharpshooter—an important plant parasite—feeds on the xylem fluids of plants and, as the insect carries other bacteria, notably *Xylella fastidiosa*, it infects the host plant to cause both phony peach disease and Pierce's disease of the grape. Although the xylem fluid provides the sharpshooter with the carbon compounds required to manufacture essential amino acids, vitamins and other organic compounds, the insect is unable to manufacture these compounds itself. Instead, it relies on the two bacterial symbionts and, in turn, provides them with the raw carbon-based ingredients that they need. The γ -proteobacterium *B. cicadellinicola* synthesizes vitamins and cofactors, whereas the Bacteroidetes species *S. muelleri* produces most or all of the essential amino acids. The two bacteria complement each other: *B. cicadellinicola* devotes much of its genome to the biosynthesis of the cofactors and vitamins and lacks the amino-acid biosynthetic pathways, whereas *S. muelleri* is dedicated to amino-acid production and lacks the ability to make some of the other essential molecules.

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...such tripartite relationships can be expected within many other combinations of organisms of different sizes, in which one organism is host to another and resident in the third

Xylem fluid contains chiefly non-essential amino acids, such as glutamine and asparagine, and as yet no animal species has been identified that is able to synthesize essential amino acids from these. Consequently, xylem-feeding insects require the help of bacteria to perform this synthesis in obligate symbiosis. In other examples of such tightly coupled relationships, mostly with aphids, only one bacterial partner has been identified. The case of the sharpshooter, first described by Nancy Moran from the University of Arizona (Tucson, USA) and colleagues, is a genuine tripartite relationship in which each partner cannot exist without the others (Wu *et al.*, 2006). The two bacteria act in obligate symbiosis with the sharpshooter, and phylogenetic studies have shown that the three partners are co-evolving. The bacteria often co-exist in the same cell in adult sharpshooters, are vertically transmitted together in eggs and are housed in a dedicated 'bacteriome' within developing sharpshooter nymphs.

This is the first example of a tightly coupled three-way symbiosis in which the genomes of the two lesser partners have been fully sequenced. There are also examples of three-way mutualistic symbioses in which all the partners are at different organizational levels, with one known case involving a virus, a bacterium and an insect. All aphids require a primary endosymbiont, the bacterium *Buchnera*, to synthesize the nutrients missing in their xylem food source. Some aphids, however, also contain a secondary symbiont, such as *Hamiltonella defensa*, to confer defence against natural enemies, such as other bacteria. Unlike the example of the sharpshooter, these two bacteria might not be reliant on each other. Research has identified an associated bacteriophage virus called APSE-2 in this obligate symbiosis (Moran *et al.*, 2005). Moran and her colleagues found that the APSE-2 genome contained intact homologues of the gene encoding cytolethal distending toxin, which disrupts the eukaryotic cell cycle. A number of pathogenic bacteria, including *Salmonella enterica*, also encode this toxin as a way of interfering with the host immune

response. The authors suggest that the phage-borne toxin provides defence against eukaryotic parasites for the aphid host; the phage gains indirectly as a result of the improved fitness and survival chances of the aphid, even though its immediate host is the bacterium *H. defensa*.

Examples of such tripartite relationships can be expected within many other combinations of organisms of different sizes, in which one organism is host to another and resident in the third. A good place to start looking is among the nematodes, which are the simplest and most numerous phyla of animals and occur in almost every freshwater, marine and terrestrial habitat. Nematodes often have mutualistic relationships with bacteria and, in turn, are common parasites of many larger organisms, including insects. In this capacity they participate in a three-way symbiosis, although it is unusual for insects to benefit from such a parasitic relationship.

Bacterial colonies or films might comprise a nested hierarchy of relationships, with the waste products of one providing the food source for another

In a few cases, however, nematodes have evolved beneficial relationships with insects, while retaining their obligate symbiosis with endosymbiont bacteria. Such a relationship has been found between various nematodes and insects associated with the maritime pine tree *Pinus pinaster* (Penas *et al.*, 2006). The nematodes have forged an alliance with their immediate insect hosts, in this case wood-boring beetles. When the beetle bores into the tree to feed on sap and reproduce, the adult nematodes release toxins that cause the pine tree to wilt and dry out inside. This assists the beetles by causing bark wounds and softening the tree tissue. The beetles are then attracted back to the pines by volatile chemicals produced by the nematodes. By this stage the nematodes have produced larvae that

are carried away and that develop inside the beetle for the next life cycle.

Not all tripartite relationships involve endosymbiosis, in which smaller partners are contained within larger ones. In some cases, bacteria live outside their host and are called episymbionts. Single-celled eukaryotes, such as protozoa, can host bacteria in this way, providing shelter within the external flagellae used for propulsion. This arrangement allows the bacteria to contribute directly to their immediate host—the protozoa—and to the larger host, which could, in principle, be virtually any multicellular eukaryote.

Such a relationship exists between the wood-feeding termite *Neotermes cubanus*, a flagellate protozoa from the genus *Staurjoenina*, and bacteria from the Bacteroidales lineage under the provisional name *Candidatus Vestibaculum illigatum* (Stingl *et al*, 2004). A research team from the University of Konstanz in Germany applied transmission electron microscopy to confirm that the cell envelope of the flagellate was populated by large numbers of the rod-shaped Gram-negative bacteria housed in special attachment sites (Stingl *et al*, 2004). In turn, the flagellates populate the gut of the termites and digest the cellulose of the wood through a fermentation process, in return for receiving xylem fluid as food.

Although Brune—one of the authors of this study—conceded that the exact functional relationships between the bacteria, protozoa and termite are poorly understood, there are some clues. Wood contains little nitrogen; therefore, the flagellates might require a source of organic nitrogen as they are unable to fix it themselves. Various prokaryotes are able to fix gaseous nitrogen from the air through biocatalytic conversion, and the bacteria in this case might well donate this to the flagellates. Another clue is that many bacteria among the Bacteroidales are polysaccharide-fermenting anaerobes, in some cases producing cellulases and other enzymes

that degrade fibre.

It is likely that the bacteria participate directly in cellulose digestion on behalf of

the termite host, as well as perhaps supplying nitrogen to the flagellates, although this remains to be shown.

In the case of viruses and bacteria, multiple mutualistic symbioses can also exist between species of the same size and belonging to the same genus, although the relationships are very different. Bacterial colonies or films might comprise a nested hierarchy of relationships, with the waste products of one providing the food source for another. Some will fix nitrogen on behalf of others, whereas other bacteria are able to degrade compounds that are not directly available to most prokaryotes.

Symbiotic viruses have a less tight relationship, given that they only come together and replicate within a host cell. The classic example of viral mutualism occurs in potato mosaic disease, involving co-infection by potato virus X, and one of the potyviruses, typically potato virus Y. Plants infected by either virus on its own experience only low levels of disease, but the two viruses together cause devastating symptoms including widespread leaf necrosis. Vicki Vance and colleagues at the University of South Carolina (Columbia, USA)

identified the two key points of this synergy (Pruss *et al*, 1997). First, the potyvirus expresses a helper-component protease that stimulates replication of other viruses

by interfering with a general anti-virus pathway in the host. Vance's team subsequently found that the presence of potyvirus enables a

broad range of unrelated viruses to accumulate in the cell. But the second key point is that this protease does not lead to increased accumulation of the potyvirus itself, which is why it takes another virus acting in synergy to produce severe infection.

Melcher suspects that multiple symbioses involving more than two viruses are also common, and that such synergistic relationships have a vital role in viral evolution. "Evidence of the importance of such interactions comes from looking at genome structures of viruses," he said. "Some are multipartite and many would argue that each segment may have had a distinct evolutionary origin." In fact, viral genomes look almost like a series of protein-encoding modules strung on a replicon string. "In many cases, each module has its own evolutionary history, suggesting recombination in the distant past of genes from distinct viruses. Where there is recombination, there had to be cohabitation," Melcher added.

It is important to recognize that the distinction between mutualism and parasitism is not always clear-cut. In some cases, symbiosis might be a double-edged sword for an organism, providing some advantages but also disadvantages—a bacterial symbiont, for example, normally confers benefits on a larger eukaryotic host but can occasionally cause disease.

The host might also betray its partner by not providing benefits. Some plants exploit fungi in this way: in the normal mutualistic relationship, the fungi obtains carbon compounds produced by the plant, which in turn benefits from the fungi through increased availability of water and soil nutrients. However, sometimes non-photosynthetic plants, known as myco-heterotrophs, obtain some or all of their food from the fungi, without contributing carbon compounds in return. As a result, the fungi either perish or must adapt to manufacture their own carbon compounds without assistance.

This recent increase in knowledge of mutualistic relationships could be used to combat disease or pests in agriculture. A team led by Thomas Miller, Professor of

Entomology at the University of California in Riverside, USA, is now working on a cure for Pierce's disease in grapes, exploiting the fact that the disease-causing bacterium is in close proximity to symbionts in the carrier leafhopper. "The vehicle to deliver the anti-Pierce's reagent is none other than your friendly symbiotic bacteria that occupies the exact same niche as the pathogen and has ready access to it," said Miller.

... understanding symbiosis offers huge potential for many applications in medicine, biotechnology and agriculture

This principle of co-existence could also be harnessed to fight human diseases by using symbiotic bacteria to deliver reagents against Crohn's disease or HIV, according to Miller. "In both cases, *Lactobacillus* or some related organism

has been chosen as the delivery vehicle," said Miller. To treat Crohn's disease, they could deliver interleukin 8 or 10 to disrupt the inflammatory pathway. In the case of HIV, the *Lactobacillus* or a related harmless bacterium could carry a surface element that binds the coat protein of HIV and thus prevents the virus from attaching to its host cell. It could be applied as a vaginal treatment to protect women against HIV, although such applications are still a long way from being realized.

Nevertheless, as Miller noted, understanding symbiosis offers huge potential for many applications in medicine, biotechnology and agriculture. However, to apply beneficial organisms efficiently, much more fundamental research is needed to unravel how different organisms coexist, particularly in the complex relationships involving more than two partners. The current trickle of such examples might therefore be just the starting point for identifying many more mutualistic symbioses.

REFERENCES

- Moran NA, Degnan PH, Santos SR, Dunbar HE, Ochman H (2005) The players in a mutualistic symbiosis: insects, bacteria, viruses, and virulence genes. *Proc Natl Acad Sci USA* **102**: 16919–16926
- Penas AC, Bravo MA, Naves P, Bonifácio L, Sousa E, Mota M (2006) Species of *Bursaphelenchus* Fuchs, 1937 (Nematoda: Parasitaphelenchidae) and other nematode genera associated with insects from *Pinus pinaster* in Portugal. *Ann Appl Biol* **148**: 121–132
- Pruss G, Ge X, Shi XM, Carrington JC, Bowman Vance V (1997) Plant viral synergism: the potyviral genome encodes a broad-range pathogenicity enhancer that transactivates replication of heterologous viruses. *Plant Cell* **9**: 859–868
- Stingl U, Maass A, Radek R, Brune A (2004) Symbionts of the gut flagellate *Staurojoenina* sp. from *Neotermes cubanus* represent a novel, termite-associated lineage of *Bacteroidales*: description of '*Candidatus* Vestibaculum illigatum'. *Microbiology* **150**: 2229–2235
- Wu D *et al* (2006) Metabolic complementarity and genomics of the dual bacterial symbiosis of sharpshooters. *PLoS Biol* **4**: e188. doi: 10.1371/journal.pbio.0040188

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