probably because of putrefaction of the brood food.

But is this really farming? Menezes *et al.* [10] persuasively argue that it is. The farmers (the bees) and the farmed (the fungus) are mutually dependent for nourishment and the bees ensure the fungus's propagation between and within nests. The innards of a stingless bee nest are constructed of cerumen, a mixture of worker-secreted wax and plant resins. The workers build elaborate structures with the cerumen, including brood cells and food storage pots, and the scaffold of connecting pillars that helps the bees get around efficiently in the dark confines of their nest. In *S. depilis*, the cerumen is inoculated by fungal hyphae, but the hyphae only grow when in contact with regurgitated brood food. After a bee emerges, the workers tear the cell down and recycle the cerumen. In this way the bees propagate their fungus horizontally. Vertical transmission, to daughter nests, occurs because stingless bees pre-provision daughter nests with cerumen and other resources from the parent nest. However, it remains to be determined how dependent the cultivated fungus is on the interaction with the bees. The finding that the fungus is genetically 100% identical with non-symbiotic strains in internal transcribed spacer sequences suggests that the fungus is not yet highly specialized.

The bee's fungus belongs to the genus *Monascus*, which is used to produce fermented 'red' rice products in Asia [19]. *Monascus* produce antibiotic metabolites and pigments that have anti-spoilage properties. This raises the question whether the bees benefit primarily from the consumption of the fungus, or from the fungus's ability to prevent spoilage of the brood food. The authors' movie [10] leaves little doubt that the bee larva eats little else but the fungus. It seems inefficient to feed nutritious brood food to a fungus, and then eat the fungus. Perhaps, however, the bee can exploit lower-quality pollen, and formulate its brood food with a lower pollen content because of its mutualism with the fungus.

It will be interesting to see if fungiculture is more widespread in stingless bees, and if so if its benefits are primarily antimicrobial or nutritional. We can look forward to this story unfolding further.

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Plant Immunity: A Little Help from Fungal Friends

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Yew trees are famous for production of the anti-cancer drug Taxol® (paclitaxel). A new study sheds light on why endophytic fungi that live inside Yew trees also make the same drug.

Taxol (a registered trademark of Bristol Myers Squibb) is one of the most widely used anti-cancer drugs, employed in the treatment of ovarian cancer, breast cancer, pancreatic cancer and a number of other carcinomas [1]. Taxol has a market value in excess of $2 billion per year and has shown enormous promise

as a cancer treatment [2]. The drug works by preventing the breakdown of microtubules and, as such, is particularly potent against rapidly dividing tumour cells [3]. Taxol was first discovered as a natural product in the bark of Pacific Yew trees (*Taxus brevifolia*), where it is synthesised in the living phloem tissue [3]. Indeed, for many years the main source of the drug was the bark of Yew trees, which was harvested in very large amounts, or Yew needles, which make a precursor of the drug from which Taxol can be derived [1]. Even today, Taxol is predominantly made by a proprietary plant cell fermentation method rather than by chemical synthesis [1–4].

Taxol is produced by Yew trees as a plant immunity compound and deployed against a wide range of wood-degrading fungi, which would otherwise be able to colonise bark tissue [5]. Taxol is, indeed, a potent anti-fungal [6] and anti-oomycete [7] drug and is able to kill the most widespread and important pathogens of conifers [6]. A real conundrum that has fascinated biologists, however, is why endophytic fungi, which naturally colonise Yew trees, can also produce Taxol [8,9]. This observation, which has been made in more than twenty fungal species, was initially disbelieved [10,11], but has now been comprehensively and independently verified by many research teams [12]. The endophytic fungi that colonise Yew trees, therefore, make the same secondary metabolite as their host plant. This is intriguing and raises many questions. Why do these fungi make Taxol? Are they immune to the Taxol that they produce, a well as that produced by the plant in which they live? Do these fungi use Taxol in the same way as the plants do? And, if they synthesise Taxol during colonisation of plant tissue, where, when and why is it being made? A recent study published in *Current Biology* [13] has provided answers to these questions and new insight into the role of endophytic fungi in these long-lived conifers.

Yew trees are very vulnerable to attack by fungal pathogens, because their bark can crack easily, especially at places where new branches emerge [14]. Yew trees are susceptible to bark cracking because they make sub-surface buds that can survive in a dormant stage for

Figure 1. Yew trees possess novel forms of plant defence enabling them to live for over 1000 years.
Yew trees undergo excessive bark cracking during their long lives as lateral buds emerge to produce new branches. This photograph shows an English Yew tree (*Taxus baccata*) believed to be more than 1000 years old in the graveyard of St. Michael's Church, Northchapel, West Sussex, England. The longevity of Yew trees was noted, even in the Bronze age, where they were often planted around burial sites as a symbol of immortality [15]. The longevity of Yew trees may be partly due to their ability to ward off fungal diseases by accumulation of Taxol in their bark and due to help from endophytic fungi, which also produce Taxol at the points where these bark cracks emerge. (Photograph reproduced under creative commons license from Miss Steel.)

decades, before a new branch is made [14]. During branch formation, the bud emerges and cracks develop before new bark is made on the newly formed branch. Yew trees undergo continual branching and bark cracking throughout their long lives, which means they are potentially particularly susceptible to pathogen attack. Deep cracks in bark allow pathogenic fungi to enter the nutrient-rich vascular tissue, with catastrophic consequences.

Yew trees have clearly, however, found a way to combat this type of attack because they live for a very long time. Indeed, one of the reasons why Yew trees were often planted in graveyards throughout Europe (this occurs in many countries) was because their long lives were thought to be symbolic of immortality in many cultures [15]. Yew trees exceeding 1000 years in age are, for example, found in some English churchyards (Figure 1) and reports of trees living for up to 3000 years have been made [15]. The plant defence mechanisms of these long-lived

trees must, therefore, be extremely effective.

An intriguing correlation was initially observed by Soliman and co-workers [13]. They found a Taxol-producing endophytic fungus, *Paraconiothyrium* sp., growing at the points at which Yew trees (of species *Taxus x media*) undergo bark-cracking during branch formation [13]. They wondered whether this correlation was associated with a role for the endophyte in plant defence. Consistent with this idea, they found that Taxol produced by the endophyte was able to prevent the growth of wood-decaying fungal species, such as *Heterobasidion annosum*, *Phaeolus schweinitzii* and *Perenniporia subacida* — all important pathogens of conifers. However, the endophytic fungus itself was also susceptible to Taxol. How then, they asked could *Paraconiothyrium* sequester and deliver Taxol in such a way that the fungus could protect itself within plant tissue? Careful ultrastructural analysis revealed the presence of hydrophobic bodies within hyphae of

Paraconiothyrium that were found to be rich in Taxol. These hydrophobic bodies were also secreted from the fungus and, interestingly, could be seen at potential points of pathogen entry where plant branching (and bark cracking) occurs. This pointed to a role for the endophyte as a potential guard against fungal infection.

To investigate the role of the endophyte *Paraconiothyrium* in plant defence, the authors studied the effect of the fungus on potential fungal pathogens. They found that the presence of an invading wood-decaying fungus induced Taxol accumulation by *Paraconiothyrium* and formation of the hydrophobic bodies that contain Taxol by the endophyte [13]. Furthermore, pathogenic infection was associated with induction of fungal genes involved in Taxol synthesis. Taxol is a diterpenoid and is made from precursors synthesised in the mevalonate and shikimate pathways. Expression of HMGCoA synthase and 3-dehydroquinate synthase genes was induced by the invading pathogen [13,16]. How could an invading fungus induce Taxol formation in this way within wood tissue? One possibility is that the process involved a volatile effector, with chloromethane a prime candidate, because it is made by wood-decaying fungi, or alternatively from chitin released during fungal growth by the invading pathogens. Chlorinated organic compounds are commonly produced during degradation of lignin during wood decay [17], and the authors therefore tested whether chloromethane could stimulate production of Taxol and also its release in hydrophobic bodies. Strong induction occurred, both by chloromethane and the presence of chitin, suggesting that growth of invading wood-decaying fungi stimulates the native endophyte to produce and sequester Taxol, specifically at branch points of the tree that are the most vulnerable to infection [13].

The endophyte, therefore, protects its own ecological niche by deploying Taxol extracellularly in hydrophobic bodies, where it is highly toxic to invading fungi. At the same time, Taxol deployment in extracellular hydrophobic bodies also protects the rapidly dividing Yew cells of the new branch from being adversely affected by the presence of the drug. Meanwhile, the host plant itself

sequesters Taxol in its bark tissue more broadly, as a barrier to fungal infection. Remarkably then, both the host plant and its endophytic colonisers use the same secondary metabolite to ward off microbial attack, in a spatially regulated manner, suggesting a long association between them. Taxol-producing endophytes are also associated with other ancient gymnosperm species such as *Ginkgo* and *Wollemia* pines — long-lived, but also anciently evolved species that have been present for more than 100 million years [18]. Recent evidence suggests their endophytic communities share similar phylogenetic histories [13]. It may be worth speculating, therefore, that there is an ancient evolutionary origin to the production of Taxol by endophytic fungi that may be associated with their colonisation of conifers.

The key insight provided by the study, however, is that an endophytic fungus is essentially able to occupy the same type of niche as mobile autonomous immune cells in the adaptive immune system of animals [19]. The endophyte not only responds to pathogen ingress, but just like circulating immune cells in an animal, the fungus can be deployed specifically to sites of infection where it releases a potent anti-microbial compound. The non-motile nature of plants provides a specific need for this type of sentinel function, whereby they can target immunity to specific locations, particularly in woody tissue, and evidently this can occur by a wide variety of mechanisms [20], including soliciting help from endophytes. Very useful assistance to ensure a long life.

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