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Quick guide

Truffles

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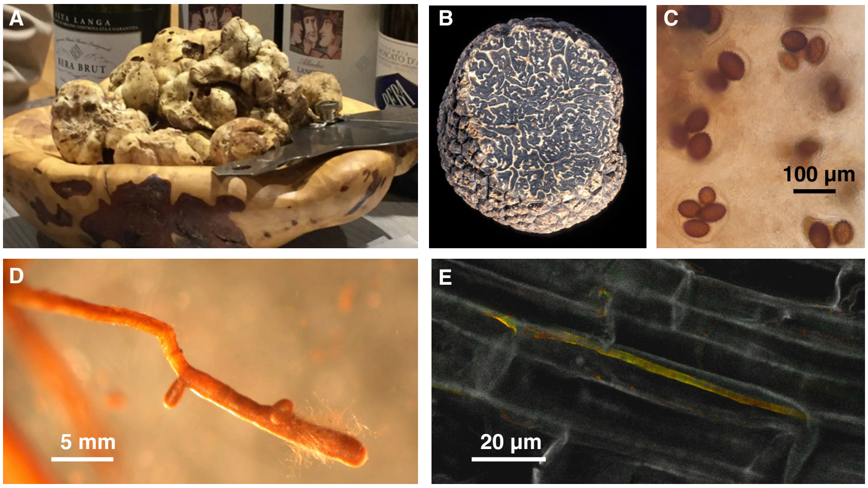

Figure 1. Truffle fruitbodies and vegetative hyphae.
(A) White Piedmont truffle (*Tuber magnatum*) fruitbodies on sale at the Alba market, Italy (photo credit: P. Bonfante). (B) Section and (C) light microscopy of a black Périgord truffle (*T. melanosporum*) fruitbody, both displaying sterile, nutritive tissues in white and meiotic spores with thick, black cell wall (photos by M.-A. Selosse). (D) An oak mycorrhiza: that is, a lateral root densely colonized by vegetative hyphae of *T. melanosporum* (photo credit: F. Le Tacon). (E) A vegetative *T. melanosporum* hypha stained by fluorescence *in situ* hybridization, colonizing a *Geranium robertianum* root as an endophyte (roots cells in grey; photo credit: L. Schneider-Maunoury).

What is a truffle? In the broadest sense, truffles are fungi that produce their meiotic spores in an underground fleshy organ. This adaptation, which allows sporulation to occur in dry sites where aerial sporulation is difficult, arose independently in more than 100 lineages during the course of fungal evolution. In the strict, taxonomic sense used here, truffles are the >86 species of a lineage belonging to the Ascomycetes: the genus *Tuber*, which has diversified over the Northern Hemisphere (Figure 1). Some European *Tuber* species have been gastronomic stars since antiquity — like *T. magnatum* (the white Piedmont truffle) and *T. melanosporum* (the black Périgord truffle), whose price can reach one to two thousand dollars per kilogram. Nowadays, the production of Chinese truffles (*T. indicum* and related species) is increasing in Asia, and some European species (especially *T. melanosporum*) have been introduced for production in Australia and South America.

Why do truffles have intense smells? The spores produced in the underground fleshy organ (called a fruitbody) cannot rely on wind for dispersal like other fungi. At the time of spore ripening, the emission of rich and volatile fragrances attracts animals (for example, mammals or soil invertebrates) who locate the fruitbodies in the soil and consume them. These fragrances are based on an elaborate metabolism of sulphur compounds (such as dimethyl sulphoxide), which are sometimes modified by the microbiota of the fruitbody. Recent genome sequencing has shown that the genes involved in odour synthesis are not innovations of *Tuber* species, but rather rely on the differential expression of a gene repertoire existing in related ascomycetes. The molecules conferring truffle fragrance have been copied in ‘truffé’ — industrial products containing between four and six dominant molecules. However, the resulting products are less complex than the 15+ main compounds produced by natural fruitbodies. The spores are protected by a thick cell wall that prevents their digestion by gut enzymes, so that they are dispersed in the faeces. This explains why all *Tuber* species are edible for mammals, although their fragrances are not all equally pleasant to humans. Notably, the nutrient gain remains limited for animals eating truffles, and spore dispersal is a kind of cheating interaction. One may even consider the worldwide production and sales of truffles as a gigantic fungal manipulation!

Why do truffles need trees? This was the question the Prussian ministry of agriculture put to the plant biologist Albert Bernhard Frank, 140 years ago. Frank found in soil a link between the vegetative fungal filaments (hyphae) of truffles and trees. This research led him to the discovery of a major form of symbiosis that exists between plants and fungi — the mycorrhiza. Frank introduced the term mycorrhiza in 1885 to describe the dual symbiotic organ where fungal hyphae colonize tree roots, covering them with a thick hyphal sheath and entering the root between the cortical root cells. Approximately 90% of plant species form mycorrhizas. The mycorrhiza is a nutritive organ, where the plant receives mineral nutrients collected in soil by the fungal hyphae, and in exchange the fungus receives plant sugars. The hyphae of truffles form mycorrhizas with trees that feed them. Currently, tree seedlings inoculated in nurseries with truffle species of commercial interest, such as *T. melanosporum* or *T. aestivum* (the Burgundy truffle), are sold in order to enhance the probability of producing truffles. Whereas 80% of production of the former species is from planted truffle grounds, inoculations with *T. magnatum* are in their infancy. In general, we do not manage or predict well the persistence and production of the inoculant after outplanting.

Do truffles only need trees? It was recently discovered that truffle hyphae also colonize the roots of surrounding plants of many herbaceous and shrubby species, but do not form mycorrhiza. This colonization, with a low density of hyphae and lacking true morphogenesis, is called endophytism. Experiments in pots show that endophytic interactions with *T. melanosporum* decrease plant growth by 30%, whereas truffle growth is increased up to 2-fold. Moreover, the germination of weeds is reduced 1.3-fold by the presence of a truffle. This interaction likely explains the somewhat mysterious ‘brûlé’ — the zone around truffle-mycorrhizal trees where truffle hyphae occur in soil and herbaceous and shrubby plants are smaller and less abundant. In the future, management of the plant cover in truffle grounds may enhance truffle production.

How is the edible fruitbody formed? Initiation of a fruitbody requires mating between two haploid individuals. Although truffles are hermaphroditic, a mating locus with two alleles prevents self-fertilization, because individuals are interfertile only with a partner of a different mating type. In the fruitbody, the spores result from meiosis and mixing of genes from the two haploid parents. One of the parents forms the flesh of the fruitbody and provides part of the genes found in the meiotic spores. This individual, called maternal, ‘is detectable on surrounding plants as the mycorrhizal or endophytic partner, where it collects nutrients for fruitbody development. The other individual, called paternal, is only present in the form of genes in the meiotic spores. Unlike maternal individuals, paternal ones are rarely observed over two successive years; they cover reduced areas and produce few (if more than one) fruitbodies. In addition, they have never been detected on surrounding plants’ roots. It is speculated that ephemeral germination of soil spores provides the paternal contribution. Indeed, the high inbreeding observed in truffles also supports mating of established individuals with their unremoved sporal offspring from previous years. In *T. melanosporum* truffle grounds, >40% of fruitbodies are not removed. We do not know whether these fruitbodies go undetected or never produce the required fragrance. However, their decay allows truffles, like most other fungi with subterranean fruitbodies (truffles *sensu lato*), to establish a spore bank in the soil. In addition, the resistant spore wall likely favours spore survival.

Why are truffles so costly? Truffle production is still not fully controlled or predictable: in essence, truffles are not fully domesticated, but rather their establishment and growth are simply promoted. For example, in France, the production of black truffles (*T. melanosporum*) is now at only 10% of its value in the 1900s, despite the planting of inoculated trees. In the wild, where they were mainly collected in previous centuries, European edible species colonize successional forests, that is, pioneer trees recolonizing open areas after disturbance. Such a situation was common when rural activities were at their highest, but is now rarer in Europe due to changes in land management practices and rural exodus. Moreover, fruitbodies grow slowly from spring to fall or even winter, but are susceptible to summer drought. Irrigation of trees in summer has paved the way to better yields, but a better understanding of truffle ecology (such as endophytic growth, nature of the paternal individual or link to disturbance) may help in the future. For this, growers and scientists will have to share knowledge, research and experimental efforts.

Where can I find out more?

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Primer

Durotaxis

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For an embryo to develop, for a tumor to metastasize, or for the immune system to eliminate pathogens, living cells must be able to undergo directed cell migration. Directed cell migration is often guided by spatial gradients in a physicochemical property of the cell microenvironment, such as chemical concentration (chemotaxis), electrical fields (galvanotaxis) or light (phototaxis). More than one century after the discovery of the first forms of gradient-guided migration, we came to learn that cells are also able to direct their migration following gradients in the stiffness of their extracellular matrix (ECM), a phenomenon called durotaxis, after Latin *durus* (hard) and Greek *taxis* (regular arrangement). Durotaxis was first reported in 2000 with the observation that isolated fibroblasts tend to move from the soft to the stiff region of an ECM-coated substrate (Figure 1A). Since then, several laboratories have reproduced findings of durotaxis using a diversity of cell types, including fibroblasts, mesenchymal stem cells, human cancer cell lines and immune cells. Although in most of the experiments durotaxis is positive (i.e. towards stiff regions), some studies have reported that durotaxis can also be negative (i.e. towards soft regions). Such negative durotaxis occurs during the development of the *Xenopus* optic pathway, when retinal ganglion cell axons grow towards softer tissue. These experiments suggest that durotaxis is much richer than initially thought. In this Primer we will review the strategies and main challenges in studying durotaxis *in vivo* and *in vitro*, the current understanding of the underlying mechanisms, and how these mechanisms can be generalized to explain other forms of directed cell migration. Finally, we will focus on collective durotaxis, a new mode of migration in which multicellular clusters exhibit efficient durotaxis even if their isolated constituent cells do not.