The Largest and Oldest Living Organism

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A fungus hidden beneath the soil surface in forest, estimated to be 1500-2400 years old, is the largest and oldest living organism.

If asked which is the largest and the oldest living organism, the California redwood tree, scientifically named *Sequoia sempervirens*, comes to mind. This tree grows to heights of 300 feet or even more (as a comparison the Qutab Minar in Delhi is 238 feet high). The oldest redwood tree is 2,200 years of age. Some might say that it is the blue whale, *Balaenoptera musculus*, which has an average length and life span are 23 feet and 110 years, respectively. In 1992, a fungus, *Armillaria bulbosa* (synonym *Armillaria gallica*) was discovered in the forests of Michigan in North America which was stated to be the largest and oldest living organism. Subsequently, another species, *Armillaria ostoyae*, was discovered in Oregon, USA, with a spread of approximately 890 hectares, and 2400 years old! These findings were startling because fungi generally are microscopic organisms, the exceptions being the fleshy mushrooms often seen in lawns and pastures, or the leathery bracket fungi growing on fallen trees and decaying timber.

The Fungi

Fungi lack chlorophyll and are therefore non-photosynthetic. The discovery of a giant fungus is therefore a testimony of the success of fungi, whose vegetative body is composed of thread-like filaments called hyphae, which secrete digestive enzymes and breakdown the complex polymeric constituents of living or dead plant remains. A part of these compounds is absorbed by the fungus for its own growth, whereas the major portion is made available for growth of plants and other microorganisms. The fungi play a vital role in sustaining the carbon cycle in the

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biosphere. An American mycologist, B O Dodge (1872-1960), who discovered *Neurospora crassa* (a fungus used by George W Beadle and Edward L Tatum for their experiments which led to the formulation of the one gene-one enzyme concept for which they were awarded the Nobel Prize) summed up his view of fungi as: "The fungi…are progressive, ever changing and evolving rapidly in their own way, so that they are capable of becoming readily adapted to every condition of life. We may be rest assured that as green plants and animals disappear one by one from the face of the globe, some of the fungi will always be present to dispose of the last remains." The fungi are the ultimate winners.

Figure 1. Hypha of a fungus is divided by transverse walls into multinucleate compartments (cells) . The septa have a pore through which protoplasmic movement occurs.

The hyphae generally have a diameter of 2-10 μm (*Figure* 1), and are concealed in the substratum, such as soil or a dead tree. How then was this fungus discovered, its age and mass estimated?

Discovery

In the 1990s aerial survey at the boundary of Michigan (USA) and Canada showed yellowing, wilting of leaves and killing of mature forest trees [1]. The culprit was a fungus that infected the roots and extended into the trunk of mature, killed trees. The fungus, scientifically named as *Armillaria bulbosa*, is commonly called the 'honey fungus' because it forms honey-coloured, edible, mushroom-like fruit bodies (basidiocarps) at the base of the killed tree (*Figure* 2). It is classified in the Phylum Basidiomycetes (or Basidiomycotina) of Kingdom Fungi (Eumycota).

Figure 2. A postage stamp showing the mushroom-like, spore-bearing fruiting bodies (basidiocarps) of Armillaria mellea.

Figure 3. Rhizomorph of Armillaria mellea growing beneath the bark of a pine tree.
Source: http:journeytoforever.org

A characteristic feature of *Armillaria* is that hundreds of hyphae aggregate into strands or cord-like structures, called the rhizomorphs (*Figure* 3). These structures tap a living tree for nutrients; extend through soil until they find a new victim. Although an individual fungal hypha is barely visible to the naked eye, the rhizomorphs are visible as black cords or shoe-laces, growing beneath the bark of the infected tree (*Figure* 3) or in the soil.

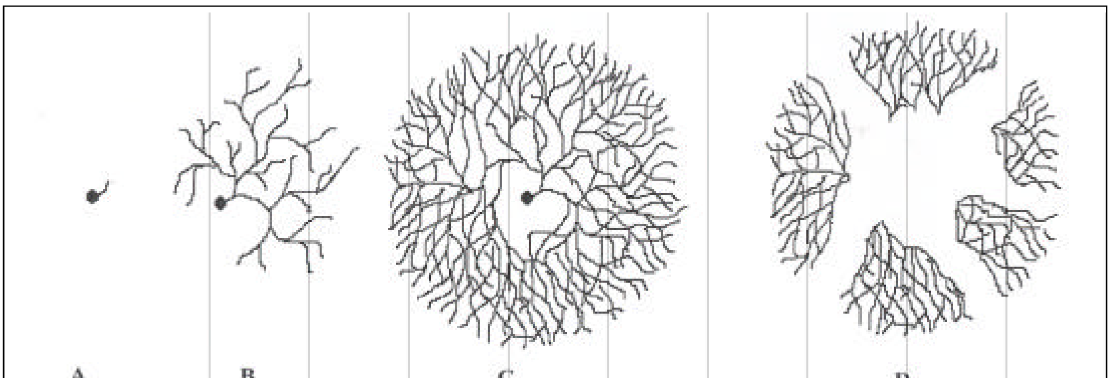
A Fungal Individual

Was the large area of the forest killed by a single fungal individual that had begun its growth from a single spore (*Figure* 4)? Mapping of the mycelial spread by microscopic examination was precluded because soil is an opaque medium, and the area very large. Therefore, microbiological and molecular methods were used to answer this question. Rhizomorphs were sampled from the infected area and portions thereof cultured on nutrient agar medium to induce the component hyphae to fan out on medium, and the pure mycelium (mass of hyphae) to be obtained for genetic and molecular analyses. Were all isolates (mycelial growth) those of a single individual?

Figure 4. Diagram of a fungal individual. The mycelium may become fragmented but the colony is regarded as a single individual.

Testing Individualism

One method to test this was to place the inoculum (a tiny amount of mycelium from which the culture develops) adjacent to each other on nutrient medium. If they are genetically the same, then



the growing mycelium from the inocula will come together and anastomose (a compatible reaction). If not, there will be a zone of confrontation with 'killed' cells in between (a 'barrage reaction'). The results showed that a single individual had parasitized trees in approximately 15 hectare area.

The nucleotide sequence of DNA is a constant feature of the genome of a particular organism. To confirm that all isolates were genetically the same, molecular analyses called restriction fragment length polymorphism (RFLP) and rapid amplified polymorphic DNA (RAPD) were used. In the RFLP method, the DNA of the isolates is cut with a bacterial enzyme, called restriction endonuclease which has a defined target sequence and the resultant DNA fragments are separated according to their size by electrophoresis. If the isolates are different even by one nucleotide difference in their DNA, then the distance between specific target cut sites on the DNA by restriction endonuclease will differ. On the other hand, if the DNA of isolates came from those of a single genetic individual, then every one of them will have band(s) at the same position (*Figure* 5). The products were separated on an agarose gel by electrophoresis and the RFLP patterns visualized by molecular hybridization with a labeled complimentary DNA strand as a probe. The pattern of restriction fragments served as a fingerprint of a DNA molecule. In the RAPD method, an arbitrarily designed 10-base-pair

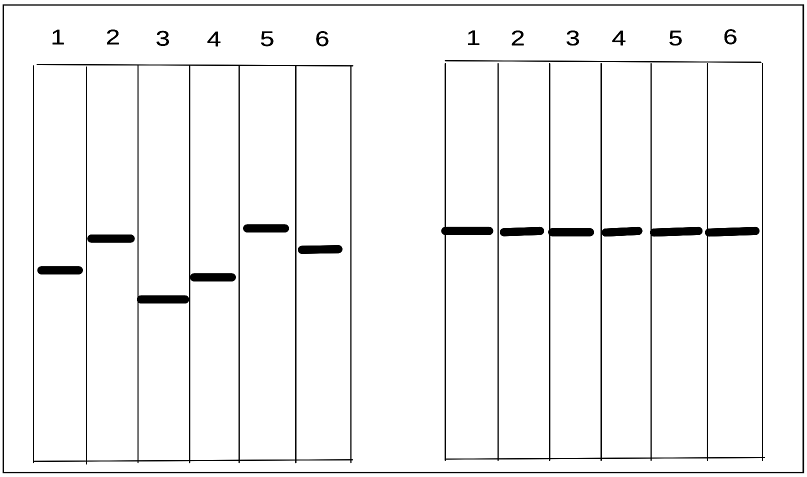


Figure 5. (left) Diagram of RFLP of genetically different isolates. (right) Diagram of RFLP of genetically same isolates.

sequence was annealed to DNA isolated from the strains and a polymerase chain reaction is carried out. Let us suppose that DNA extracted from six isolates showed different bands, the inference would be that the DNA came from different *Armillaria* individuals. However, the surprising finding was that DNA fingerprints of all isolates from an extensive area were identical! It was inferred that rhizomorphs sampled from the area, were parts of an individual fungus, regardless whether its mycelium had remained intact, or become fragmented because of predation by microfauna or due to physicochemical regions in soil.

A feature of fungal growth is that different parts of a genetically identical mycelium may reunite by hyphal fusion and advance, exploiting resources in the direction of available organic food. Another feature is that the mycelium is an interconnected system of hyphae which have a pore in their transverse walls (septa) which can be sealed by a proteinaceous material, called Woronin bodies. Thus the pores can be selectively closed, as for example the valves in a city water supply system, allowing cytoplasmic flow and redistribution of nutrients in any direction. The success of fungi is based on cooperation, not competition, among hyphae – an obvious message for the human beings!

Age and Mass of Giant Fungal Colony

What might be the age of this fungal colony? From the rate of growth of rhizomorphs on wooden posts buried in the ground, as well as from the rate of expansion of mycelium on nutrient media in Petri dishes, the age of the colony was estimated to be approximately 1500 years! And what might be the mass of this colony? From the numbers of rhizomorphs in a representative area of the forest, the mass of this colony was conservatively estimated to be 10,000 kg. Remarkably, RFLP and RAPD analyses also indicated that these two fungal colonies have remained genetically stable!

Presently, approximately 70,000 species of fungi are known. Therefore, we may ask: What is unique about the genus *Armillaria* that has contributed to the giant size of its colony. Its rhizomorphs grow beneath the bark of the tree, and tap xylem vascular tissue for water, and the phloem vascular tissue for organic compounds synthesized by leaves by photosynthesis and translocated downwards. With a water and nutrient supply base in a tree, the rhizomorphs can extend several meters through soil, even if the terrain is inhospitable, until it encounters another victim. Additionally, the rhizomorph is resistant to heat and can survive forest fires. Furthermore, this fungus is opportunistic - it is a facultative parasite which means that it lives as a saprophyte, breaking down organic polymeric compounds present in dead leaf litter or dead wood in soil and absorbing them for its growth requirements; but it also has the capability of parasitizing a living host. These fungal colonies may still be growing, and may continue to do so indefinitely, unless there is some holocaust.

Suggested Reading

[1] **M Smith, J N Bruhn and J B Anderson, The fungus *Armillaria bulbosa* is among the largest and oldest living organisms, *Nature*, Vol. 356, pp.428-431, 1992.**

[2] **http://www.anbg.gov.au/fungi/mycelium.html**

[3] **http://journeytoforever.org/**

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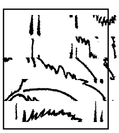

We are glorious accidents of an unpredictable process with no drive to complexity, not the expected results of evolutionary principles that yearn to produce a creature capable of understanding the mode of its own necessary construction.

Stephen Jay Gould