



ADAPTED PEER REVIEWED

# LITERATURE

## The mushroom forming fungus, *Armillaria bulbosa*, is one of the oldest and largest living organisms.<sup>1</sup>

ADAPTED BY PERMISSION FROM MACMILLAN PUBLISHERS LTD: SMITH, M.L., J.N. BRUHN & J.B. ANDERSON. (1992) THE FUNGUS *ARMILLARIA BULBOSA* IS AMONG THE LARGEST AND OLDEST LIVING ORGANISMS. *NATURE* 356(2) 428-431.

**ABSTRACT:** Smith and colleagues used the RAPD DNA fingerprinting method to determine the size of an individual fungus of the species *Armillaria bulbosa*.<sup>2</sup> They estimated that one genetically identical clone can cover 15 hectares, weigh 10,000 Kg and survive 1,500 years. *Armillaria bulbosa* forms mushrooms related to the “honey mushroom”, and members of this group act as necrotrophic parasites, first infecting and eventually killing their host, then feeding off the dead tissue.

### BACKGROUND:

*Armillaria bulbosa* is a **necrotrophic parasite** of a broad range of tree roots, causing root rot of oaks. When oaks were cut in the area of this study, pines were planted in their place, but the economically important pine seedlings were killed by *Armillaria bulbosa*. (Volk, 2002)

*Armillaria* fungi live as hyphae in the soil and under the bark of the trees they infect, but also produce black, rootlike structures called **rhizomorphs** that can grow quickly across the forest floor in search of new sources of nutrition.

Researchers have long been interested in how large an individual fungus might be. This has been a challenge because it is difficult to directly observe the beginning or end of fungus **mycelium** in the soil. To estimate the size of *Armillaria* individuals, Smith et al. used **DNA fingerprinting** techniques to identify how far one fungus **clone** (genetically identical organism) could reach. The question remains whether this clone, if it is at all broken or does not share nutrients throughout all its parts, is really an individual.

In this study, Smith et al. used RAPD DNA fingerprinting to identify genetically identical clones from *Armillaria* mushrooms and rhizomorphs in Crystal Falls, MI.



*Armillaria bulbosa* mushroom. Photo used with permission from Ray Hamblett. <http://homepage.ntlworld.com/ray.hamblett1/>

<sup>1</sup> This article is based on published, peer reviewed science. We make every attempt to remain as accurate as possible in the process of making this science accessible to younger students, however we acknowledge that simplifications and omissions may occur.

<sup>2</sup> The accepted name of this fungus is now *Armillaria gallica* (Volk, 2002).

## MATERIALS AND METHODS:

Smith et al. grew **cultures** of *Armillaria bulbosa* taken from sterile mushroom tissue and rhizomorphs in a 30 hectare site, extracted DNA from the cultures, then performed **PCR** with the R25 primer. PCR products were visualized by **agarose gel electrophoresis** with ethidium bromide to make fragments fluoresce under ultraviolet light. **RAPD** profiles of individual clones were identified by the pattern of 6 PCR fragments of different size that appeared on the gel. Spores were also cultured and tested to determine if what appeared to be a clone might really be an offspring.

The weight of clone 1 was determined by digging soil from 25 pits (15cm)<sup>3</sup> in size, sorting *Armillaria* rhizomorphs, averaging their net mass per square meter and multiplying by the area covered by the clone.

The age of clone 1 was determined by measuring growth of rhizomorphs on (1) poplar stakes buried in the area of the clone, (2) cultures in the lab and (3) wood blocks with clone 1 soil in the lab. The growth rate was used to determine the length of time to colonize the area covered by clone 1.

## RESULTS:

Smith et al. determined that the area under study was occupied by two unique clones (Figure 1) by analyzing the results of RAPD PCR from each culture. Figure 2 shows how all cultures from mushrooms and rhizomorphs (diploid cultures) fall into one of two patterns. Furthermore, the majority of the site was colonized by clone 1.

When they tested single spore offspring from clone 1 mushrooms, they found most to have a different RAPD patterns from either of the clones.

They estimated that *Armillaria* rhizomorphs grow 0.2 meters per year and be 635 meters across. Therefore, the age of clone 1 is approximately 1500 years.

The y estimated the weight of the rhizomorphs to be 64.4grams per square meter, and the rhizomorphs to cover an area of approximately 15 hectares. At 10,000 square meters per hectare they calculated the size of clone 1 to be 9,660,000 grams or about 9,700 kilograms.

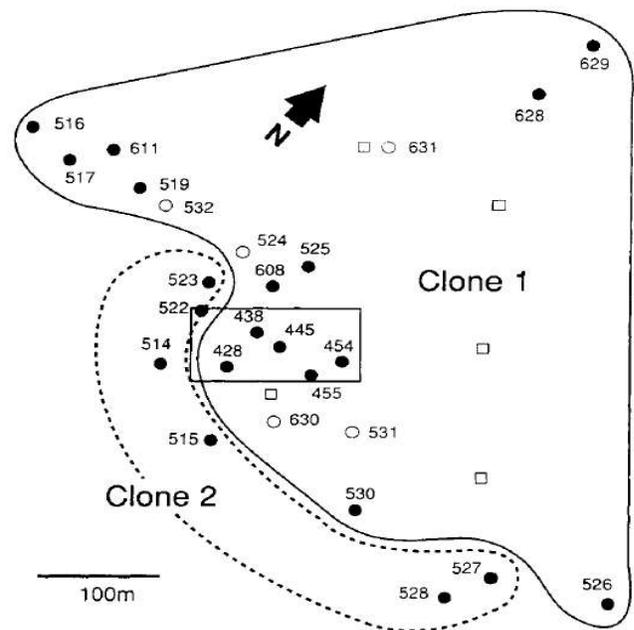
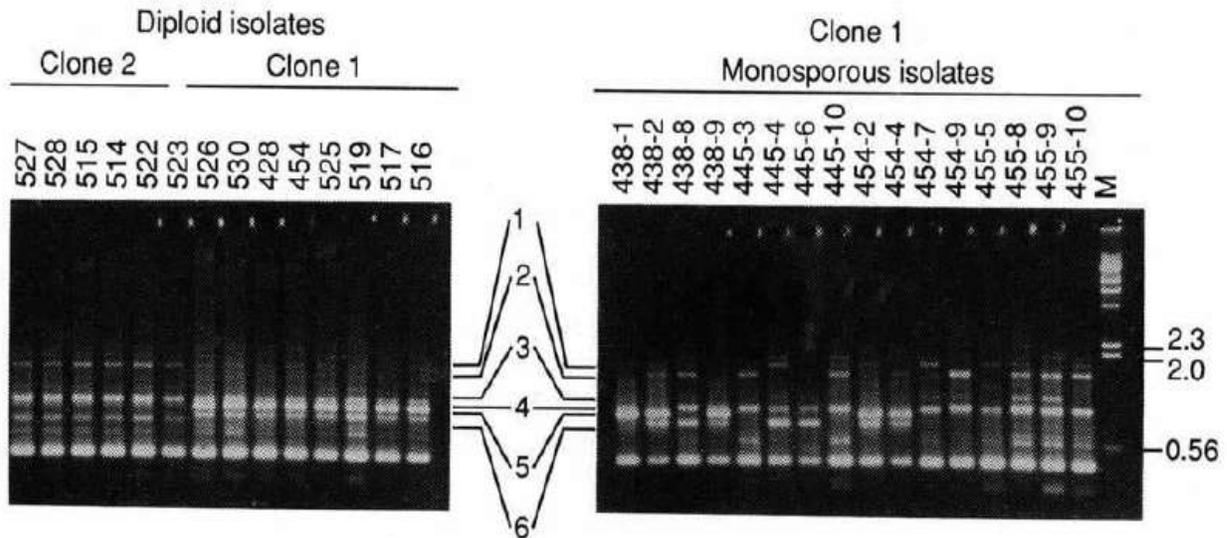


Figure 1. This is a map of the samples taken from the study site. Circles represent where cultures were taken. Squares represent where soil pits were dug. The rectangular area in clone 1 shows from where spores were taken for cultures. Reprinted by permission from Macmillan Publishers Ltd.:Smith, M.L., J.N. Bruhn & J.B. Anderson. (1992) The fungus *Armillaria bulbosa* is among the largest and oldest living organisms. [Nature](#) 356(2) 428-431.

## DISCUSSION:

The RAPD patterns suggest that there are only 2 clones that make up all of the rhizomorphs and fruiting bodies in the 30 hectare study plot. When spores were germinated from one of these clones, they were shown to have very diverse RAPD patterns that did not match the parent clone. This suggests that these are true clones and not merely genetically similar sibling fungi.

The estimated age of the fungus is remarkable at 1500 years, especially because this site burned to the ground 60 years ago. For this “individual” fungus to survive forest fires and perhaps many other



*Figure 2.* Agarose gel electrophoresis of RAPD PCR products with six identifying bands marked. The gel on the left demonstrates distinct “fingerprints” for each *Armillaria bulbosa* clone from each of the numbered locations. The gel on the right shows the “fingerprints” of the spores that are the offspring of a clone 1 mushroom, suggesting that the consistent clone 1 “fingerprint” region is not a result of several closely related fungi. Reprinted by permission from Macmillan Publishers Ltd.:Smith, M.L., J.N. Bruhn & J.B. Anderson. (1992) The fungus *Armillaria bulbosa* is among the largest and oldest living organisms. [Nature](#) 356(2) 428-431.

environmental changes over this period of time implies it is very adaptable and hardy. This may be of concern to the forest industry because the most economical trees, such as red pine are killed as saplings by the fungus, while hardwoods such as oak and maple survive with varying degrees of root rot.

The estimated size of the fungus clearly puts it in league with the world's largest organisms<sup>3</sup>. The estimate of 9,700 Kilograms (a mass greater than a blue whale) for one clone does not even take into account the fine hyphae in the soil and throughout the wood of infected trees. The true size of the fungus is likely many times this conservative estimate.

-Adapted by Jason Slot

## REFERENCES:

Smith, M.L., J.N. Bruhn & J.B. Anderson. (1992) The fungus *Armillaria bulbosa* is among the largest and oldest living organisms. [Nature](#) 356(2) 428-431.

[Volk, Tom.](#) 2002. The Humongous Fungus—Ten Years Later. [Inoculum](#) 53(2): 4-8.

<sup>3</sup> Since the publication of this study, a far larger relative of *Armillaria bulbosa* (now *Armillaria gallica*) was discovered in Oregon. *Armillaria oostoyae* is estimated to cover an area of 3.4 square miles and to be 2,400 years old.

## **GLOSSARY:**

**agarose gel electrophoresis-** DNA is put in a gelatin-like agarose gel and the gel placed in an electrical field. Fragments of DNA travel according to their size (number of nucleotides), with smaller fragments travelling faster.

**clone-** A genetically identical organism. In the case of these fungi, the clone comes from the continuous growth from one mating of two spores.

**DNA fingerprinting-** Using the DNA patterns that are unique to individuals to identify exact matches.

**mycelium-** The “body” of a fungus usually seen as a mat of threadlike hyphae.

**necrotrophic-** Living by first killing an organism (in this case with toxins), then absorbing nutrients from the dead tissue.

**parasite-** An organism that gets nutrition by digesting the tissues of living organisms.

**PCR- Polymerase Chain Reaction.** Multiple copies of discrete sequences of DNA are made by attaching “primers” that attach to the DNA pointing toward each other in the direction of DNA synthesis. By repeating the process of 1. allowing the primers to stick to the DNA, 2. using polymerase to copy DNA from the primer forward, then 3. Melting the New strands to allow primers to stick to twice as many copies as the previous cycle. This allows for exponential magnification of DNA.

**RAPD- Random Amplified Polymorphic DNA.** PCR is performed using short primers that match multiple locations in the genome and therefore create multiple fragments of DNA of different sizes. Because of slight differences in every genome (due to mutation and sexual recombination), the locations where the primers attach is different and therefore, the size and number of DNA fragments produced are unique to each individual.

**rhizomorph-** Specialized, pigmented structures made of fungal hyphae that resemble plant roots. Rhizomorphs are able to grow over surfaces to explore new feeding territories.