The In Vitro Production of Abortive Fructifications of Lycoperdon Pusillum Pers.[1]

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The Gasteromycetes are a group of 1500 species of higher fungi (Basidiomycetes) that include such organisms as the Bird's Nest fungi, Stinkhorns, Earth Stars and Puffballs. These fungi are worldwide in distribution and are found growing in lawns, pastures and woodlands.

The term Puffball or Earth Star, etc., refers to the fruit body which has developed from an extensive underground root system. This structure is the birthplace of the sexual spores called basidiospores. At maturity these spores are released and theoretically they eventually land in a suitable substrate, germinate and grow into root systems which, under ideal conditions, produce fruit bodies.

Historically, the spores of these organisms have been refractory to germination under laboratory conditions. Until recently, in fact, the spores of less than 12 species had been germinated (Bulmer, 1960). To date, a few species of the Bird's Nest fungi have been fruited *in vitro* and, as a result of further studies, we know that their sexuality is heterothallic and multiplealleiomorphic (Brodie, 1962). The life cycle of the remaining hundreds of species is still a mystery.

Thus, the study of these phylogenetically significant fungi has been hampered by two major problems:

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(1) *Inability to Germinate the Basidiospores.* In this regard, the problem has recently been at least partially solved. Over the past seven years I have germinated the spores of approximately 50 species of puffballs using a yeast co-culture method (unpublished). This technique has been successful with the basidiospores of every species I have studied.

(2) *In Vitro* Fructification: To my knowledge, no one has reported experiments with any species of Puffballs, Earth Stars or Stinkhorns that have led to fruiting. If this could be accomplished, these organisms would be available for extensive investigations into their sexuality, genetics and evolutionary significance.

Figure 1 shows a section prepared from the puffball, *Lycoperdon marginatum* Vitt., which grew in nature. Note tissue differentiation e.g. peridia, cavities, basidia, spores and origins of capillitia.

During the past few years I have attempted to grow these structures in my laboratory. In various media the vegetative mycelium proliferates; however, I have never induced the production of a completely mature fruit body as seen in Fig. 1.

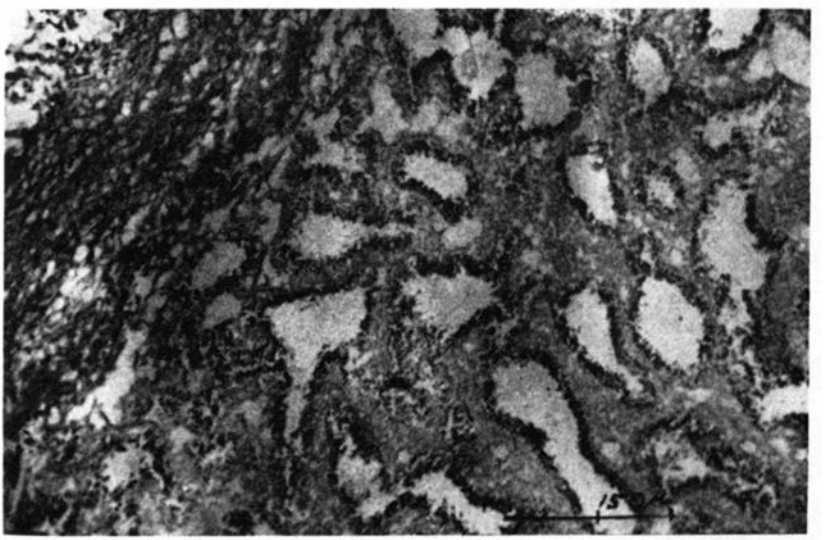
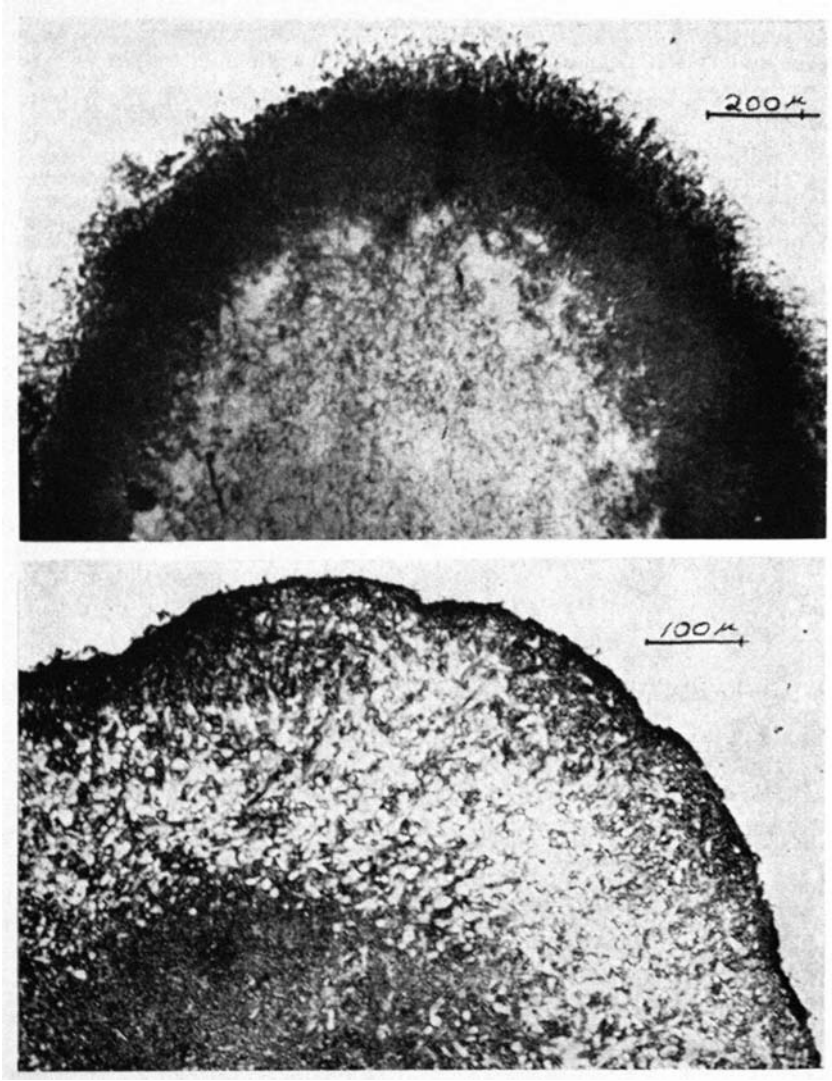


Figure 1. Section through fruit body of *Lycoperdon marginatum.* Note peridium, cavities and hymenial layers.

However, on numerous occasions I have observed structures that I interpret as being abortive fruit bodies. These structures were produced in Petri dishes containing either Brodies (1948) Bird's Nest medium, 2% malt extract, or a modified Czapek's Dox (Bulmer, 1960). In some instances, small strips of sterile filter paper were placed on these media. These fruit bodies generally appeared to develop from monosporus cultures, first grown in the dark at 26C for 3-4 weeks and then subjected to light for an 8-hour day under Sylvania Gro-lux fluorescent lights.

The species used in these experiments is *Lycoperdon pusillum* Pers., which, in nature, produces a round and white puffball when young, brown at maturity. It has a smooth peridium and rarely exceeds 2-3 cm in diameter.

The structures I have grown in Petri dishes in my laboratory are also round and white and have a smooth peridium. They are firm and approximately 1 cm in diameter. However, they do not mature to produce spores. In addition, they lack basidia and capillitia are rare. Figures 2, 3, and 4 show their tissue differentiation; e.g., peridia, a few cavities and possibly capillitia.



Figures 2 and 3. Sections of abortive fructifications of *Lycoperdon pusillum* grown *in vitro*. Note distinct tissue differentiation into peridium and gleba.

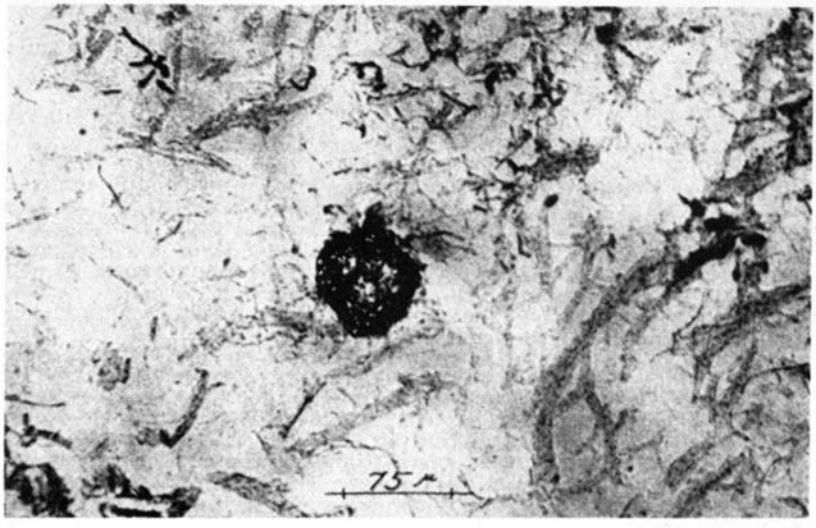


Figure 4. Section of abortive fructifications of *Lycoperdon pusillum* grown *in vitro* showing abortive cavity. Note capillitial appearance of some hyphae.

Apparently these structures develop only to a critical point and then cease further differentiation. This point appears to coincide with the stage of development at which karyogamy takes place in nature.

This abortive development may be due to one of several factors: 1) lack of proper environmental conditions, e.g., light, humidity or possibly some physical factor; 2) lack of stimulation, e.g., hormones, vitamins or other factors possibly essential for the sexual processes; 3) a mating problem, e.g., these organisms may be heterothallic, however, incompatability between the strains I have used has resulted in an inability by the organism to produce genetically compatible tertiary mycelia.

Despite the fact that these structures do not develop to maturity, it is felt that such *in vitro* studies may at least be a step in the right direction. In current investigations other media are being used together with the incorporation of additional growth factors and plant hormones.

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