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Ascomycota truffles: Cup fungi go underground

by

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Fungi pursuing the truffle strategy by producing underground fruiting bodies have long been recognized as an artificial group with representatives in Zygomycota, Ascomycota and Basidiomycota. Those producing asci (a sac-like structure where the spores are produced) were at one time all placed in the order Tuberales (Ascomycota) (e.g. from Tulasne & Tulasne 1851 to Korf 1973). Similarities between tuberalean truffles and above ground fruiting bodies of cup-fungi (Pezizales) have been recognized for over a century and various hypotheses about the evolution from above ground fruiting bodies (apothecia) to below ground truffles were discussed. It was suggested that several distinct lineages of the Pezizales had given rise to members of Tuberales independently, but a formal fusion was not made until Trappe (1979) emended several families of Pezizales to include truffles and transferred selected families from the Tuberales to the Pezizales. Since then, the close relationship between truffles and cup-fungi have gradually been confirmed and expanded upon or in some cases corrected, using cytological and ultra-structural features of asci and spores, and most recently analyses of DNA sequences.

Fig. 1. *Humaria hemisphaerica*, fruiting bodies 2 cm. in diam., deeply cup-shaped, densely covered on

In my research on the Pezizales I have studied the evolution of truffles using LSU and SSU rDNA sequences. The most recent evidence from these genealogical analyses suggests that the truffle growth form has arisen independently at least 15 times from above ground fruiting ancestors within the Pezizales in six families

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(Glaziellaceae, Discinaceae, Helvellaceae, Tuberaceae, Pezizaceae, and Pyronemataceae). The only family left with strictly underground fruiting is Tuberaceae. Thirty-eight truffle genera (or genera including truffles) are currently recognized within the Pezizales (Læssøe and Hansen, in review).

Fig. 2. Fruiting bodies of *Hydnotrya michaelis,* 3-4 cm in diam., hollow with an apical opening, two fruiting bodies cut open to show the single cavity lined with asci and paraphyses in a palisade. Photo: Jens H. Petersen.

Ascomycete truffles (the "true truffles") can be defined as producing fruiting bodies below or at ground level and having asci without a mechanism for forcible spore discharge. Above ground fruiting bodies of cup-fungi are typically open, disc- to cup-shaped (Fig. 1) and the spores are explosively ejected from the asci, through an apical, lid-like structure (the operculum) that opens. The spores are then dispersed by air. Operculate asci are cylindrical, usually with 8 spores in a row, and arranged in a palisade between tread-like sterile elements (paraphyses).

Fig. 3. *Peziza whitei*, fruiting body 2.5 cm, cut in half to show the folded inside, lined with a layer of asci and paraphyses. Photo: Michael Castellano.

Underground fruiting bodies are closed or nearly closed spherical structures. There is a continuous variation from truffles with a single cavity lined with asci and paraphyses in a palisade, often with a single opening (e.g. in *Genea* and *Hydnotrya*; Fig. 2), to truffles with intricate folding (e.g. in *Peziza whitei* and *Amylascus*; Fig. 3) or with pockets of asci in a firm, solid tissue (e.g. in *Tuber*; Fig. 4). The asci range from those that resemble asci of above ground species, being

Fig. 4. *Tuber rufum*, fruiting bodies, 1-3 cm., asci randomly imbedded in dense, firm tissue, with lighter colored sterile veins. Photo: Jan Vesterholt.

cylindrical with spores in a row, to those that are completely globose and often having a reduced number of very large spores. The paraphyses may extend above the asci, often branching and growing together toward the apices forming a tissue over the asci. In open apothecia such a tissue would impede ascus discharge. In the most derived species of truffles paraphyses have been lost.

Species with intermediary characters can be found. *Sarcosphaera coronaria* is an example of a cup-fungus that has nearly become a truffle. It forms apothecia under ground and sometimes opens by a rather small aperture, but since the spores are still actively discharged we consider it a classic cup-fungus.

Fig. 5. *Geopora cooperi*, fruiting body 5 cm. diam., remains closed, cut surface showing inside of folded walls. Photo: Brian A. Perry.

The truffle genus *Hydnotryopsis*, which produces firm potato-like fruiting bodies, shares a most recent common ancestor with *Sarcosphaera*.

Geopora cooperi (Fig. 5) has been said to be a "truffle-in-the-making." Biologically it behaves like an ordinary truffle, but has maintained its forcible spore discharge mechanism, even though the asci are enclosed within the fruiting body chambers. If a fruiting body breaks open by animal excavation, the spores could be discharged and dispersed by air. The other species of *Geopora* develop in the soil but open at the surface at maturity, splitting into vertical, irregular rays (Fig. 6) or completely expanding while still deeply immersed in the soil.

The species-rich truffle genus *Genea* produces fruiting bodies that still have an obvious apical opening, can be unfolded to strongly folded, and have cylindrical asci in a palisade, closely resembling a closed above ground cup-shaped fruiting body. Recently, the cup-fungus *Humaria hemisphaerica* (Fig. 1) was shown to be closely related to *Genea*. This is supported by the arrangement of the cells in the fruiting bodies and of the hairs on the outside. Once a fruiting body does not open fully, relaxation of selection for forcible spore discharge may permit loss of some or all of the accompanying morphological traits, such as the loss of the operculum and / or loss of the arrangement of the asci in a palisade. Highly derived truffles occur in Tuberaceae (for example *Tuber*) but also in the Pezizaceae with *Terfezia* and *Tirmania* being closely related to typical above ground species of *Peziza*. Species of *Tuber* are among the most highly prized edible fungi, with the Piedmont white truffle (*Tuber magnatum*) and the Périgord black truffle (*Tuber melanosporum*) as the best known. Inhabitants of North Africa and Middle Eastern deserts treasure fruiting bodies of *Terfezia* and *Tirmania*, and in the Kalahari Desert, *Kalaharituber* (Pezizaceae) is collected for consumption. In North America the Oregon white truffle *Tuber gibbosum* and other species are becoming popular edibles.

Fig. 6. *Geopora* sp., fruiting bodies 1-1.5 cm diam., at first globose, closed, underground or almost so, but opens at maturity, splitting into vertical rays, to actively disperse spores. Outside covered with brownish hairs. Photo: Karen Hansen.

In most truffles the operculum of the ascus is lost and the evidence is strong that the key dispersal vectors of truffles are animals.

Various small mammals, including Australian marsupials and North American voles and chipmunks, collect and often hoard fruiting bodies and by this activity play a dispersal role (e.g. Maser et al. 1978). Larger mammals such as boar and deer are also well known for their ability to locate and digest truffles and presumably act in a beneficial way to the truffles by their dispersal abilities. Truffles exude volatile compounds when ripe and in this way attract animals.

Selection for the truffle growth habit is probably driven and maintained by avoidance of desiccation, frost, and surface fires by fruiting in the soil. The high diversity of truffles in arid or seasonally dry areas favors this hypothesis. It is generally assumed that truffles form ectomycorrhiza (EM), a mutual interaction in which the fungus grows among the outer cells of plant rootlets, assisting plants in absorbing nutrients and water from the soil, while the plants provide the fungi with carbohydrates and vitamins. The direct proof has not been established in all cases, but circumstantial evidence clearly indicates the validity of this assumption. In collaboration with researchers in Estonia and Denmark, graduate student Brian Perry and I identified 33 species of Pezizales to be EM-forming, including 5 truffle genera (*Genea*, *Geopora*, *Hydnotrya*, *Pachyphloeus*, and *Tuber*) using morphology and DNA sequencing of EM root tips (Tedersoo et al. 2006).

We hypothesize that the EM lifestyle is a precondition for the switch from above to below ground fruiting. Our results indicate that the truffles are EM-forming and occur in lineages with above ground EM-forming cup-fungi. Spores of truffles probably persist for a longer time in the soil than those of air-dispersed relatives, which is likely of importance in respect to life in xeric environments. As EM-forming, it may also be an advantage that the spores are deposited in the rooting zone.

Generally, truffles seems to prefer warm, fairly dry climates and calcareous soils, but this may be slightly overstated due to the emphasis on the requirements for the edible *Tuber* species. Still, the overall species diversity appears to be highest in alkaline soils in warm temperate-subtropical climates. Also deserts around the world have a special truffle funga, which includes *Eremiomyces*, *Kalaharituber*, *Terfezia*, and *Tirmania* species (Pezizaceae). Although too little is yet known, it is clear that many endemics are to be found among pezizalean truffles.

Much has been learned of truffle biology, taxonomy and genealogical relationships since the Tuberales were abandoned as an independent order, but all three fields are still very active research areas where many exciting results will be forthcoming in the near future.

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