Close encounters with Clathrus ruber, the latticed stinkhorn

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Considerable variation in the height of the carpophores of *Clathrus ruber* Mich.: Pers. was observed, ranging from a mere 8 cm for Spanish and French collections to more than 20 cm among the Clathri growing in a park at Ouchy (Lausanne) on Lake Geneva. Chemical investigation of collections from that site confirmed that *C. ruber* accumulates manganese, just as other stinkhorns do. In all probability, this metal plays a role in the biochemistry of the fungus, notably in the enzymatic liquefaction of the gleba with simultaneous formation of odorous compounds. *Clathrus* eggs were subjected to multi-element analysis in which the gelatinous outer layer, the embryonal receptaculum and - gleba were separately investigated. The gelatinous layer proved most rich in potassium, calcium, manganese and iron. Calcium undoubtedly stabilizes the polysaccharide gel protecting the embryonal carpophore from drying out during the growth of the egg. The superior concentrations of the other elements (compared to those in the developing carpophore) suggest a placenta-like function of the gelatinous layer. The significance of the various elements in the biology of the *Clathrus* is briefly discussed.

Key words: *Clathrus ruber*, multi-element analysis.

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Byly pozorovány značné rozdíly velikosti plodnic *Clathrus ruber* Mich.: Pers. pohybující se od 8 cm u sběrů z Francie a Španělska až do více než 20 cm plodnic rostoucích v parku Ouchy (Lausanne) u Ženevského jezera. Chemické výzkumy sběrů z těchto oblastí potvrdily, že *C. ruber* koncentruje v plodnicích mangan stejně jako ostatní hadovkovité houby. Tento kov hraje pravděpodobně roli v biochemii houby, zvláště v enzymatickém zkapalnění gleby za současné tvorby vonných sloučenin. Vajíčka mřížovky byla podrobena multielementární analýze a samostatně byla studována vnější gelatinosní vrstva, embryonální receptakulum a gleba. Gelatinosní vrstva se ukázala jako nejbohatší na draslík, vápník, mangan a železo. Vápník nepochybně stabilizuje polysacharidový gel, který chrání mladé plodnice před vysušením během růstu vajíčka. Je krátce diskutován význam různých prvků v biologii *Clathrus*.

Clathrus ruber is undoubtedly one of the most beautiful representatives of the large family of stinkhorns and allies. It was already described by the 16th century botanist Charles de l'Escluse, better known as Carolus Clusius. In fact, in his large work on the fungus flora of Austria/Hungary, "Fungorum in Pannoniis observatorum brevis historia (1601)", he gives a full description of the species as *Fungus coralloeides cancellatus*, complete with an illustration that is reproduced here. In all European literature *Clathrus ruber* is presented as a warmth-loving species that is rather common in countries surrounding the Mediterranean. It is

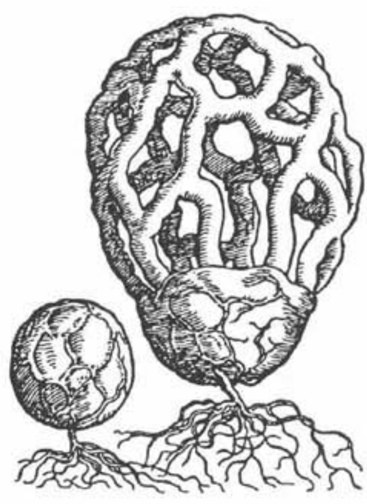

Fig. 1. *Clathrus ruber* Mich: Pers. (from Clusius C. (1601): Fungorum in Pannoniis observatorum brevis historia)

virtually absent in Holland and the Scandinavian countries, rare in Germany and Switzerland, but, surprisingly, not uncommon in the British isles, especially on the South Coast.

As a Dutch chemist with a keen interest in mycology, I published my first studies on the "flavour" of the big stinkhorn (*Phallus impudicus*) in the mid-sixties. At that time, I would have loved to extend my modest research to the *Clathrus,* but alas, this species proved extremely rare in my country. From the literature I learned that it had been found in 1735 by the great Linnaeus (the founding father of the modern botanical nomenclatural system) along a road between Amsterdam and Haarlem. After that it had only been observed a few times in gardens and hothouses, presumably introduced with soil or leafmould. For a very long time I knew *Clathrus* only from pictures and photos until I found it in a garden on the Spanish island of Mallorca. It formed a small colony there, and the eggs had only the size of a ping-pong ball. Of course, I was thrilled to watch those eggs burst, and see the beautiful red receptacle emerge. This process took only a few hours.

French authors describe the fruitbody as a "fenêtre treillisée" (window with bars), which is about the translation of the Greek word *Clathrus*. English and American mycologists speak of a latticed stinkhorn, which amounts to the same thing.

For those readers who are not familiar with the *Clathrus,* it may be useful to give a brief description. The egg of this particular mushroom can already be recognised by the network markings that become more pronounced during development. The wall of the egg consists of three layers, the inner and outer ones are thin, the middle is a thick gelatinous mass that protects the embryonal mushroom from drying out. This mass also contains the minerals and chemical compounds necessary for the development of the stinkhorn. Upon eclosion the holes in the emerging lattice are still small, but they rapidly grow bigger upon expansion of the receptacle. Finally, the pink to coral red *Clathrus* stands upright, somewhat loosely connected to the remainders of the egg.

Subsequently, the olive brown spore mass on the inner side of the receptacle starts liquefying, whereupon a particular fetid smell is produced, which readily attracts flies which feed on the sugar-containing mucus, thus assuring the dissemination of the spores. After about 24 hours the lattice structure collapses, but by this time the spore mass has been completely removed and the offensive smell greatly diminished.

I observed that my Spanish collection had indeed a cadaverous smell, but it was not as strong as that produced by a mature *Phallus impudicus*. This was also the case with the *Clathrus* I found some years later in a neglected garden in the French town of Lyon. The owner of the garden had rather negative feelings about these "clathres en réseau". He looked on with disgust, while I dug out a few eggs to take them along, assuring me that it was dangerous even to touch those fungi. Indeed, Ramsbottom in his classic "Mushrooms and Toadstools"(London, 1953) mentions that *Clathrus* has a bad reputation in France, e.g. people in Gascogne believe that – what they call – the Cancru causes cancer when handled. If they find one, they bury it carefully and deep. In other French departments touching the *Clathrus* is supposed to give you eczema, or even convulsions! In Spain the population does not love the *Clathrus* either. Folknames like "Gita de bruixa" (witches' egg) and "Cranc" (cancer) speak volumes...

Although in several countries eggs of *Phallus impudicus* are eaten and sometimes considered a delicacy, I have not found any information about culinary or medicinal use of *Clathrus ruber*.

My third encounter with this fungus took place in 1987 during a visit to the Barla Museum in Nice (South of France). Jean Baptiste Barla (1817–1890), a well-known mycologist, had not only written a voluminous guide to the fungi found in the Nice area, but had also made a series of most realistically looking wax models of the *Clathrus* in all stages of development and with variously shaped receptacles. I noticed that these models were far bigger than my collections of this particular mushroom, and I asked myself if this reflected reality. In 1988 this question was positively answered when I found in the parc d'Elysée in Ouchy (Lausanne, Switzerland) some ghost eggs being as big as an average apple. At first I thought that these were eggs of *Phallus impudicus*, which is a common species in this country. However, imagine my surprise and joy when one of those eggs – which I had taken home for further study – produced after a while a most beautiful *Clathrus*! The colour of the lattice work was not as red as that of my earlier finds, but the receptacle measured not less than 5 inches which was twice as big as that observed in the Spanish and French collections.

Finding *Clathrus ruber* in Ouchy can probably be explained by the almost Mediterranean climate there. The park is situated on the side of Lake Geneva that receives most sunshine. Clearly, *Clathrus* must feel itself at home there, since further investigation at the site revealed two more colonies, which produced carpophores two to three times a year. The occurrence of *Clathrus* here is probably just a manifestation of what is called " the advance of the stinkhorns in Europe." These highly specialised, non-mycorrhizal gasteromycetes are apparently not affected by environmental degradation. Svrček (1983) has pointed out that during the last 30 years *Phallus impudicus* has been widely diffused, even to the South of Sweden, whereas in the beginning of the century it was a fairly rare mushroom there. *Clathrus ruber* may also be conquering new territories. Indeed, about ten years ago, it was repeatedly reported in the Berlin area. Even more exotic stinkhorns turn up with increasing frequency in Europe, e.g. *Anthurus archeri*, the octopus stinkhorn, which was accidently brought into France by the Australian army during WW I. It is now already a common species in Ticino, the Italian-speaking part of Switzerland. Recently, it was even observed as far north as Holland.

I learned from further observations in Ouchy that the dimensions of the latticed stinkhorn are variable, but it was clear that Barla had not exaggerated when making his wax models. Late autumn 1993, at a temperature of 4 °C, with a strong wind blowing, I found a colony that was really thriving: when approaching the park from a 100 yards distance I saw a really enormous red receptacle. It was 8 inches high and 5 inches broad, and it was accompanied by half a dozen large eggs. Two of those, weighing 110 and 195 gms were taken along and put in a bin of garden soil under glass jars of respectively 0.6 and 1 litre (to avoid being surprised by the stench of the expanded carpophore). The skin of the biggest egg was already torn during the evening of the next day showing the orange-red colour of the embryonal *Clathrus*. During the next 24 hours it emerged as a bulging sphere with holes, which permitted to see the olive-black gleba on the inside. The typical latticed form was only achieved on the 4th day: the 1 litre jar proved too small and was removed whereupon the receptacle proceeded to grow into a fine orange-red lantern, measuring 4 × 4 × 6 inches! Somewhat surprisingly, the cadaverous odour proved weak enough to permit measuring and photographing the fungus, and to show it to interested persons. When placed outside, the smell still proved strong enough to attract flies, in spite of the low temperature. After one day the lattice work collapsed, and was dried to be preserved as an herbarium collection. The other egg only opened after 8 days producing a pink receptacle of 2.8 × 3.3 × 4.5 inches, which was also too big for the glass jar covering it. The dimensions of the receptacles proved about proportional with the weight of the eggs. This specimen also had a rather weak odour.

Chemical investigations

Stinkhorns are not only characterised by their peculiar Jack – in – the – box way of growth, but they also have in common that, after eclosion, a number of chemical reactions are initiated to liquefy the gleba and produce the cadaverous odour. *Phallus impudicus* has repeatedly been the subject of chemical investigations, which even resulted (during the 60ies) in two doctoral theses. The German scientist Johannes Schmitt found that during eclosion of *Phallus impudicus* and *Anthurus* *archeri* a considerable amount of carbon dioxide gas is produced, simultaneously with the carrion-like stench. Carbon dioxide and the "flavour" components (methyl sulfides, aldehydes and amines) are probably produced by enzymatic decarboxylation of keto- and amino acids, but such a process will work only in presence of certain metals, such as manganese. Now every mushroom contains detectable amounts of this trace element, but in most gilled fungi, boletes and puffballs the concentration seldom exceeds 60 mg kg^{-1} on dry matter. Interestingly, Schmitt found in a number of Hysterangia, and especially in stinkhorns, exceptional high levels of manganese. The concentrations of this metal were even higher than those of the closely related essential element iron. See Table 1.

Table 1. Manganese and iron concentrations in Hysterangiales and Phallales (as reported by Schmitt et al., 1977)

Species	Manganese in mg kg^{-1}	Iron mg kg^{-1}	Ratio Fe: Mn
Hysterangiales			
Hysterangium coriaceum	100	557	5.6
Hysterangium stoloniferum	13–25	75–78	3.2–5.8
Hysterangium nephriticum	14–46	393–702	15.3–28.1
Hysterangium rubricatum	225	116	0.5
Hysterangium calcareum	18	295	16.4
Gauteria otthii	10	138	13.8
Phallogaster saccatus	448	135	0.3
Phallales			
Clathrus ruber	447	573	1.3
Anthurus archeri Egg	1956	226	0.1
Receptacle	538	297	0.6
Mutinus caninus Egg	230	335	1.5
Phallus impudicus Egg	218	224	1.0
Gelatinous layer	447	270	0.6
Egg without outer layer	168	132	0.8

All values expressed on dry matter

These interesting results invite a number of comments. Among the Hysterangia there are species with a low as well as a high manganese content. Some of these subterranean gasteromycetes apparently exclude the element, since the soil contains on the average 1000 mg kg^{-1} (0,1 %), whereas the iron content fluctuates between 1 and 6 percent. The above — ground growing *Phallogaster saccatus*, a rare fungus representing a bridge to the "true" stinkhorns and which contains, just as them, more manganese than iron! Some of the stinkhorns, e.g. *A. archeri* (bio) concentrates manganese, since its content, 2000 mg kg^{-1}, is higher than that of the average soil.

Our *Clathrus ruber* contains both much manganese and iron, but since Schmitt examined herbarium material, it is not clear what part of the fungus he analysed. The figures listed for the different parts of the big stinkhorn indicate that the outer part of the egg contains more manganese than the embryonal gleba! Such differences are also observed in the results for the various parts of *A. archeri*.

Table 2. Manganese and iron concentrations in dried eggs of two Phallales species compared to soil levels (values expressed on dry matter)

	Manganese [mg kg^{-1}]	Iron [mg kg^{-1}]
Phallus impudicus from la Foret de Jorat, Lausanne, CH	725 —1118	108–143
Soil samples	430–1220	24000–36000
Clathrus ruber from the Parc d'Elysée, Ouchy, CH	450–1900	180–570
Soil samples	650–1250	13500–50000

Table 3. Essential chemical elements in *Clathrus ruber* (values expressed on dry matter)

	Na mg kg^{-1}	K %	P %	Ca mg kg^{-1}	Mg mg kg^{-1}	Zn mg kg^{-1}	Mn mg kg^{-1}	Fe mg kg^{-1}	Cu mg kg^{-1}
Whole eggs after drying & grinding	170	4.03	0.72	1137	1953	13	488	229	20
Gelatinous layer and outer skin	413	8.65	0.51	3490	2045	37	1454	261	17
Receptaculum	431	5.62	0.82	289	2230	20	621	97	22
Spore mass	223	2.84	0.62	111	2094	23	236	127	26

To check these interesting findings, we decided to analyse a few stinkhorns in our own laboratory. For this purpose, comparative analyses of dried eggs of both *Clathrus ruber* and *Phallus impudicus*, as well as corresponding soil samples were carried out. Table 2 shows that both stinkhorns prefer manganese. The much more abundant iron is only taken up in minor quantities. To study the distribution of these metals and those of other essential elements in the different parts of *Clathrus* *ruber*, we gathered a number of eggs in July 1993 of the afore-mentioned colony in Ouchy. Half a dozen were cut in thin slices, whereupon we isolated with a sharp knife the reddish embryonal receptacle and its blackgreen gleba, and dried these parts overnight separately in a draft oven set at 55 °C. The remaining gelatinous layer and its adhering skin were treated in the same way. Subsequently, the dried parts were ground to a fine powder, sieved and stored in glass vials until carrying out the multi-element analyses of which the results are given in Table 3.

The concentrations listed for the various elements should not be taken too absolutely, since we analysed biological material, subject to considerable variation. However, the high levels of potassium, calcium, manganese and iron in the gelatinous layer are striking. These are undoubtedly those elements that are most essential to the fungus. Potassium is a component of the cells regulating their osmotic pressure. It is foremost necessary for the growth of the carpophore. There is not only a correlation between the potassium concentration and the water content of the fungi, but also the velocity of growth depends on the metal. The slowly growing polypores contain seldom more than 2 percent potassium, but in the rapidly evolving Coprinaceae 10 to 12 percent is found (Stijve 1996). The gelatinous layer contains 8.65%. It is therefore not unthinkable that the receptacle obtains its potassium from this source.

The calcium concentration of 3490 mg kg^{-1} is much higher than that reported in literature for gilled fungi and puffballs (Seeger and Hüttner 1981). Calcium plays a role in the metabolism of the mushroom, stabilising intercellular membranes. In our *Clathrus* calcium undoubtedly stabilises the gelatinous layer which protects the embryonal carpophore during the growth of the egg, which takes between 2–4 weeks for its full development. The concentrations in the receptacle and gleba are rather modest. It has been established (Bindler 1967) that the gelatinous layer consists of polysaccharides just as the vegetable gums that are used as thickeners in the food industry. Indeed, the slimy part of the egg has characteristics similar to those of alginic acid and pectine that also need calcium to produce a gel. The amount of manganese in the gelatinous layer suggests again that this part plays the role of a reservoir, even as a placenta, because the receptacle as well as the gleba contain more than average concentrations of the metal. The level in the spore mass (236 mg kg^{-1}) suggests the presence of manganese-containing enzymes that produce the sugars and odorous compounds necessary to attract the flies. Although the ratio iron: manganese in Stinkhorns is smaller than 1, it cannot be said that these fungi are poor in iron. In our *Clathrus* the amount in the gelatinous layer is well above the average value of 158 mg kg^{-1} reported by Manfred Lupper in 1988 who examined not less than 500 fungi. An antagonism between the two metals – as observed in animal metabolism – does not seem to exist in higher fungi. In all stinkhorns analysed so far, manganese predominates, but the iron content is also appreciable.

The other elements listed in Table 3 do not invite much comment. The sodium content is less than 1 percent of the potassium concentration. It apparently does not play a role in the fungal metabolism. It is curious that the levels of zinc and copper are significantly lower than those measured in many other mushrooms (Mutsch et al. 1979). Perhaps the uptake of these metals is inhibited in presence of much manganese. Magnesium is evenly distributed among the different parts of the *Clathrus* and its levels are in agreement with those reported in literature for other stinkhorns (Seeger and Beckert 1979). The reader having some knowledge of biochemistry will not be surprised that the metals are accompanied by a considerable amount of phosphorus (P), just as is the case in green plants. The element is largely present as phosphate (quantitatively the major anion) and it plays a key role in the transport of metals through the cell membranes. Of course, phosphate is also necessary for buffering the acid compounds formed during the metabolism of *Clathrus*.

There is little doubt that the chemistry of *Clathrus* is interesting enough to be investigated more thoroughly. We know now that the mushroom takes up much manganese, but the supposed role of this metal in the enzymatic reactions occurring during the liquefaction of the gleba has still to be elucidated. The isolation and characterisation of the manganese-containing enzymes would be a fine subject for a doctoral thesis, especially for a biochemist having an interest in mycology.

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