

OBSERVATIONS ON THE BOLBITIACEAE—IV

Developmental studies on *Conocybe* with particular reference to the annulate species¹

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(With one Text-figure)

The macroscopic characters of the pileus, veil, and stipe of members of *Conocybe* subgenus and section *Pholiotina* are related to the microscopic structure and development of the fruit-body. Differences between various authors' descriptions are explained by results from observations made in the field and in the laboratory. The colour of the pileus and the position of the veil is shown to be more variable in these same fungi than at first supposed. The development of the fruit-body in subgenus *Pholiotina* is compared with subgenus *Conocybe*.

The evaluation of the macroscopic characters utilized in distinguishing annulate members of *Conocybe* subgenus *Pholiotina* outlined in an earlier paper by Kits van Waveren (1970) is fully supported by the observations which have been made on over one hundred and fifty British collections of this same group and certain other closely related North American members of the Bolbitiaceae.

PILEUS COLOUR

Usually in agaric taxonomy great emphasis is placed on the colour of the fruit-body, particularly that of the pileus, so much so that it would be certainly rebellious to suggest otherwise. Kits van Waveren's and my ideas do not strictly oppose this view but observations indicate that a rather more careful appraisal is required, as to when and under what conditions the colour of the pileus is considered significant. Over several collecting seasons now, specimens have been found which differ one from the other simply in the colour of the pileus; field observations and experimentation have led to the belief that this is simply a reflection of the environmental conditions rather than of differences in genotype.

The development of the fruit-body, from before the veil begins to fragment until full maturity, has been studied in several species but most extensively in *Conocybe aporos* Kits van Wav. and *C. teneroides* (J. E. Lange) Kits van Wav. Under field conditions the colour of the pileus varied between rich red-brown or chestnut to

¹ Some of the results in this paper are taken from a thesis accepted for the degree of Ph. D. in the University of Edinburgh.

bay when fresh but due to the hygrophanous nature of the pileus these colours soon pale, changing to ochraceous honey, clay-buff, etc.; the rich red-brown colours can, however, be retained if the fruit-body is transferred to a damp-chamber immediately on collecting and allowed to develop to maturity there. Frequently under dry weather conditions specimens of members of subgenus *Pholiotina*, indeed many species in other subgenera of *Conocybe* are found dry, non-striate, and ochraceous honey to dark straw-colour. Fruit-bodies of all species of *Conocybe* so far studied appear to be unusually sensitive to changes in their water balance and this appears also to be applicable to all the species so far fructified in culture, or brought to maturity in damp-chambers. It has been found that if water is withheld, either by accident or under controlled conditions, by restricting or damaging the mycelium about the base of the stipe, the colour of the pileus soon fades; similar results are obtained by partially severing the stipe and keeping the cut ends apart by using a glass cover-slip. This damage one does, unconsciously it is true, when collecting a specimen; after all one cannot help but sever the multitude of fine mycelial connections between the base of the stipe and the substrate even though great care is taken when collecting to remove some of the substrate along with the specimen. Even the few hours in which a specimen may be in transport to the laboratory is sufficient time to allow distinct colour changes to take place.

Hora (1957) in *Panaeolus* revived the colour of the pilei of his specimens simply by placing the base of the stipe of an intact fruit-body in water in a closed container and allowing the water to be taken up by the hyphae of the stipe and transferred to the pileus trama; he considered that the final colour obtained in this group of agarics by this method was a true record of the original colour. It can be easily demonstrated that water is transported to the pileus tissue by using a hypodermic syringe and injecting a vital stain such as Janus green into the liquid column in the stipe cavity. Similarly an aqueous solution of Eosin can be located in the pileus trama and certain cells of the hymeniderm when it is placed in the medulla of the stipe. The stain may also be found at the tips of the cheilocystidia often in the small cap of mucus (possibly mucopolysaccharide) which is frequently present there in members of the Bolbitiaceae. However, whenever Hora's technique was applied to the annulate species of *Conocybe*, and to a lesser extent to some of the other species of *Conocybe*, the pileus never seemed to return to the bright rich red-brown colour found in the fresh fruit-bodies or primordia, the colours found after treatment were more 'greyed'. There is little doubt that in the field under drying conditions water tensions are set up between substrate and fungus such that the cells of the pileus trama are not fully saturated and so the colour of the pileus begins to pale; this process is continued if the tension is not released. When the soil becomes moist and the conditions in the soil more favourable water is once again taken up by the fruit-body, but the colour does not return to the original intensity and clarity. Thus it would appear in the field that a single mycelium could produce fruit-bodies with pileus colour red-brown (under ideal conditions), ochraceous brown (under drying conditions) and snuff-brown (under the re-establishment of more favourable conditions after an initial,

short period of drying); surely *C. filaris* var. *ochracea* Singer (1955) is a reflection of this phenomenon. If the length of the drying period is over a certain value, perhaps only a few hours for some delicate species of agaric, the fruit-bodies never revive. These observations might well explain the discrepancies in and differences between some of the descriptions of our common annulate species of *Conocybe* which appear in various texts, indeed between some of the illustrations which have been discussed at some length by Kits van Waveren (1970).

By using dyes directly injected into the stipe trama movement of water can be demonstrated but Schutte (1956) has carried out many more extensive experiments and these would lead one to believe that water is transported to and lost from the entire surface of the pileus. During unfavourable conditions probably due to the thinness of the pileus trama in species of *Conocybe* and due to the pileal surface being made up of large, thin-walled cells, a rapid loss of water is experienced such that basidia begin to collapse; however, under exactly similar conditions or even more stringent ones species of *Agrocybe*, which normally have a much thicker pileus trama and therefore larger source of water, continue to disperse spores.

STRIATION OF THE PILEUS

The pileus becomes striate in the annulate species of *Conocybe* as soon as the veil breaks and the pileus commences to expand, for it is then that the pileus trama thins sufficiently to show the base of the gills visibly through the tissue; this character is lost as the pileus dries out but on reviving as described above the striations may return, but are never exhibited as strongly as when the fruit-bodies are freshly picked, perhaps due to the development of air pockets within the pileus tissue. In some robust specimens the striation never seems to return and this may be due to nothing more than that the pileus which is a little thicker in these specimens contains such an extensive net-work of air-spaces that the movement of water through the tissue is so hindered that complete saturation never takes place.

RUGULOSITY OF THE PILEUS

Rugulosity of the pileus only occasionally enters descriptions of annulate European species of *Conocybe* but when one examines the literature pertaining to foreign representatives of the same group it is a prominent character; however it is certainly not because the character is absent in European material, indeed *C. filaris* (Fr.) Kühner is frequently rugulose, at the disc anyway. Mycologists are more conscious of this character in the related genus *Bolbitius*, for some specimens of *B. vitellinus* (Pers. per Fr.) Fr. growing on dung or straw may be strikingly wrinkled and rugulose at the pileus disc. Atkinson (1918) described an olivaceous coloured species of *Bolbitius*, *B. varicolor*, which was marked by the strongly veined pileus, but collections of even this fungus may be found completely, or almost completely, smooth; much discussion

has been conducted on the real differences, if any, between *B. reticulatus* (Pers. per Fr.) Ricken (with reticulate-veined pileus) and *B. aleuriatus* (Fr. per Fr.) Singer (with smooth pileus). Thus it would seem that under favourable environmental conditions the pilei in some species may become wrinkled or even rugulose but there appears little or no other difference between these rugose-capped specimens and those that would be considered normal, i.e. smooth.

The rugulose nature of the pileus can be related to its structure and development. The 'cuticle' of species of *Conocybe* consists of a palisade of stalked vesiculose, pyriform to ellipsoid cells. Although these cells are very variable in size and originate at quite different levels in the hypoderm (Kühner, 1935; Disbrey & Watling, 1967), the pileal cells of members of the subgenus *Pholiotina* originate at a greater number of levels than those of members of the *Conocybe tenera* group. However, both groups are marked by the development of pockets of similar, but smaller, cells below the hymeniderm surface which expand during maturation and push up into the limiting layer of the pileus to increase its surface area; this increase in the cell number which makes up the surface allows the pileus to expand but if the change of shape of the pileus is not rapid enough, or the differentiating parts are not in phase, then the cells throw the pileus surface into small irregularities. These irregularities are composed of thin ridges of stalked, vesiculose to pyriform cells of the hymeniderm; a complexity of these irregularities gives the rugulose surface. A similar pattern is seen in some members of the genera *Coprinus* and *Psathyrella* and is accentuated when the fruit-bodies dry. Extensive cracking exhibited in *Agrocybe* can also be related to the hymeniform structure of the pileal surface.

The cells of the hymeniderm in *Conocybe* are variable in size even in a single pileus, generally those at the margin being slightly smaller than those at the disc; this may be related to the age of the cells and the availability of water. The size of the cell in no way seems to be related to the size of the fruit-body.

COLOUR OF THE STIPE

Maturation of the fruit-body is also of importance when one considers, in the annulate species of *Conocybe*, the colour of the stipe base and the colour of the stipe flesh, for although commencing dark ochraceous in the primordium and young fruit-body, the flesh in the stipe base gradually darkens to become cigar-brown or snuff-brown, even umber; during maturation not only is there a darkening but there is an extension of that tissue which becomes darkened (metachroic), so that in fully and over mature specimens all the tissue below the ring is dark brown. Thus in a single collection individuals with dark and light stipe bases can be found growing together in juxtaposition. Also during maturation the silky covering to the stipe breaks up or separates to expose the darker 'context' and this shows through more and more strongly with age.

VELAR CHARACTERS AND THEIR RELATION TO DEVELOPMENT

The veil when present in *Conocybe* is formed as part of the paravelangiocarpic development, a type of development which appears to typify all the members of the Bolbitiaceae so far studied, but as in other agarics with this type of development e.g. *Panaeolus semiovatus* (Sow. per Fr.) Lundell, the veil may be completely annulate, or annulate and dentate, or only dentate. True the *Conocybe arrhenii-blattaria* group is normally thought of as a complex of annulate species but specimens can be found with a distinct marginal veil, with or without accompanying remnants of a ring on the stipe; it is only when in some individuals remnants are present on the stipe one receives clues as to where the veil originated. A similar and parallel phenomenon is found in the *Agrocybe praecox* and *Agrocybe erebia* complexes.

Whether the veil is marginal or annulate may be considered an expression of the environment for if the fruit-bodies develop rapidly, they tear the veil into fragments and these are either all left on the pileus or some are left on the pileus and some on the stipe. Damage to the veil and repositioning of the veil may result when during development the fruit-body rubs against vegetation, soil particles, etc.

Little is known about the stimulus required to initiate fruit-body development, the first signs of which to the naked eye are small loose knots of hyphae. These structures are found at the junctions of those groups of hyphae which come into close proximity to one another during growth. Lateral branches branch rapidly by what appears to be a simultaneous, yet random, system to form a tight group of hyphae, the innermost of which divide more regularly and become much more compacted than those towards the outside. Finally a pseudoparenchymatic tissue is formed and it is from this tissue that the fruit-body develops.

In culture hyphae of members of the Bolbitiaceae frequently grow in close contact with each other, indeed the intertwining to form small knots is common. In these intertwining hyphae the cells close together divide transversely and then branch, later branching again but at right angles. The fruit-body at its earliest stage is covered by these loosely branched and irregularly arranged hyphae and to the naked eye the primordium appears to be enveloped in a mass of pale ochraceous or dirty whitish hyphal strands often giving the primordium a woolly appearance when seated on the substrate. If more than one primordium develops in close proximity usually all but one abort early in development, or they remain checked in formation at this same stage until those close-by have matured. Often it is these primordia which are found after careful searching in the field and it is they which act as suitable material for study. They are particularly important in the study of the annulate species of *Conocybe* which have not been cultured. There is no indication that these primordia are either different in structure or will develop differently from the mature fruit-bodies.

Some of the loose hyphae on the primordia branch sympodially, the tips ballooning out to form drumstick-shaped terminal cells. The apical button in these cases frequently resembles the apical proliferation found in the lecythiform cheilocystidium

which characterizes many species of *Conocybe*. By subterminal branching larger, more inflated cells are formed from the pseudoparenchyma beneath the filiform, capitate or subcapitate cells. During further expansion of the primordium these former cells inflate still further and become fully exposed and rapidly form a continuous layer; this is the hymeniderm. The capitate cells wither and may become sloughed off, some remain attached to the base of the stipe, margin of the pileus and randomly, yet frequently, on the pileus disc; such cells may remain on the pileus until the fruit-body is fully mature. The presence of such cells may explain why Kühner (1935) recorded colourless filaments and capitate cells on the pilei in some of his collections of *C. spicula* (i.e. *C. rickeniana* Orton, 1960). It is here suggested that the clamp-connected hyphae with capitate end-cells and/or capitate lateral branches found in the *C. brunnea* group are formed in this way; similar hyphae appear to be

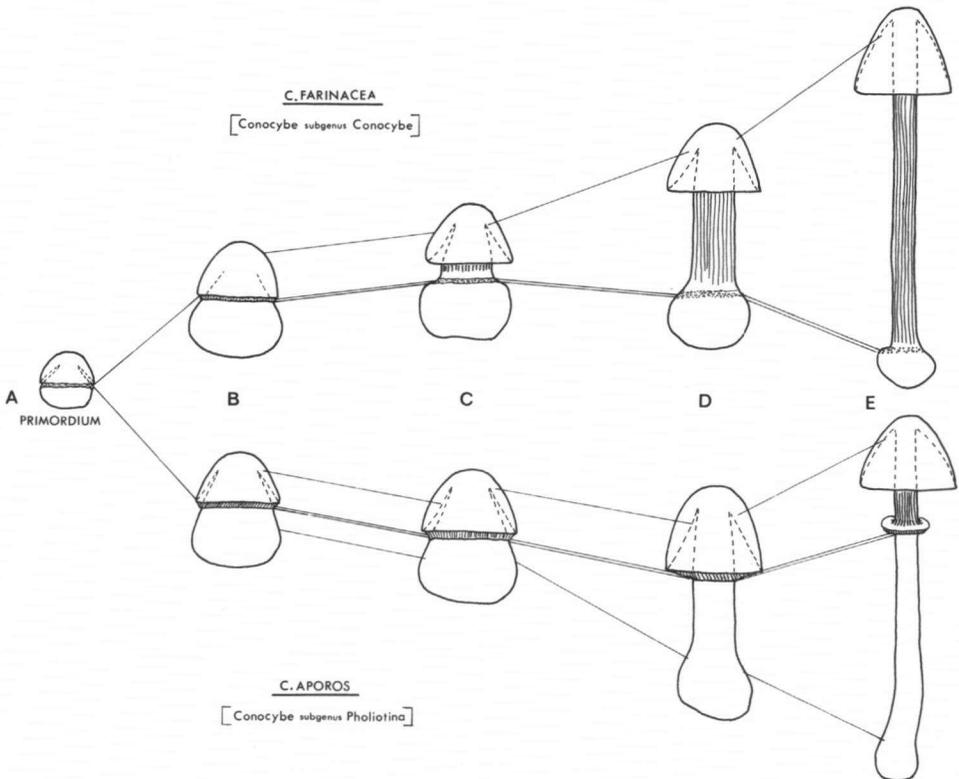


Fig. 1. Median sections of primordia and developing fruit-bodies showing diagrammatically the original and final positions of the caulocystidial zone in *Conocybe* (subgenus *Pholiotina*) *aporos* and *C.* (subgenus *Conocybe*) *farinacea*.

rare in *C. aporos*, *C. arrhenii* (Fr.) Kits van Wav., *C. blattaria* (Fr.) Kühner, and *C. filaris*.

In addition to such cells in *Conocybe* subgenus *Piliferae* and *Conocybe laricina* (Kühner) Kühner, as well as *C. brunnea*,² some of the hymeniform units are also converted into dermatocystidia (= pileocystidia). The development of members of *Conocybe* subgenus *Pholiotina* has not been studied in as great detail as other members of the genus *Conocybe* because of difficulties in culture but many observations indicate that in *C. aporos* and *C. filaris*, as well as *C. coprophila* (Kühner) Kühner in subgenus *Piliferae* the picture during the early stages of development of the fruit-body is very much the same as in *C. farinacea* Watling.

However, in later stages of development it is found that in primordia of *C. aporos* and several other members of the subgenus *Pholiotina* the stipe of the fruit-body is not completely homologous to the stipe in *C. farinacea*. In the former group the basal part of the primordia elongates to form the greater part of the stipe (see diagram); this is in contrast to *C. farinacea* where the expanding tissue which ultimately forms the stipe is that tissue at first hidden from sight above the junction of the margin of the immature pileus and stipe. This would explain why in *C. aporos* groups of cystidia analogous to the cheilocystidia are not found below the ring, for the cystidia on both stipe and gill-margin are formed in association with the lipsanenchyma which does not envelop the basal area of the primordia. The stipe in the lower region in subgenus *Pholiotina* is ornamented simply with long, clamp-connected hyphae with cystidioid tips; at most these end-cells are clavate to torpedo-shaped. True caulocystidia are found above the ring and in sections of primordia and young fruit-bodies they can be actually found on the ridges of the upper surface of the ring. In fact there appears to be a positive correlation between the ridges or striations on the ring and the number of secondary gills, for as far as field observations allow the number of ridges on the ring equals the number of gaps between the major gills. The development in *C. farinacea*, *C. tenera* (Schaeff. per Fr.) Fayod, *C. pubescens* (Gillet) Kühner, and *C. subpubescens* P. D. Orton, etc. observed in culture is parallel to that described above but the striations of the stipe in these species are due to the development of lines of caulocystidia; these striations are in fact analogous to the ridges above the ring in the annulate species, and those on the basal area of the stipe equivalent to those actually on the ring. Although this can be confirmed in culture for exannulate members of *Conocybe* subgenus *Conocybe*, fructification in pure culture of annulate members of subgenus *Pholiotina* has not as yet been achieved and observations are based on material collected in the field.

The type of veil produced during the paravelangiocarpic development of an agaric fruit-body is dependent on at least two factors: (1) the degree of mutual adherence of the individual hyphal elements constituting the velar tissue and (2) the

² Nomen nudum which will be validated in a later paper in this series.

degree of adherence of the velar tissue, lipsanenchyma, and associated tissue to the pileus and/or stipe. Thus from (1) one can obtain different textures of veil, e.g. filamentous, membranous and from (2) different positionings of the veil. If adherence to the pileus is strongest then a marginal veil is formed, whereas if adherence to the stipe is greatest an annulate veil results; intermediate types might be expected and are indeed found in the field. It is felt that mycologists incorrectly have tended to think only in terms of the extremes of the morphological series existing between annulate and exannulate specimens; perhaps this is left over from Elias Fries' classification where the presence of a ring is of paramount importance.

Conocybe peronata Kühner & Maire remains an anomaly; Maire's description in Kühner's monograph (1935) is based on a single collection from near Blida, Algeria (8 xii 1932, under *Cedrus atlantica*). The cheilocystidia are close to those found in *C. blattaria* and *C. filaris* but the spore dimensions are wrong for the former and the spore shape slightly different from that of the latter; there are also differences in veil pattern, stature, etc. It appears to be a good species but it is unfortunate that no more information on microscopic characters is available other than that in Kühner's original account because it would be most interesting to observe whether cystidia are present all down the stipe to the top of the peronate ring, as they should be if the observations discussed above are general, or whether cystidia cease to develop below a certain point; the actual development of this species would be intriguing to study.

In our studies the character of the presence or the absence of a veil in a single species of *Conocybe* has always been found to be constant, although its position may be variable, and in some cases its development may be reduced; cultural conditions are ideal for examining these characters. Even when fully annulate the adherence of the veil to the stipe is often tenuous, at first being superior or median on the stipe, although later during maturation it may well become inferior; such a veil is called mobile and may even fall to the bottom of the stipe and appear volvate (see above under *C. peronata*) or be lost altogether, particularly when badly collected. Herbarium material itself may be deceptive because the very act of collecting these annulate species of *Conocybe* may alter the position of the ring; certainly drying can modify the position even further by destroying the tenuous connection between ring and stipe.

The one factor however, which does remain constant is the character of the hyphae which make up the veil; unfortunately as yet no anatomical differences can be seen to distinguish the veils of the more critical species. Some species can be separated by their hyphal characters but these can also be separated on other more easily observable characters, e.g. on basidiospore shape and structure in *C. vestita* (Fr.) Kühner and *C. brunnea*.³

When a veil in an annulate species develops under very favourable conditions it may fragment, the remnants becoming irregularly distributed on the pileus margin;

³ Nomen nudum which will be validated in a later paper in this series.

they do not appear to form a distinct series of denticles. In contrast when fruit-bodies of typically marginal-veiled species develop, the veil splits up more regularly to give a distinctive and regular pattern; however, suspected annulate forms of *Conocybe appendiculata* are recorded (Kühner 1935)) as are similar forms of *Conocybe brunnea* (see below). It must at this point be strongly stressed that veils formed in other agarics during other types of development (e.g. bivelangiocarpic development, see Reijnders, 1967) do not have the same type of structure as those found in the Bolbitiaceae and may be much more constant in their external appearance. Thus although the presence of a veil is important as a character in *Conocybe* the position of the ring is of less importance in this same group, whereas in another group of agarics the presence and position of the veil may be equally important.

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