Studies on the morphogenesis of Agaricus bisporus: the dilemma of normal versus abnormal fruit body development

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Development of mushrooms is driven by genetic and epigenetic factors in a continuous interaction with the environment. It is assumed that each successive stage of morphogenesis depends on specific sets of signals arising at the appropriate time and place during the growth process. Morphogenetic dynamism proceeds in a time dimension through a cascade of signal-effect associations. Developmental errors may occur when such signals originate in the wrong place and/or at the wrong time. As a result various abnormalities such as ectopic tissues can develop and morphogenesis can be severely disturbed. Both endogenous genetic disturbances and exogenous factors can cause developmental errors. Lamellar dysplasia, which is a pore-like proliferation of the gills, forms an example; it may be induced experimentally. Both lamellar dysplasia and rosecomb disease of Agaricus bisporus result from endogenous genetic instability, whereas the developmental errors observed in wet bubble disease, which is caused by the infection of Mycogone perniciosa, originate from an exogenous factor. Morphogenesis normally leads to a symmetrical form of primordia of A. bisporus. Asymmetry is very frequently associated with an underlying pathological situation. Defining exact criteria of and sharp borderlines between normal and abnormal development seems infeasible. Fungi may readily tolerate morphogenetic imprecision. In this report, various macro- and microscopic features of normal and pathological development are illustrated; the dilemma of normal versus abnormal fruit body development has been discussed.

Mushroom growing in The Netherlands is dominated almost exclusively by the cultivation of the white button mushroom, Agaricus bisporus (J. E. Lange) Imbach. The mushroom growers of this country produce 12% of the world’s annual output of 2 x 10^6 t. The world’s most widely grown Agaricus strain, Horst® U1, was developed by Gerda Fritsche in the late seventies at the Mushroom Experimental Station at Horst (Fritsche, 1982, 1983) and has been commercially available since then. Because vegetative cultures of A. bisporus are genetically unstable (Loftus, Moore & Elliott, 1988; Li et al., 1994; Horgen et al., 1996) extreme care is taken by breeders, suppliers of mother cultures and spawn companies both to prevent morphological and physiological changes, which may result in strain degeneration, and to retain the characteristics of high yield and high product quality (Van Griensven et al., 1998). In addition to continuous monitoring for biological performance of mushroom strains, morphological studies of fruit body development are of great importance. We have long ago focused our attention on the determination of objective criteria for normal versus abnormal fruit bodies. This, at first glance simple task, is in fact an intriguing problem requiring substantial, interdisciplinary research on fungal morphogenesis and gross anatomic and microscopic studies of fruit bodies in health and disease (Umar & Van Griensven, 1995). Previous studies on the early development of primordia (Umar & Van Griensven, 1997a), on the hyphal regeneration and histogenesis (Umar & Van Griensven, 1997b), on the life span, senescence and developmental stages (Umar & Van Griensven, 1997c), and on the role of programmed cell death in the histogenesis of the mycelial cord and in the development of macrofungi (Umar & Van Griensven, 1998) have already been reported. Together they form an introduction to this aspect.

Rosecomb is a well-known and rather frequently occurring disease in mushroom cultivation (Lambert, 1930); morphological features of rosecomb disease are illustrated and used in this study to explain morphogenetic disturbances of endogenous origin. Wet bubble disease, caused by Mycogone perniciosa (Magnus) Delacr. is characterized by dramatic developmental errors (Chaze & Sarazin, 1936). Some relevant aspects of the experiments performed using this pathogen as an exogenous factor capable of modifying morphogenesis in A. bisporus are presented. The pathogenesis of cluster formation is still not fully understood (Umar & Van Griensven, 1995); the developmental errors observed in clustering may be exo- and/or endogenous of origin. Our view on the role of morphogenetic signals in normal growth process as well as in various types of developmental errors like ectopic tissues and organs are explained. Using A. bisporus as a model organism, we also discuss morphological landmarks to describe a normal fruit body and try to illustrate the borderlines between normal and abnormal development.

MATERIALS AND METHODS

Fruit bodies of A. bisporus, strain Horst® U1, used throughout this study were cultivated in our climate-controlled growing rooms as described by Van Gils (1988). In addition, an
unusually large specimen of rosecomb disease was supplied by a local mushroom grower. It was explicitly the only abnormal fruit body found in a large, environmentally controlled growing room.

Freshly harvested mushrooms from different flushes were randomly collected and routinely studied for the morphological criteria of normal and diseased fruit bodies. For analytical and comparative reasons, histological sections, gross anatomical and pathological specimens, including cluster forming fruit bodies of earlier studies, were re-evaluated. The symmetrical growth pattern and the occurrence of asymmetry were morphometrically investigated using 1437 randomly taken fruit bodies; 529 from the first flush, 577 from the second flush and 331 from the third flush. About 800 freshly harvested fruit bodies with different kinds of developmental errors such as cluster formation and rosecomb disease were gathered from our growing rooms and morphologically studied between 1993 and 1998.

Focal defects of the partial veil were experimentally created by small incisions and by cutting the lateral edge and the base of the hymenophoral organ of young primordia of about 1 cm tall. Wet bubble disease of *A. bisporus* was induced by spraying a mixture of spores and vegetative cells by *Mycogone perniciosa* (approx. $3.5 \times 10^3$ conidia and $3.5 \times 10^3$ bicellular spores ml$^{-1}$) on the casing soil at the growth stage of primordial initiation. The causal pathogenic fungus has afterwards been re-cultured from the previously inoculated, diseased mushrooms.

Tissue samples were processed for LM and TEM as described by Umar & Van Griensven (1997b). For the counting of hyphal reserve cells, 100 different tissue sections of 3 µm thick obtained from the fruit bodies of various developmental stages and they were stained with aqueous crystal violet (0.1% w/v) for 10 s. The decolourization of these stained sections was achieved with dipping in 50% ethyl alcohol. The hyphal segments still retaining the crystal violet stain were counted at the magnification 40 x. Statistical data were analyzed using the software programme, MicroCal Origin 3.0. Counting the primary lamellae of the fruit bodies was performed on 150 specimens with a cap diam. of 4–6 cm using a stereomicroscope, Zeiss SV11.

**RESULTS**

Primordial initials of healthy *A. bisporus*, are observed at the beginning of morphogenesis as tiny spherules attached to a mycelial cord (Umar & Van Griensven, 1998). They remain in a globular shape till they reach the size of about 5 mm (Fig. 1). A cut surface passing through the centre of such primordia reveals two, more or less equal, parts. Further development is characterized by a polarized growth resulting in formation and elongation of the stipe and steadily enlarging cap (Fig. 2). A medially bisected mature and healthy fruit body of *A. bisporus* gives a mirror image of both halves, a feature comparable to bilateral symmetry. Consequently, the transverse sections of both the stipe and pileus exhibit radial symmetry for any conceptual line crossing through the centre. Depending on the flushes and cultivation conditions, pilei were found to be asymmetrical in 2–8% of fruit bodies. The majority of asymmetrical pilei displayed retraction and a defect of the pileal margin (Fig. 3) mostly in combination with focal atrophy of the partial veil, lamellar dysplasia or ectopic tissues in descending order. The hymenophoral organ, stipe and pileus were grossly involved in various kinds of pathological changes when asymmetry was a prominent feature. The number of primary lamellae of *A. bisporus*, which connect the
Figs 4–8. LM features of the reserve cells in a mature fruit body of A. bisporus and its mycelial cord showing hyphal reserve cells. Sections are stained with crystal violet and slightly decolourized. Some of the nuclei are arrowed. Scale bar for all figures, 20 μm. Fig. 4. Upper part of the stipe. Figs 5 and 6. Pileal tissue. Figs 7 and 8. Mycelial cord. Note the non-transparent, darkly stained hyphal segments.

Fig. 9. TEM of a mycelial cord showing cross section of normally growing hyphal cells surrounded by a fine granular extracellular matrix (*). At the centre there is a reserve cell with densely packed and heavily osmiophilic cytoplasm. The arrow indicates an inclusion body. Scale bar, 1 μm.

Stipe to the cap margin, was found to be about 126 (mean 125·97, median 127, s.d. 6·79, n = 150).

Using crystal violet staining, non-decolourizable hyphal segments are shown scattered in the stipe mainly as narrow hyphae, in the pileus and in the mycelial cord (Figs 4–8) of A. bisporus. The counting of hyphal reserve cells which were defined by Umar & Van Griensven (1997b) of young primordia revealed a mean number of 0·99; median 1, and s.d. 0·87 (n = 100) per microscope field. In a 36 d. old fruit body, the result of the counting was: mean 0·03, s.d. 0·17 and median 0. In addition, the highest number of hyphal reserve cells was observed both in the pileal tissue and lamellar trama of growing primordia whereas the lowest value was found in the partial veil that composed of parallelly arranged and overlapping, hydrophobic hyphae. In the case of cluster formation, hyphal regeneration or lamellar dysplasia the number of hyphal reserve cells was three to four times higher than that found in a normal young primordium. TEM revealed that hyphal segments were distinctly osmiophilic and possessed a large amount of free ribosomes in the cytoplasm and also large glycogen rosettes (Fig. 9).

The severely malformed unusually large fruit body (21 cm x 16 cm x 13 cm) illustrated in Fig. 10 is an example of ‘rosecomb disease’ of A. bisporus (Lambert, 1930; Fletcher et al., 1989; Umar & Van Griensven, 1995). Its weight was 1·48 kg and its stipe 7·5 cm wide and 5 cm tall. A thick mycelial
cords was centrally attached at the base. The cap consisted of numerous, very irregular, sometimes cup-shaped structures up to 4 cm. Most of them were confluent and the usual radiating gill pattern was not present. Islands of irregular gill tissue were observed inside the cap in various vertical sections. Near the stipe, at the possible site of the stipo-hymenial junction, there were radiating lamellae only in a small area of about 3 cm wide. Histological sections made from the pileal surface of this fruit body revealed an ectopic hymenium with a warty appearance.

Morphologically, two different types of rosecomb disease of *A. bisporus* could be identified using a large series of collected specimens from growing rooms: the majority of cases (86%) was found to be of the **hymenophoral type** in which the partial veil is always focally absent and dysplastic lesions have an indisputable connection with the rest of the radiating gills through the opening of the hymenial cavity (Fig. 11). In the second form, the **pileal type**, there is no connection between mostly cup-shaped, but sometimes flat and verrucous, lesions and the hymenial cavity (Fig. 12). This occurred in 14% of the cases.
Figs 13, 14. Experimentally induced lamellar dysplasia (arrowed). A small lateral portion of the pileus was excised together with the partial veil when these primordia were about 14 mm long. **Fig. 13.** The largest cap diam. is 7 cm. **Fig. 14.** A close-up view. P, pileus; v, partial veil. Scale bar, 1 cm.

Figs 15, 16. Experimentally induced lamellar dysplasia. Note the absence of lamellae and an excessive, pore-like proliferation of the hymenium. v, partial veil; Scale bars, 5 mm.

We observed various developmental errors of the gills and pileal margin almost exclusively at the site of focal defects of the partial veil. We could experimentally induce abnormal lamellar morphogenesis and dysplasia simulating rosecomb disease in developing young primordia by an excision of a small piece of the partial veil (Figs 13–16). The results are always reproducible.

We also encountered some exceptional examples of *A. bisporus* primordia which were several centimetres in size but still in an undifferentiated state. These abnormal, pear-shaped primordia showed no signs of programmed cell death, formation of hymenial slit, or stipeal, pileal, basidial and lamellar differentiation (Figs 17–19).

Infection by *Mycogone perniciosa* caused various malformations of *A. bisporus*. Large (sometimes about 16 cm wide), very irregular, confluent, tumorous fungal structures were formed (Fig. 20) instead of the usual morphological patterns of a healthy fruit body. Most of the affected fruit bodies gained a cerebriform aspect. Microscopically, the histogenogenesis was found to be seriously disturbed in such pathological primordia. Cell and basidial differentiation was
Fig. 19. Fruit body on the right hand is 17 mm tall and pear-shaped and represents an exceptional primordial growth in an undifferentiated state. It is compared with a normally growing fruit body (on the left hand) of the same length.

Fig. 21. Cluster formation of A. bisporus. Five fruit bodies developing from a compact mass of fungal tissue. Scale bar, 1 cm.

generally absent, lamellae and hymenophoral organ usually did not develop or, if present, appeared as rudimentary structures. Extensive cell death and tissue necrosis became quickly visible during the development of nodular, hyperplastic, undifferentiated hyphal tissue. Non-viscous contents of degrading necrotic cells and extracellular fluid drained through small openings onto the surface as light yellow drops, hence ‘wet bubble’ disease.

Cluster mycelium characteristically produces a massive, undifferentiated, pseudosclerotal tissue instead of mycelial cords (Umar & Van Griensven, 1995). From this basal mass numerous fruit bodies develop in all directions (Figs. 21–23). Several new fruit bodies are frequently generated again in all positions from the stipe of already developed fungi. The growth process leading to cluster formation is typically very accelerated and clusters containing of tens of fully grown mushrooms appear actually before the regular first flush. The majority of the remaining primordia on the same culture bed are found still to be at the beginning of the histo-organogenetic growth stage as described by Umar & Van Griensven (1997c).

DISCUSSION

This investigation was started in 1993 and aimed at giving a scientific definition to a ‘normal’ fruit body of A. bisporus using morphological criteria. It is still not possible to define satisfactorily the normal fruit body despite the simplicity of the question and the intensive studies carried out. We briefly overviewed here our previously unpublished data and the results of gross anatomical, microscopical and pathological observations.

In A. bisporus, there is a long period of vegetative growth as a heterokaryotic mycelium followed by a relatively short period of fruiting during which the onset of primordia and their development into mushrooms take place. Fruit bodies of A. bisporus are attached to the mycelium through a linear organ, the mycelial cord, from which they originate (Umar & Van Griensven, 1997b, c, 1988). Fundamentally, they have the property of being organized in a symmetrical fashion from the initial stage on. Bilateral and radial symmetry dominate as an anatomical landmark except for the last stage of life, senescence, in which asymmetrical forms of organs develop. We observed asymmetry only in a small percentage (2–8%)

Figs 22, 23. Clustering fruit bodies of A. bisporus growing in all directions. Scale bars, 1 cm. Fig. 22. The largest one has a pileal protrusion. Fig. 23. A median cut surface of this fruit body.
of the harvested mushrooms. The coincidence of various pathological conditions in asymmetrical fruit bodies indirectly indicates that symmetrical development is the norm. Symmetry can, therefore, be used as a morphological criterion for the definition of normal fruit body of *A. bisporus*.

After this conclusion we have to explain how developing primordia realize the symmetrical form. Since LM consistently revealed a compact, darkly stained area in the middle portion of newly formed spherical primordia we presumed that (i) some kinds of morphogenetic signals may exist and that (ii) they may originate at the centre of primordia and may radiate equally and centrifugally towards all directions effecting the symmetrical development.

In animals, aggregated cells with the same grade of differentiation form a tissue together with the extracellular matrix. Cell-to-cell contact and firm intercellular connections are the rule. Accordingly, histogenesis in filamentous fungi such as *A. bisporus* (Umar & Van Griensven, 1997b, 1998) is characterized by hypha-to-hypha interaction via interhyphal spaces in the presence of the extracellular matrix through which morphogenetic signals should be able to propagate. In addition, signals may also spread through a hypha from one segment to neighbouring segments.

One of our most difficult tasks is the determination of the nature of such morphogenetic signals. In recent years, extensive studies were performed on morphogens and pattern formation during development especially in animals and plants (Scott, 1997) and to a lesser degree in fungi (Harold, 1995). Stillman (1997) has noted one of the most interesting conclusions drawn from sophisticated studies: ‘One of the great scientific accomplishments of the past decade was the recognition that the mechanisms used for patterning of tissues and organs during development are remarkably similar among species. What works for flies and frogs also serves human beings very well as embryos acquire their form and identity’. This citation may certainly be valid for fungi as well and supports our hypothesis.

We are not able yet to ascertain the nature of presumptive morphogenetic signals in fungi. Nevertheless, they must be originated at the right time of the development and at the right place inside a primordium. If such signals do exist and really play a key role in morphogenesis and cell differentiation, any inaccuracy in their origination in time and space must lead to visible developmental errors, for example, to an asymmetrical growth or to an ectopy.

Lamellar morphogenesis in *A. bisporus* becomes observable when primordia are about 6 mm tall (Umar & Van Griensven, 1997). This provided us with an alternative possibility for the defining of the normal fruit body. Establishing the number of primary lamellae first appeared as a reliable parameter for this goal. It turned out however, to be a variable number between 108 and 139, whereby the statistical mean value was 126. Because this large numerical variability is obviously tolerable in *A. bisporus*, and surprisingly enough does not interfere in its development, form and patterns, the number of primary lamellae cannot be the right criterion for the assessment of the normal.

One may expect that the radiating pattern of the lamellae, which are usually vertical and curtain-like hymenium-bearing structures in *A. bisporus*, would be a more reliable and constant feature of fruit bodies. There are several circumstances, however, that lead to ‘lamellar dysplasia’, in which the perpendicular form and gross patterns of the gills are severely disturbed without any impairment of spore forming and shedding. Thus, an anatomically malformed fruit body may still function normally. Apparently, fungi can easily tolerate the imprecision of lamellar morphogenesis to a great extent (Moore, 1998b).

‘Lamellar dysplasia’ defines a gross morphogenetic abnormality consistent with imprecision and formation of pore-like patterns. In the pathogenesis of lamellar dysplasia, the phylogenetic relatedness of *A. bisporus* to poly porous fungi or the expression of ancestral traits like pore formation deserve to be mentioned as evolutionarily conserved mechanisms. According to our observations the presence of the partial veil is needed for a normal, radiating pattern of lamellae and its absence results in developmental errors. This conclusion has been proven true by cutting off a portion of the cap margin together with the partial veil. The visible effect of this manipulation was perfectly consistent with lamellar dysplasia (Figs 13–16). Here the interruption of radiating morphogenetic signals can thus play a crucial role in the genesis of lamellar dysplasia. Obviously, the presence of the partial veil may be used as a morphological criterion for the normal fruit body before the developmental stage of spore shedding in *A. bisporus*.

The prototype of lamellar dysplasia is the so-called rosecomb disease of *A. bisporus* (Fig. 11). There have been several suggestions as to the cause of rosecomb disease (Flegg, 1983; Geels, van de Geijn & Rutjens, 1988). It is highly probable that exposure to diesel oil vapours and other agents cannot be etiologic but rather a co- incidental promoting factor. Records of the growth conditions for the unusual specimen of rosecomb disease (Fig. 10) revealed that there was no possibility for any contamination with agents which were previously suggested as inducers of rosecomb disease. It is tempting to speculate that some unknown endogenous factors played a fundamental role in the pathogenesis of developmental errors in this particular case.

Morphogenesis and histo-organogenesis can only be accomplished correctly if the governing genetic and epigenetic (Harold, 1995) factors function harmoniously in a time dimension (Moore, 1996, 1998a). According to our postulate the developmental errors are due to the absence or abundance and/or inaccuracy in timing and origin of the morphogenetic signals. When they originate they effectuate new aspects of the structure and/or function of the organisms. It is thus appropriate to speak about a signal/effect couple. If the former partner cannot be made easily demonstrable, the latter partner may provide a clue for an indirect way of explanation. We have thus far considered a continuous signal/effect couple originating from a central site inside the primordium to explain different aspects of the development. There are, however, still other questions to be answered in this context: What happens if NO morphogenetic signals originate inside a primordium and what happens if morphogenetic signals originate multicentrically or ectopically, in the wrong place and at the wrong time?
Figs 24–26. Fig. 24. Barely visible tumescence of the pileus (arrowed). Fig. 25. A vertical section through this small swelling reveals an intrapileal hymenial tissue with lamellae. Fig. 26. A small ectopic hymenial tissue with an imperfect simulation of lamellae is visible. Scale bars, 10 mm.

A cell is considered differentiated when it is able to express a new function and, consequently but not necessarily, a new shape, size and form. Cells frequently pass successive steps of a differentiation process to become finally radically different from the parental cells. If no morphogenetic signals are generated, cell differentiation and organogenesis would have not been accomplished. This is an extreme form of a developmental process but the dramatic effect of their absence can be observed in various circumstances. From time to time mushroom growers observe fluffy mycelial growth on the top of the casing layer and sometimes formation of stroma in large patches during the cultivation process without any identifiable cause. From this stroma no primordia initiate. In several instances, we found newly formed mycelial tissue making large, solid masses instead of cords. These masses remain inactive during the entire cultivation process. Horgen et al. (1996) reported considerable changes in the chromosome pattern of mycelial cultures derived from a variety of fluffy growing and stroma lines. These changes included chromosomal loss, length polymorphism, translocations and variations in the number of ribosomal repeats. Studies on mushroom cultivation revealed that an overwhelming majority (up to 95%) of primordia up to 10 mm in size, remains undifferentiated without any signs of further development. They are suppressed and deemed to regress and decay through the interaction of microorganisms. In other words, only about 5% of primordial initials are normally able to grow into mature state. A maximum of 1700 fruit bodies m⁻² suitable for harvesting was found to be produced by Amsing & Gerrits (1991).

Ectopically originating morphogenetic signals should result in a visible new tissue formation in the wrong place inside the body. If they are strong signals and appropriate to effectuate, for example, formation of gill tissue, then spherical islands of lamellae should be found outside the hymenophoral organ since the signals are ectopic and radiate centrifugally. Such cases do happen though infrequently (Figs 24–27). Fruit bodies that exhibit ectopical tissues are consequently asymmetrical in form by swellings and protrusions.

Thus, examples of duplicate stipe or pileus as complete organs at an unexpected site (Figs 28, 29) may be evidence for ectopic signals. Complete renewal of fruit bodies, a well-known ability of fungi (Rammel & Webster, 1995; Van der Aa, 1997), can start as well as a consequence of ectopic morphogenetic signals. The gross abnormalities shown in Fig. 10 may be initiated by multiple ectopic signals directing hymenial genesis at very many sites in and on the cap. In this pathological event, the ectopic hymenium with features of dysplasia can be explained with the absence of the partial veil at those atypical sites.

Cluster formation of A. bisporus is a rather new phenomenon in mushroom cultivation, regularly appearing since 1993. So far, no causal agent has been identified for this disease and it is generally assumed that genetic instability forms the etiopathogenesis (Van Griensven et al., 1998). One of the parental homokaryons of the cluster mycelium appears reproducibly affected, leading to strongly modified growth on compost (Sonnenberg, pers. comm.). According to our observations the growth characteristics of clusters indicate that (i) multiple morphogenetic signals directing primordial initiation arise at different places in abnormally polarized directions within the undifferentiated fungal tissue, and (ii) similar initiation signals can re-appear in the stipe of primordia, which are already in an
advanced developmental stage. Individual mushrooms of a cluster show no higher incidence of malformations when compared to normally grown fungi in the same growing room. Clearly, the way by which the clustering fruit bodies initiate and grow is abnormal although each individual has a normal anatomical structure (Fig. 21).

In fungi, cellular differentiation is not a stable process and needs additional factors to maintain the differentiated state (Moore, 1998c; Moore et al., 1998; Umar & Van Griensven, 1997b). Even basidia, which are terminally differentiated hyphal cells may dedifferentiate under certain experimental conditions (Chiu & Moore, 1990). Dedifferentiated hyphal cells could possibly be involved in fruit body renewal. Hyphal reserve cells, the presence of which was proposed in a study of wound healing and hyphal regenerative ability (Umar & Van Griensven, 1997b), may also be involved in the renewal process. Maturing and ageing fruit bodies contain mostly inflated, vacuolated or empty hyphal segments in increasing numbers parallel to the time dimension. In such conditions it is unlikely that regenerative ability can be retained by most of the hyphal cells. The reserve cell hypothesis is, therefore, plausible and provides an alternative pathway in the explanation of fruit body renewal under favourable sets of morphogenetic signals.

The heterotrophic life style of fungi and their interaction with other organisms may cause a large spectrum of disorders which may be classified as Fungal Pathology of plants and of animals. Fungi also have the ability to modify the development and structure of their hosts. On the other hand, fungi as living organisms are understandably subjected to the same kind of disorders caused by viruses, parasites, micro-organisms as well as by fungi themselves. During our study of the pathology of A. bisporus we noticed that some infectious organisms are capable of modifying its normal development including its symmetrical form and patterning. Especially, Mycogone perniciosa, causing wet bubble disease of A. bisporus (Chaze & Sarazin, 1936), has such a modifying potential. Pathological changes observed are characterized by necrosis, atrophy, local regeneration and hyperplasia of cells and tissues giving a nodular appearance to the fruit bodies (Fig. 20). Furthermore, infection by Mycogone perniciosa induces asymmetrical growth and a tumourous development of young primordia, actually a large mass of undifferentiated fungal tissue in which no differentiation of basidia, lamella formation or hymenophoral organ is recognizable. This fact is important from a morphogenetic point of view and it provides us with a powerful tool as an external modifier of the normal morphogenesis for further investigations.

With regard to cultivation of A. bisporus, the definition of normal versus abnormal development is clearly ambiguous. Since there can be considerable morphogenetic variations it is rather difficult to separate normal from abnormal with a firm borderline. Apparently, this field of research poses us a dilemma. Scientists involved in fungal biology and morphogenesis have repeatedly expressed their view that our current knowledge on the normal course of development still needs to be improved by additional research. Keeping in mind that fungi easily tolerate morphogenetic imprecision (Moore, 1998b), we wish to underline that the investigation of developmental abnormalities and of Pathology of Fungi in particular has the power to illuminate the way leading to a better understanding of normal development.

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Normal & abnormal development of *Agaricus bisporus*


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