

## Responses of lignicolous-agaric fruit-bodies to light and gravity: a study to overview the fruit-body development in hymenomycetes

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Our previous study revealed that, when exposed to light from below, fruit-bodies of humus-borne agarics grow straight downward both in the '*Coprinus Type*' and '*Non-Coprinus Type*' species and that, in the latter, gills tilt by gravitropism, caps swell and wave, and stipes twist. The present study revealed that fruit-bodies of some lignicolous agarics also grew straight downward. Among them, *Flammulina velutipes* and *Hypsizygos marmoreus* fruit-bodies showed almost the same behaviour as the '*Non-Coprinus Type*', but *Pleurotus ostreatus* (assumed *Polyporus Type*) fruit-bodies did not show the gill tilting and cap waving. Rather, it redifferentiated to produce new gills or new fruit-bodies, possibly by gravimorphogenesis, or formed spiral stipes. Based on these results, graviresponses in hymenomycetes are over-viewed.

Key Words—*Flammulina velutipes*; *Hypsizygos marmoreus*; morphogenesis; phototropism; *Pleurotus ostreatus*.

In many species of Agaricales, fruit-bodies initially grow toward light by positive phototropism, and then grow upward, opposite to the gravity vector, by negative gravitropism (e.g. Buller, 1909). While their stipes are negatively gravitropic, gills are positively gravitropic (Schmitz, 1842, 1843; Sachs, 1865, 1877).

Sensitivity of a fruit-body to light and gravity varies with the fungal species. Buller (1909) showed that *Lentinus edodes* (Berk.) Sing., a lignicolous agaric, possessed both phototropism and gravitropism, whereas *Agaricus campestris* L.: Fr., an epigeous agaric, possessed only gravitropism. He considered that, since *L. edodes* fruits on wood surfaces which are often not horizontal, the possession of both phototropism and gravitropism would allow its fruit-bodies to posture suitably for spore dispersal.

Previously we examined humus-borne epigeous agarics (Kaneko and Sagara, 2001), and here we examine lignicolous, wood-rotting agarics to compare the responses of these two groups to light and gravity. Wood-rotting agarics usually fruit on non-horizontal wood-surfaces, whereas epigeous ones usually fruit on horizontal ground-surfaces. We examined whether such difference in their fruiting habits has any significance in their sensitivity to light and gravity.

As a substratum, we previously used raw humus to obtain agaric fruit-bodies in light from below (Kaneko and Sagara, 2001). Here, we use sawdust, since it can as well be set afloat as the raw humus, and thus fruit-bodies emerging from the undersurface of the substratum can be exposed to light from below throughout their development.

Gorovoj et al. (1987) recognised the following three types of hymenomycetes as for gravitropic response.

Type 1: Most gill fungi and polypore fungi with soft, quick-growing fruit-bodies. Gills and tubes are wedge-shaped and the hymenophore has its own positive gravitropism, so that the spores may fall out freely. Formation and release of basidiospores occur simultaneously over the whole surface of the hymenophore (Buller, 1909).

Type 2: Species of *Coprinus*. Gills are parallel-sided, and formation and release of basidiospores advance gradually upward from the edge of each gill (Buller, 1909).

Type 3: Bracket fungi with tough, solid consistency. The fruit-body is considered to exhibit gravimorphogenesis rather than gravitropism; only the newly formed parts of the fruit-body assume a suitable posturing. ('Gravimorphogenesis' has been applied to morphogenesis in response to gravity: see Moore, 1991; for a typical example, see Fig. 24.) Ingold (1953) stated that gravity exerts a morphogenetic effect on bracket fungi, and that it determines the overall shape and form of the fruit-body.

Buller (1922) stated that the gills of the *Copriini* do not respond to gravity and that they are brought into approximately vertical posturing through a negatively geotropic response of the stipe. He called this type the '*Inaequi-hymeniiferous* or *Coprinus Type*'. He also called all Agaricales except the *Copriini* the '*Aequi-hymeniiferous* or *Non-Coprinus Type*'.

Hereafter, we will call the first type identified by Gorovoj et al. (1987) *Non-Coprinus Type*; the second

type, *Coprinus* Type; and the third type, *Polyporus* Type (for the former two types, we follow the terms proposed by Buller (1922)).

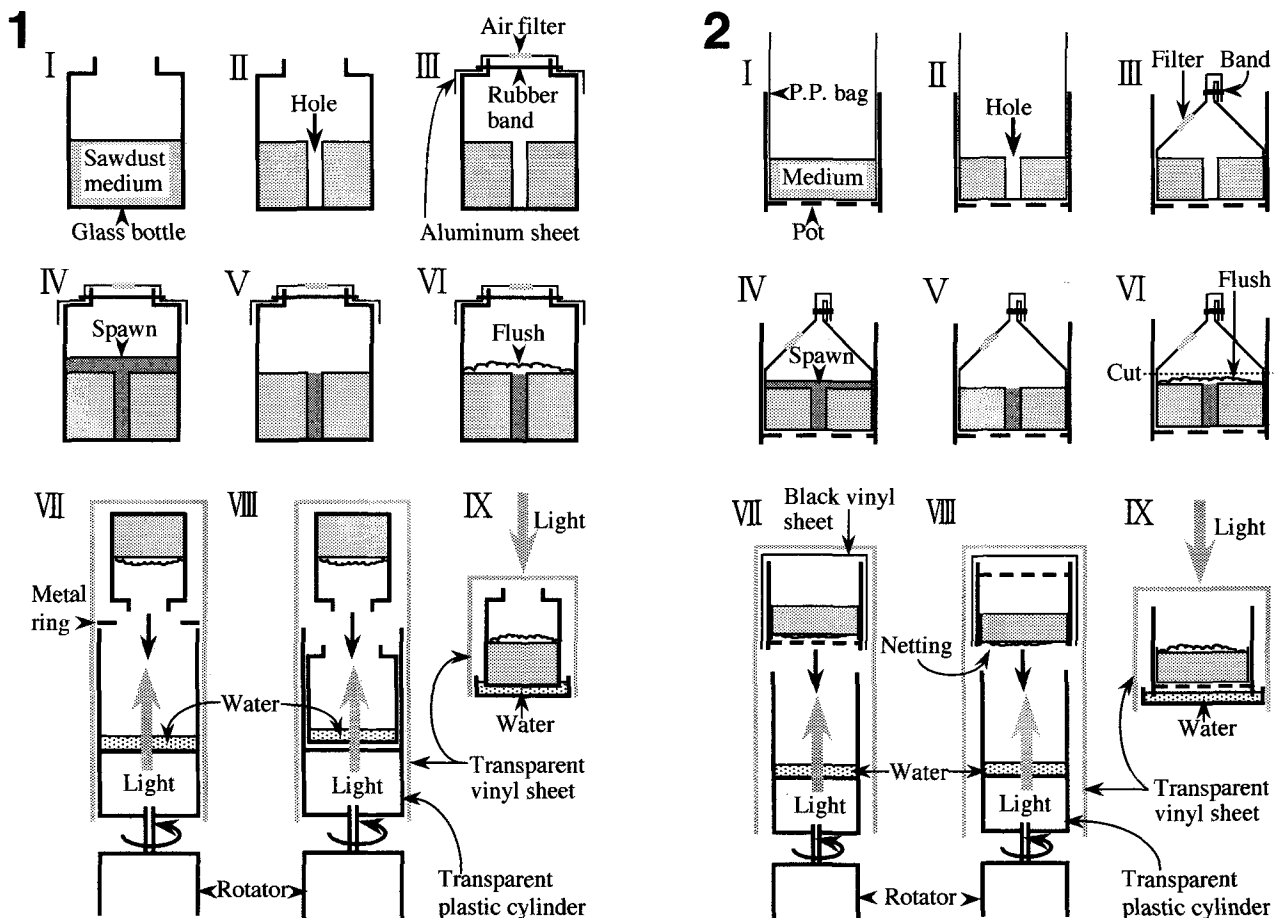
Previously we examined one *Non-Coprinus* Type species and three *Coprinus* Type species (Kaneko and Sagara, 2001). Here, we examine two *Non-Coprinus* Type species and one assumed *Polyporus* Type species, i.e., *Pleurotus ostreatus* (Fr.) Quél. The last has traditionally been classified into the agaric family Tricholomataceae because it possesses gills and white spores (Miller, 1973), but recently it is often regarded as belonging to Polyporaceae based on its hyphal system (monomitic/dimitic), DNA analyses, etc. (Moser, 1967; Singer, 1986). Here, we assume that *P. ostreatus* belongs to the *Polyporus* Type. Based upon this assumption, we will compare, in Discussion, the *Coprinus* Type, the *Non-Coprinus* Type and the *Polyporus* Type, to overview graviresponses in hymenomycetes, referring to our previous data (Kaneko and Sagara, 2001) and the literature.

## Materials and Methods

**Fungal species** *Flammulina velutipes* (Curt.: Fr.) Sing., *Hypsizygus marmoreus* (Peck) Bigelow and *Pleurotus ostreatus* which fruit on dead wood, were used. The former two species were expected to represent the *Non-Coprinus* Type, and the last the *Polyporus* Type.

*Flammulina velutipes* and *P. ostreatus* (wild types) were isolated on 29 Jan. 1995 from fruit-bodies collected in Kyoto City, Japan; *F. velutipes* (commercial cultivar 'Shinshu') was isolated on 1 Feb. 1994 from a fruit-body produced by JA Nagano Keizairen; *H. marmoreus* (commercial cultivar 'Yamabiko', Takara 2-go) was isolated on 14 Mar. 1994 from a fruit-body produced by JA Kamina. Thus, both a wild type and a commercial cultivar of *F. velutipes* were used, the latter being a pure white strain.

**Culture media** Cultures were maintained and subcultured at 10°C on malt-yeast-soytone agar (MYP agar: malt extract (Difco), 7 g; bacto soytone (Difco), 1 g; yeast extract (Difco), 0.5 g; agar, 15 g; distilled water, 1000 ml (Bandoni and Johri, 1972)). To obtain fruit-



Figs. 1, 2. Procedures for cultivation in glass bottles (Fig. 1) and bags (Fig. 2). I: Packing the medium. II: Making a hole (ca. 1 cm diam) reaching the bottom of the medium. III: Covering. (After this, the medium was autoclaved.) IV: Inoculation, followed by mycelial growth. V: Removing the surface mycelium from the medium. VI: Appearance of fruit-body primordia. VII, VIII: Setting the culture on the rotator to expose fruit-bodies to light from below. IX: Placing the culture in a container to expose fruit-bodies to light from above (control).

bodies, we used the medium composed of 68% (w/w) sawdust, 32% (w/w) rice bran and tap water (sawdust : rice bran = 3 : 1 (v/v); moisture content ca. 65%). The medium (240–400 g) was packed into 500–1000-ml glass bottles, the bottles which were then covered with aluminum sheets (Sun-Sheet12-12; Asahi Techno Glass Corp., Funabashi, Japan) (Fig. 1, I–III). The medium (300–700 g) was also packed into polypropylene bags for mushroom cultivation (Sun Bag; Santomi Sangyo Co., Ltd, Tokyo, Japan) (Fig. 2, I–III). The bags were put into pots which were the same as those used in the previous study (Kaneko and Sagara, 2001). After autoclaving at 120°C for 40 min and cooling to room temperature, the medium was inoculated with 5–15 ml of spawn grown on the MYP agar (IV in Figs. 1, 2).

**Incubation** The pots and bottles with the inoculated media were incubated at  $22 \pm 1^\circ\text{C}$  in the dark. Colonization was complete about 3–4 wk after inoculation, when the whole surface of the medium became covered with mycelium. Subsequently, the inoculated spawn and the surface layer of medium were removed (V in Figs. 1, 2), and the cultures were shifted to a lower temperature ( $15 \pm 1^\circ\text{C}$ , 60% relative humidity) to enhance fruiting. This treatment was done immediately after the completion of colonization for *F. velutipes*, more than 2 wk after for *H. marmoreus*, and more than 1 wk after for *P. ostreatus*. After this treatment, the cultures were placed under 12 h light/12 h dark using fluorescent tubes. The intensity of light from above, measured at the surface of the culture medium, was 800–1500 lx (1 lx = 1.46 mW/m<sup>2</sup>).

About 1–3 wk after the temperature shift, primordia

of the first flush began to appear (VI in Figs. 1, 2). At this time, the culture was placed on a rotator (VII or VIII in Figs. 1, 2). The fluorescent tubes and the apparatus equipped with the rotator were the same as those used in the previous study (Kaneko and Sagara, 2001). The intensity of light from below, measured at the undersurface of the culture, was 1200–1800 lx depending on the position in the incubator; that to study the effect of low-intensity light was set to ca. 50, 100, 150 or 200 lx.

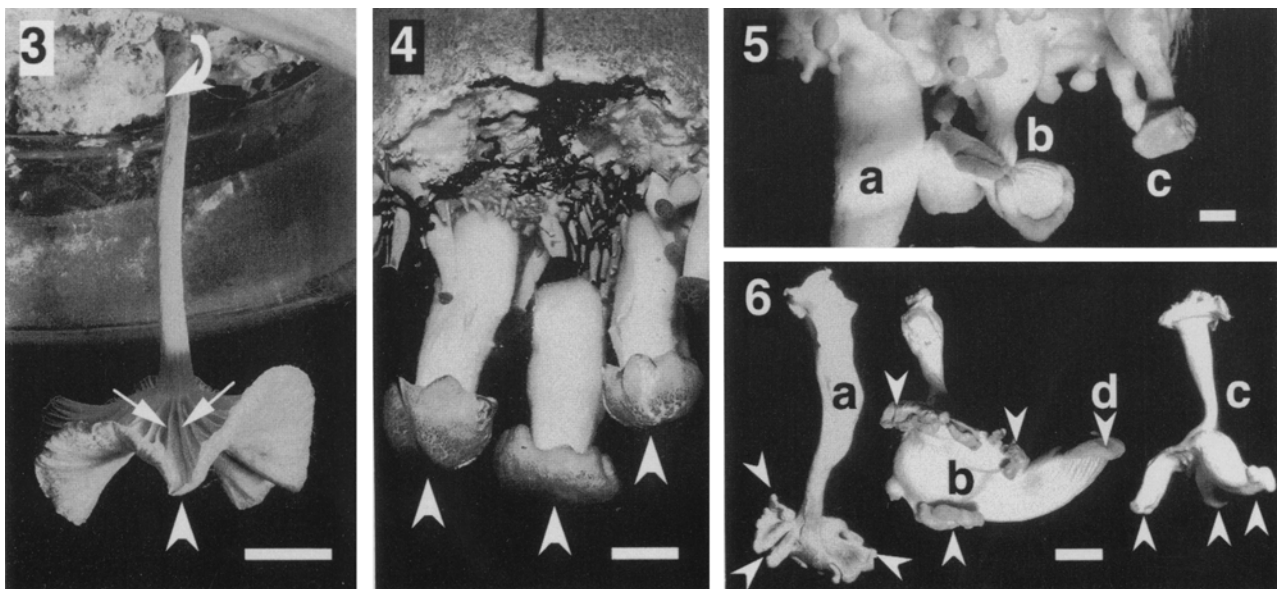
The control culture was left in the incubator without rotating (IX in Figs. 1, 2), under the same light conditions as described above.

The test fungi often fruit fasciculately, and this may cause oblique growth (by phototropism due to the shading by neighbouring fruit-bodies), resulting in gravitropic bending (Kaneko and Sagara, 2001). To avoid this, we thinned out the fasciculate fruit-bodies to expose the remaining ones directly to the light from below.

Fruit-bodies appeared mostly 1–3 mo after inoculation. In *H. marmoreus* and *P. ostreatus*, a few fruit-bodies appeared even after 6 mo.

## Results

**Posturing and development of the fruit-bodies** In all three species, most of the fruit-bodies exposed to light from below grew straight downward throughout all stages of their development (Figs. 3–5), that is, not only during the phototropic stage but also during the *presumed* gravitropic stage after the onset of basidiospore formation (for the term '*presumed* gravitropic stage', see Kaneko and Sagara, 2001). Gills faced upward.

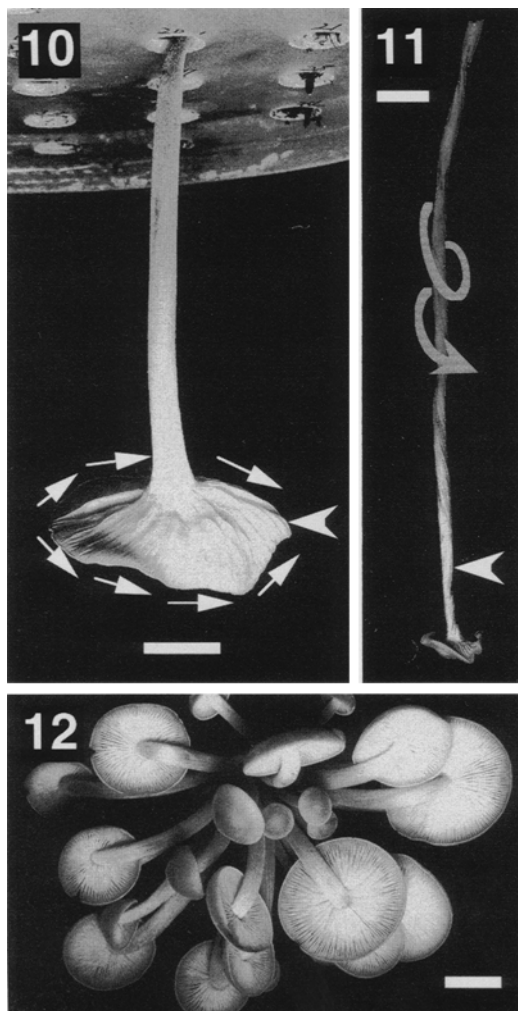


Figs. 3–6. Fruit-bodies growing straight downward in light from below. 3. *Flammulina velutipes* whose stipe twisted clockwise (arrow), cap wavy and gills tilted (arrows) toward a low part (arrowhead) of the wavy cap. This fruit-body formed basidiospores. 4. *Hypsizygus marmoreus* with wavy caps (arrowheads). These fruit-bodies formed basidiospores. 5, 6. *Pleurotus ostreatus* at successive stages of development. 5. Fruit-bodies (a–c) forming shallow gills which directed upward and formed basidiospores. 6. Fruit-bodies (a–c) of Fig. 5, 14 d later (a, c) or 16 d later (b) (composite photograph); their caps, now old, produced new fruit-bodies (arrowheads), one of which (d) grew upward and formed basidiospores. Scale bar = 1 cm.



Figs. 7, 8. Fruit-bodies of *Flammulina velutipes* (Fig. 7) and *Hypsizygos marmoreus* (Fig. 8) growing upward in light from above (control). Scale bar=3 cm.

Fig. 9. Fruit-body of *Pleurotus ostreatus* in light from obliquely above, growing toward light (arrow) in the early stage, and then upward after the onset of basidiospore formation. Scale bar=3 cm.



Figs. 10–12. *Flammulina velutipes* (wild type) fruit-bodies in low-intensity light from below. 10. Fruit-body growing straight downward with gills tilted (arrows) toward the lowest part (arrowhead) of the tilted cap. 11. Fruit-body growing straight downward; it first twisted anticlockwise (arrow), then clockwise (arrowhead). 12. Fruit-bodies in a fascicle, which emerged in all directions from one hole and bent upward when thinning was not carried out. Scale bar=1 cm.

Basidiospores were formed normally and projected from basidia, but they accumulated between gills without dispersing abundantly. Thus, the light from below disturbed spore dispersal owing to the inverted posturing of the fruit-bodies, but disturbed neither the formation of primordia nor the differentiation of cap, stipe and gills.

*Pleurotus ostreatus* fruit-bodies often dedifferentiated and redifferentiated after the straight-downward growth. As a result of redifferentiation, new fruit-bodies were produced on the old caps (Fig. 6). Most of the new fruit-bodies grew downward again (see Fig. 16), but some of them grew first obliquely downward, then bent upward and eventually dispersed spores (Fig. 6, d). *Pleurotus ostreatus* gills directing upward (Fig. 5, b) were shallower than those directing downward in the control (see Fig. 9).

In all species, some fruit-bodies bent upward under the same experimental conditions. This response will be discussed later.

All fruit-bodies exposed to light from above (control) grew upward as usual (Figs. 7–9). Their caps were first convex, then flat. Finally, some of them became repand. In all cases, gills directed downward and basidio-

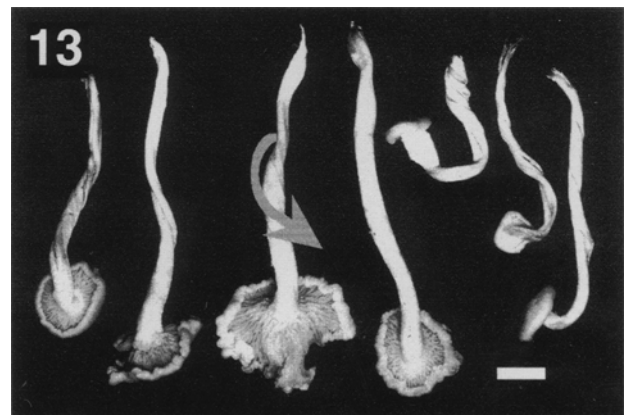


Fig. 13. *Flammulina velutipes* (commercial cultivar) fruit-bodies in light from below, showing anticlockwise twisting (arrow) of the stipes, and swelling and waving of the caps and gills. Three fruit-bodies to the right have bent due to strong twisting. Scale bar=1 cm.

Table 1. Direction of stipe twisting in *Flammulina velutipes* in light from below and from above (control), represented by the number and percentage of fruit-bodies showing each response.

Kind of strain and direction of light	Total no. <sup>a)</sup>	No. twisting clockwise	No. twisting anticlockwise	No. twisting clockwise and then anticlockwise	No. twisting anticlockwise and then clockwise
Wild type					
From below	134	53 (40) <sup>b)</sup>	67 (50)	7 (5)	7 (5)
From above	209	60 (29)	80 (38)	40 (19)	29 (14)
Commercial cultivar					
From below	74	27 (36)	41 (55)	3 (4)	3 (4)
From above	284	124 (44)	120 (42)	23 (8)	17 (6)

<sup>a)</sup> Including those exposed to low-intensity light.

<sup>b)</sup> Values in parentheses show percentage of total.

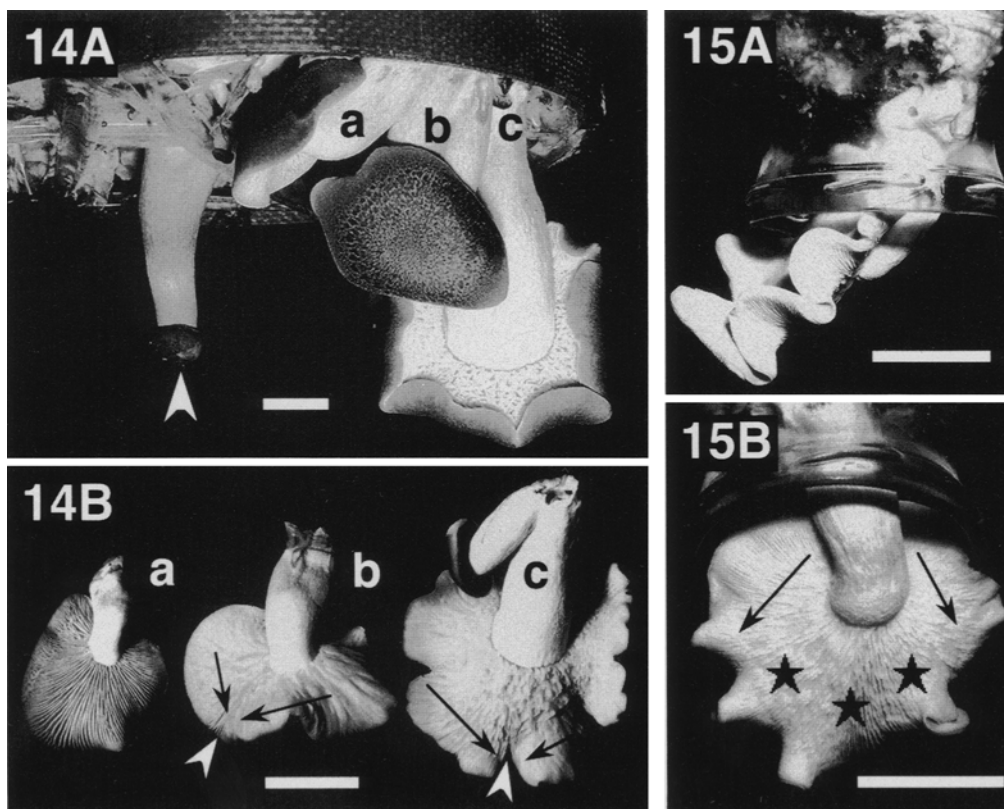
spores dispersed as usual.

The amount of the medium did not affect these responses, although it affected the yield of fruit-bodies and the period of fruiting (more medium gave a greater yield and a longer fruiting period).

**Factors affecting the behaviour of fruit-bodies: low-intensity light; fruit-body thinning; the second flush** In low-intensity light below 200 lx, all fruit-bodies of *H. mar-*

*moreus* and *P. ostreatus* bent upward after growing downward. In *F. velutipes*, however, a few fruit-bodies grew straight downward when thinning was carried out (Figs. 10, 11); when it was not carried out, they grew in all directions and bent upward, in the form of a chandelier (Fig. 12).

In all species under the same light conditions, caps tended to become smaller, and stipes thinner and longer



Figs. 14, 15. *Hypsizygus marmoreus* fruit-bodies in light from below, showing tilting, swelling and waving of the caps and gills. 14. A: One fruit-body that emerged singly and grew straight downward (arrowhead), and three that emerged in a fascicle (a-c). B: Ventral view of the three fruit-bodies (a-c), 18 d later. a: Fruit-body that emerged obliquely downward and has bent upward, with gills growing downward normally. b: Fruit-body that emerged slightly obliquely downward, with the cap and gills tilted. c: Fruit-body that grew downward, with the cap and gills swollen and waved. Gills of b and c tilted (arrows in B) toward the lowest part (arrowhead) of the cap. 15. Fruit-body which emerged obliquely downward from the edge of the culture and bent upward (the bending was incomplete, obstructed by the bottle). A: Side view. B: Ventral view. Higher gills tilted (arrows), and the lower gills (stars) and lower part of the cap swelled and waved. Scale bar=3 cm.

when thinning was not carried out. Such tendencies were also observed in fruit-bodies of the second to fourth flushes (Fig. 11 shows a fruit-body in the third flush from the medium shown in Fig. 10). When thinning was carried out, caps tended to become larger, swollen and waved, and stipes thicker and shorter as in Fig. 3.

**Responses of the caps, gills and stipes in *Flammulina velutipes*** The caps often tilted (Fig. 10) or swelled entirely with their margins waved (Figs. 3, 13). The gills sometimes tilted toward the lower parts of the caps (Figs. 3, 10), or swelled and waved (Fig. 13). The stipes sometimes became twisted (Figs. 3, 11, 13), and strong twisting caused their bending (Fig. 13). Viewed from the base of a fruit-body, twisting was anticlockwise (Fig. 13) or clockwise (Fig. 3), and sometimes reversed direction during growth (Fig. 11). The predominant direction was anticlockwise in both the wild type and the commercial cultivar (Table 1).

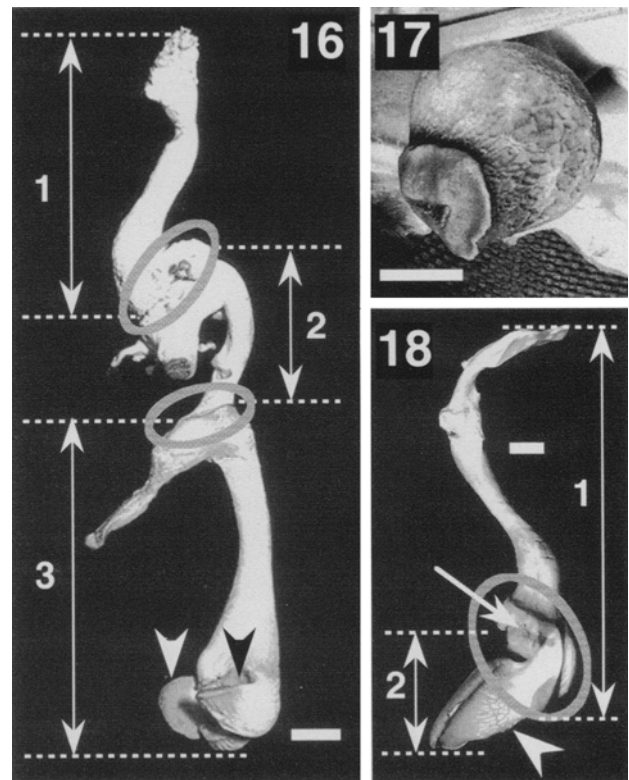
Morphologically abnormal developments such as swelling of the caps and gills, and twisting of the stipes were more remarkable in the commercial cultivar (Fig. 13) than in the wild type (Figs. 3, 10–12).

**Responses of the caps, gills and stipes in *Hypsizygus marmoreus*** The caps often tilted (Fig. 14, b) or swelled entirely with their margins waved (Figs. 4; 14, c; 15). The gills sometimes tilted toward the lower parts of the caps (Figs. 14B, b and c; 15B) or swelled and waved (Figs. 14B, c; 15B). As the caps grew larger, both caps and gills tended to swell and wave, and the stipes tended to grow thicker (Figs. 14, c; 15).

**Responses of the caps, gills and stipes in *Pleurotus ostreatus*** De- and redifferentiation of the caps often occurred when the fruit-bodies grew downward, but not when they bent completely upward or grew upward (in control). Figure 16 shows repeated de- and redifferentiation, where only those fruit-bodies having redifferentiated for the second time (arrowheads) grew upward to disperse basidiospores.

When fruit-bodies grew downward, the gills sometimes became shallow and mesh-like at the early stage of differentiation (Fig. 17), whereas this response was scarcely seen when fruit-bodies grew upward. Figure 18 shows mesh-like gills (arrowhead) formed after dedifferentiation of old gills and before redifferentiation into new gills.

A fruit-body (a) in Fig. 19 emerged obliquely downward and bent upward, finally drooping under its own weight. At this time, new primordia formed at the edge of the cap (Fig. 19, arrowheads) and gills tilted downward. Two other fruit-bodies (b and c) in Figs. 19 and 20 grew in the same way as (a), and formed funnel-shaped caps and new shallow gills on the morphologically upper surfaces of the caps (Figs. 21, 23, open stars). On their morphologically lower surfaces, gills directing upward were shallow (Fig. 22), while gills directing downward were almost normal (Fig. 23). Fruit-body (d) in Figs. 19 and 20 bent upward before the onset of basidiospore formation, then spiralled anticlockwise (Fig. 20).



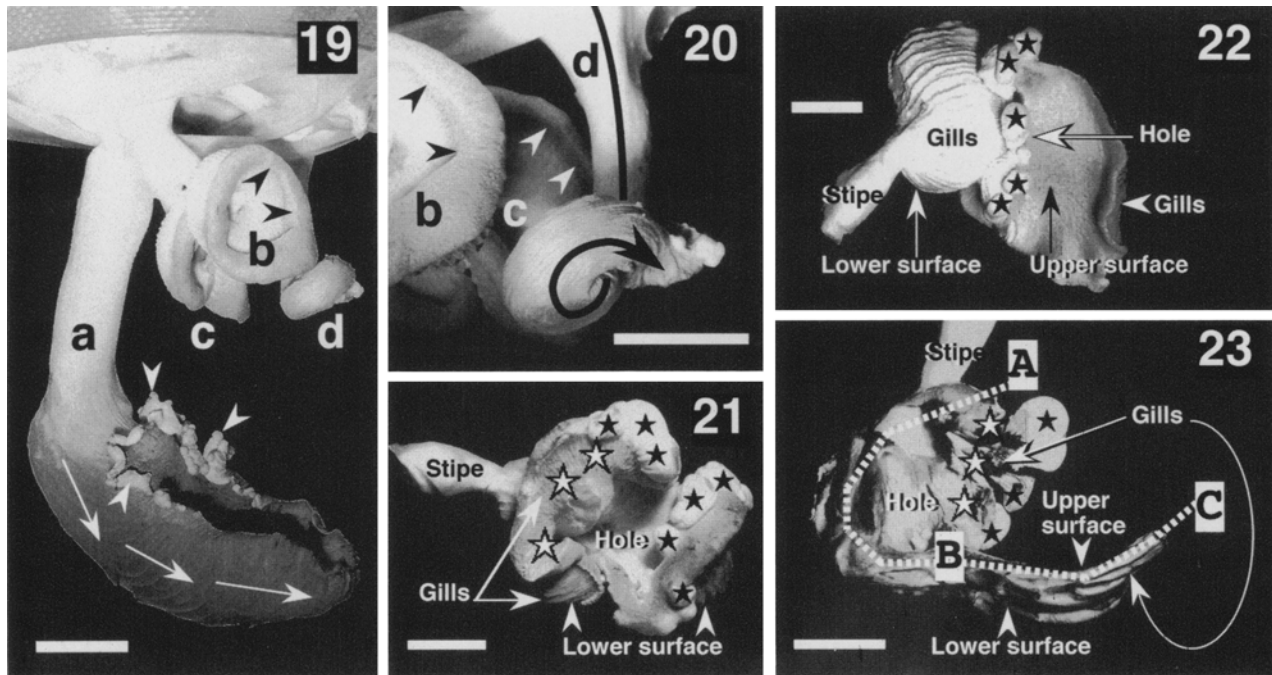
Figs. 16–18. *Pleurotus ostreatus* fruit-bodies in light from below, showing formation of new fruit-bodies or mesh-like gills. 16. Fruit-body which has redifferentiated twice, with fruit-bodies of the third stage (arrowheads) growing upward. 17. Fruit-body with swelled stipe, forming shallow, mesh-like gills, 9 mo after inoculation. 18. Fruit-body which has redifferentiated to produce a new cap with shallow, mesh-like gills (arrowheads). The upper surface of the first cap (half-tone ellipse) became uneven (arrow) before the redifferentiation; the lower surface, i.e., the previously normal gills, has become covered with dedifferentiating hyphae. In Figs. 16 and 18, the stages of fruiting are indicated by numbers: 1, first stage; 2, second stage, after the first redifferentiation; 3, third stage, after the second redifferentiation. Half-tone ellipses indicate the old caps from which new fruit-bodies have redifferentiated. Scale bar = 1 cm.

## Discussion

Table 2 summarises the results of the previous (Kaneko and Sagara, 2001) and present studies. Fruit-bodies of all species exposed to light from below showed the common behaviour of growing straight downward, but the morphogenesis of fruit-bodies and the graviresponse of gills of the *Coprinus* Type, *Non-Coprinus* Type and *Polyporus* Type differed. Differences between epigeous and lignicolous agarics in graviresponse were less remarkable than those among the above three types.

**Posturing** In light from below, fruit-bodies of all three species examined in the present study grew straight downward even after the onset of spore formation, as did





Figs. 19–23. Fruit-bodies of *Pleurotus ostreatus* in light from below, showing tilting of the gills, formation of new gills and spiralling of the stipe. 19, 20. a: Fruit-body with gills tilted downward (arrows) and new fruit-bodies formed at the edge of the cap (arrowheads). b and c: Fruit-bodies with initials of shallow gills on the cap (arrowheads). d: Fruit-body spiralling anticlockwise (arrow in Fig. 20). 21–23. Fruit-bodies (b) and (c) of Figs. 19 and 20, 18 d later. 21. Top view of the fruit-body (b). 22, 23. Top view of the fruit-body (c) (Fig. 22) and its vertical section (Fig. 23). (The vertical section of (b) had almost the same structure as that of (c), although the hole was shallower.) The caps redifferentiated to produce new, shallow gills (open stars) and primordia (solid stars). Gills formed first on the morphologically lower surface of the cap (Figs. 21–23), and subsequently (by redifferentiation) on the morphologically upper surface facing downward (the upper side of the hole in the cap, on the dotted line A–B in Fig. 23), but not on the surface facing upward (the lower side of the hole, on the dotted line B–C). Scale bar: 19, 20=3 cm; 21–23=1 cm.

those of *T. tesquorum* and *Coprinus* spp. in the previous study (Kaneko and Sagara, 2001). Therefore, this straight-downward growth without gravitropic bending can be said to be common to the *Coprinus* Type, *Non-Coprinus* Type and *Polyporus* Type as identified by Gorovoj et al. (1987), i.e., three different types of hymenomycetes as for gravitropic mechanism. Thus, our assumption presented in the previous study (Kaneko and Sagara, 2001) for *T. tesquorum* and *Coprinus* spp. can be extended as follows: hymenomycete fruit-bodies

principally grow straight downward throughout all stages of their development if they are exposed to vertically-directed light from below, and if they emerge vertically downward and grow straight without twisting.

After the straight-downward growth, however, only *P. ostreatus* fruit-bodies often de- and redifferentiated to produce new fruit-bodies and/or gills. We consider this (de- and re-) differentiation as a process of gravimorphogenesis (morphogenesis in response to gravity). Occurrence of gravimorphogenesis in *P. ostreatus* suggests

Table 2. Growth direction and behaviour in light from below of hymenomycete fruit-bodies, with reference to their 'Types' *sensu* Gorovoj et al. (1987).

	<i>Coprinus</i> Type	<i>Non-Coprinus</i> Type	<i>Polyporus</i> Type
Species	<i>Coprinus</i> spp. <sup>a,b)</sup>	<i>Tephroclybe tesquorum</i> <sup>a)</sup> , <i>Flammulina velutipes</i> , <i>Hypsizygus marmoreus</i>	<i>Pleurotus ostreatus</i>
Growth direction	Straight downward	Straight downward	Straight downward
Gill	Not tilting (non-gravitropic) Normal as in the control	Tilting downward (positively gravitropic) Sometimes swelling and waving	Rarely forming newly on the cap (gravimorphogenetic) Shallow and sometimes mesh-like
Cap	Normal as in the control	Swelling and waving	De- and redifferentiating
Stipe	Normal as in the control	Sometimes twisting	Sometimes spiralling

<sup>a)</sup> Fungal species used in the previous study (Kaneko and Sagara, 2001).

<sup>b)</sup> *Coprinus neologopus*, *C. phlyctidosporus* and *C. cinereus*.

that this fungus is closer to Aphylophorales than to Agaricales (see below). The redifferentiated fruit-bodies may bend upward by negative gravitropism if they emerge obliquely downward (Fig. 6, d); if none of them bend upward, they may redifferentiate a second time to produce fruit-bodies (Fig. 16). Namely, *P. ostreatus* fruit-bodies may respond to gravity by gravitropism when a fine adjustment is required as in the former case, or by gravimorphogenesis when a rough adjustment is required as in the latter case. It seems that their graviresponse, either gravitropism or gravimorphogenesis, is determined by their posturing. Thus, even when they grow downward, they are more likely to bend upward than the fruit-bodies of the other species.

De- and redifferentiation in *P. ostreatus* fruit-bodies have been known to occur under a high concentration of CO<sub>2</sub> (Kinugawa et al., 1986), but it does not seem to apply here for the following reasons. The de- and redifferentiation here occurred only when the fruit-bodies grew downward (Figs. 6, 16, 18) or when they did not bend upward completely (Figs. 19, a; 19–23, b and c); it never occurred when they grew upward or bent completely upward. Even when the fruit-bodies appeared from the same culture at the same time (i.e., when they can be assumed to have been under the same CO<sub>2</sub> concentration), those bending upward never de- and redifferentiated. Even within one fruit-body, new gills were formed only on that part of the cap facing downward (Figs. 21, 23, open stars). These observations suggest that the de- and redifferentiation here resulted mainly from inadequate posturing of fruit-bodies or parts of them.

**Upward bending in light from below** Even in the light from below, some fruit-bodies bent upward after growing downward. This was observed in all species. Possible explanations for this atypical growth are as follows.

(1) Light was not actually provided from directly below, being partly intercepted by neighbouring fruit-bodies (Figs. 12; 14, a). Alternatively, light intensity was too low (below 200 lx; Fig. 12) (see Table 1 in Kaneko and Sagara, 2001).

(2) The fruit-bodies were mechanically forced to emerge obliquely downward and thus had opportunity to bend (Figs. 12; 14, a; 15; 19, a). In *P. ostreatus*, redifferentiating fruit-bodies emerged obliquely downward and similarly had opportunity to bend (Figs. 6, d; 16, arrowheads).

(3) The fruit-bodies developed asymmetrically as a result of internal factors and thus had opportunity to bend (Figs. 13; 20, d).

Such atypical results were previously discussed from a more general (theoretical) viewpoint (Kaneko, 2001).

**Gravitropism or gravimorphogenesis in gills** Gills of *F. velutipes* and *H. marmoreus* tilted toward lower parts of the caps (Figs. 3, 10, 14, 15), in the same way as those of *T. tesquorum* (Kaneko and Sagara, 2001). This behaviour can be regarded as positive gravitropism in gills. In *P. ostreatus*, gill tilting by gravitropism (Fig. 19, a) may occur only when fruit-bodies bend upward or grow upward with gills growing downward; when fruit-bodies

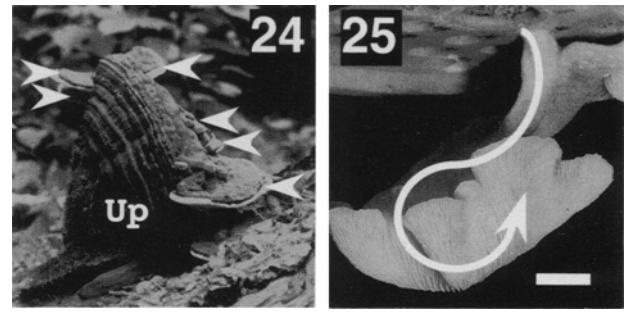


Fig. 24. Fruit-bodies of *Ganoderma applanata* (Pers.) Karst showing gravimorphogenesis: new fruit-bodies (arrowheads) are developing on an old one after fall of the dead tree, with pores directing downward, independent of the posturing of the old one. Up: morphologically upper surface of the old fruit-body. Tree top to lower left. Photograph on 1 Oct. 1984 in Kyoto Prefecture, Japan.

Fig. 25. Fruit-body of *Pleurotus salmoneostramineus* L. Vass. (SJAL 43) in low-intensity light, spiralling anticlockwise (arrow) and eventually growing upward. Scale bar = 1 cm.

grow downward with gills growing upward, redifferentiation of gills by gravimorphogenesis may occur (Figs. 19–23, b and c). Thus, in *P. ostreatus* gills, as in the fruit-bodies, posturing seems to determine the graviresponse of gravitropism or gravimorphogenesis.

The new formation of gills on the upper surface of the cap occurred only in *P. ostreatus* (Figs. 21, 23, open stars) among the seven species so far studied. The newly formed gills directed downward. This can be explained, as Gorovoj et al. (1987) suggested for bracket fungi, as follows: the newly formed parts of the fruit-body assume a suitable posturing by gravimorphogenesis. Examples of such gravimorphogenesis are shown in Fig. 24 and in Buller (1922). The formation of shallow gills in *P. ostreatus* on (a part of) its cap facing downward (on line A–B in Fig. 23) or on (a part of) the cap's lower surface facing upward (on line A–B in Fig. 23, see also Figs. 5, b; 22) suggests that, for normal development of gills in this species, adequate posturing of the fruit-bodies is required, with caps facing upward and gills downward (on line B–C in Fig. 23, see also Fig. 22). Mesh-like gills in *P. ostreatus* formed at the beginning of cap differentiation (Fig. 17) or of redifferentiation (Fig. 18) resemble, in shape, the shallow pores of *Polyporus* (see below).

Abnormal morphogeneses in *P. ostreatus*, such as gill formation on the cap (absence of dorsiventrality) and shallow or mesh-like gill formation (Figs. 17–23), have been reported for *Polyporus Type* species, which suggests that *P. ostreatus* should be classified into *Polyporaceae*: new shallow pores formed on the cap of *Trametes gibbosa* (Pers.) Fr. fruit-bodies growing into an abnormal, symmetric form but not into the bracket form (Ingold, 1953); labyrinthine pores (similar to mesh-like gills) formed in *Phellinus contiguus* (Fr.) Pat. in inverted dishes (Butler and Wood, 1988); tubes formed on the whole surface of pulvinate fruit-bodies in *Pycnoporus cinnabarinus* (Jacq.: Fr.) P. Karst. when the fruit-bodies were rotated



on the clinostat to cancel the effect of gravity (Hasselbring, 1907); shallow pores formed in *Polyporus brumalis* Pers.: Fr. under clinostat rotation (Plunkett, 1961); normal pores rarely formed in *P. brumalis* under clinostat rotation (Gorovoj et al., 1989) or under microgravity (Kasatkina et al., 1984). These abnormal morphogeneses seem to be caused by inadequate posturing of fruit-bodies (as in the present study) or certain inadequate conditions of gravity.

These findings suggest that a hymenophore of *Polyporus Type* species can change its form easily, and that no essential difference exists among gills, mesh-like gills (labyrinthine pores) and pores. Hilber (1978) expressed the same view from the observations of labyrinthine (pore-like) gills in *Pleurotus eryngii* (DC. ex Fr.) Qué. In addition, according to the hypothesis of Pegler (1983) that *Pleurotus*, *Lentinus* and *Panus* are derived from a polyporoid ancestry, the presence of gills in both *Pleurotus* and Agaricales could be a result of convergent evolution, and therefore *Pleurotus* could potentially possess the ability to form pores. Thus, as has often been suggested, the traditional classification of hymenomycetes based on hymenophore morphology may need to be revised.

**Twisting or spiralling in stipes** Stipes of *F. velutipes* sometimes twisted in the same way as those of *T. tesquorum* (Kaneko and Sagara, 2001). The *Non-Coprinus Type* species other than *H. marmoreus* twisted anticlockwise more often in light from below than in light from above (control) (Table 1; Table 2 in Kaneko and Sagara, 2001). *Hypsizygus marmoreus* did not show stipe twisting, possibly due to its thicker stipes.

Stipe spiralling as observed in *P. ostreatus* may be common to this group of fungi since it is also observed in another *Pleurotus* (Fig. 25). The spiralling may be related to the eccentricity of their fruit-body development: fruit-bodies are centric, eccentric or pleurogenous and hence probably potentially plagiotropic. By spiralling, the fruit-bodies have greater opportunity for gravitropic bending.

Stipe twisting in light from below as observed here occurs also in *F. velutipes* under microgravity (Kern and Hock, 1996); stipe spiralling occurs also in *Polyporus brumalis* under microgravity (Zharikova et al., 1977; Kasatkina et al., 1984). Thus, the stipe twisting and the stipe spiralling seem to be caused by inadequate posturing of fruit-bodies or by certain conditions of gravity.

**An overview of graviresponses in hymenomycetes** The absence of gravitropism in *Coprinus* gills may be attributed to their characteristic structure in which cystidia are firmly attached to the opposite hymenium, as discussed previously (Kaneko and Sagara, 2001). Buller (1909, 1922) suggested: it may also be related to their developmental pattern in which basidiospore maturation advances upward from the gill edge, followed by gill autodigestion (Buller 1909, 1922); this developmental process may promote the successful dispersal of basidiospores in *Coprinus* (Buller 1909, 1922); the presence of gravitropism in *Non-Coprinus Type* gills and their wedge-shaped form may promote the successful spore dispersal

in *Non-Coprinus Type* species, in a different way from *Coprinus Type* species. Gravimorphogenesis in *Polyporus Type* species may be related to the characteristics of their fruit-bodies which are mostly solid and long-lived: the solidity of the fruit-bodies makes them unable to bend gravitropically, and their long life-span increases the possibility of encountering a change of their fruiting positions. Therefore, *Polyporus Type* species should have to respond to gravity gravimorphogenetically but not gravitropically, unlike *Coprinus* and *Non-Coprinus Type* species.

The occurrence of morphologically abnormal developments such as cap swelling and stipe twisting in the *Non-Coprinus Type* species but not in the *Coprinus Type* suggests a difference in the pattern of fruit-body development between the two. Such morphologically abnormal developments rarely occurred in *Polyporus Type* (*P. ostreatus*).

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#### Literature cited

- Bandoni, R. J. and Johri, B. N. 1972. *Tilletiaria*: a new genus in the Ustilaginales. *Can. J. Bot.* **50**: 39–43.
- Buller, A. H. R. 1909. *Researches on fungi*, vol. 1. Longmans, Green and Co., London.
- Buller, A. H. R. 1922. *Researches on fungi*, vol. 2. Longmans, Green and Co., London.
- Butler, G. M. and Wood, A. E. 1988. Effects of environmental factors on basidiome development in the resupinate polypore *Phellinus contiguus*. *Trans. Br. Mycol. Soc.* **90**: 75–83.
- Gorovoj, L. F., Kasatkina, T. B. and Klyushkina, N. S. 1987. [Role of gravitation in the development of carpophores in hymenomycetes.] (In Russian.) *Mikol. Fitopatol.* **21**: 301–307. (Cited in Moore, 1991).
- Gorovoj, L. F., Kasatkina, T. B. and Laurinavichius, R. S. 1989. [Morphogenesis of mushrooms in changed gravitation conditions.] (In Russian.) Report of the N. G. Kholodny Institute of Botany, Academy of Sciences of the Ukrainian SSR. (Cited in Moore, 1991).
- Hasselbring, H. 1907. Gravity as a form-stimulus in fungi. *Bot. Gaz.* **43**: 251–258.
- Hilber, O. 1978. Biosystematische Untersuchungen zur Kenntnis von *Pleurotus* sect. *Pleurotus*. *Z. Mykol.* **44**: 31–50.
- Ingold, C. T. 1953. *Dispersal in fungi*. Clarendon Press, Oxford.
- Kaneko, A. 2001. Application of the equilibrium concept to the development of agaric fruit-bodies, with special reference to their straight downward growth in light from below. *Mycoscience* **42**: 75–82.
- Kaneko, A. and Sagara, N. 2001. Responses of agaric fruit-bodies to light and gravity: growth straight downward in response to light from below. *Mycoscience* **42**: 67–74.
- Kasatkina, T. B., Zharikova, G. G., Rubin, A. B. and Palmbach, L. R. 1984. [Development of higher fungi under conditions of weightlessness.] In: [Biological Studies on the Salyut Orbital Stations], pp. 46–49. Nauka, Moscow. (In Rus-

- sian.) (Cited in Moore, 1991).
- Kern, V. D. and Hock, B. 1996. Gravimorphogenesis and ultrastructure of the fungus *Flammulina velutipes* grown in space, on clinostats and under hyper-g conditions. *Adv. Space Res.* **17**: 183–186.
- Kinugawa, K., Takamatsu, Y., Suzuki, A., Tanaka, K. and Kondo, N. 1986. Effects of concentrated carbon dioxide on the fruiting of several cultivated basidiomycetes. *Trans. Mycol. Soc. Japan* **27**: 327–340.
- Miller, O. Jr. 1973. *Mushrooms of North America*. E. P. Dutton & Co., Inc., New York.
- Moore, D. 1991. Perception and response to gravity in higher fungi—a critical appraisal. *New Phytol.* **117**: 3–23.
- Moser, M. 1967. *Basidiomyceten II. Die Röhrlinge und Blätterpilze (Agaricales)*. Gustav Fischer Verlag, Stuttgart.
- Pegler, D. N. 1983. *The genus Lentinus, a world monograph*. Kew Bull. Add. Ser. **10**: 1–281.
- Plunkett, B. E. 1961. The change of tropism in *Polyporus brumalis* stipes and the effect of directional stimuli on pileus differentiation. *Ann. Bot. [N. S.]* **25**: 206–223.
- Sachs, J. 1865. *Handbuch der Experimentalphysiologie der Pflanzen*. Engelmann, Leipzig.
- Sachs, J. 1877. *Lectures on the physiology of plants*. Clarendon Press, Oxford.
- Schmitz, J. 1842. *Mycologische Beobachtungen, als Beiträge zur Lebens- und Entwicklungsgeschichte einiger Schwämme aus der Klasse der Gastromyceten und Hymenomyceten*. *Linnaea* **16**: 141–215.
- Schmitz, J. 1843. *Beiträge zur Anatomie und Physiologie der Schwämme. I–IV*. *Linnaea* **17**: 415–548.
- Singer, R. 1986. *The Agaricales in modern taxonomy*. 4th ed. Koeltz Scientific Books, Königstein.
- Zharikova, G. G., Rubin, A. B. and Nemchinov, A. V. 1977. Effects of weightlessness, space orientation and light on geotropism and the formation of fruit bodies in higher fungi. *Life Sci. Space Res.* **15**: 291–294.