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Responses of *Hebeloma radicosum* fruit-bodies to light and gravity: negatively gravitropic and nonphototropic growth

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Abstract Responses of the long-rooting agaric *Hebeloma radicosum* fruit-bodies to light and gravity were studied. In light from below or obliquely below, fruit-bodies grew straight downward with gills tilted and cap swollen and waved if they had emerged downward from the culture medium, or bent upward from the beginning if they had emerged obliquely downward. In light from above or obliquely above, they grew upward if they had emerged upward. Thus, they did not grow toward unilateral light from obliquely below or obliquely above, and hence their growth was nonphototropic and negatively gravitropic from the beginning of development. Even the straight downward growth seems to be latently negative-gravitropic. In the dark, fruit-bodies grew upward, forming pseudorhizas, but they remained immature; they matured only in the light. These characteristics may be related to the growth habits of the fungus colonizing deep in the ground, forming primordia there, and developing mature fruit-bodies on the ground.

Key words Agaricales · Gravitropism · *Hebeloma radicosum* · Mole · Pseudorhiza

Introduction

In many species of Agaricales, the fruit-body grows initially toward light by positive phototropism, and then upward by negative gravitropism (Buller 1909). The stipe is negatively gravitropic and gills positively gravitropic (Schmitz 1842, 1843; Sachs 1865, 1877).

The sensitivity of fruit-bodies to light or gravity varies with the species. Buller (1909) showed that *Agaricus campestris* L. : Fr., an epigeous fungus, possessed only gravitropism, whereas *Lentinus edodes* (Berk.) Sing. possessed both phototropism and gravitropism. He considered that because *A. campestris* fruits on the ground, which is usually horizontal, its fruit-body does not have to adjust its posture substantially to effect spore dispersal; and that because *L. edodes* fruits on dead wood, which is often not horizontal, its fruit-body has to adjust its posture by both phototropism and gravitropism. He also observed that, when the *A. campestris* cultures were hung upside down, the fruit-body appeared from their undersurface and bent upward, although not completely, with cap facing sideways.

Hebeloma radicosum (Bull. : Fr.) Ricken is known to colonize deep in the ground in deserted latrines of the mole, forming primordia there, growing upward through the soil, developing pseudorhizas (rooting bases), and fruiting on the ground (Sagara 1995, 1999). Buller (1934) stated that the upward growth of the pseudorhizas in *Xerula* (under the name *Collybia*), *Squamanita* (under the name *Tricholoma*), and *Coprinus* is due to negative geotropism, and that their longitudinal growth is inhibited in the light but never in the dark. However, his statement was based mostly on field observations. We studied the responses of the *H. radicosum* fruit-body to light and gravity in the laboratory using pure cultures to elucidate what stimulus leads it from the depths to the surface of the ground.

To clarify whether these deep-colonizing and pseudorhiza-forming habits have any relevance to sensitivity to light and gravity, we compared this fungus with “epigeous agarics” that colonize shallow soils and fruit on the ground (Kaneko and Sagara 2001a) and with “lignicolous agarics” that colonize dead wood and fruit on its surface (Kaneko and Sagara 2001b). Because *H. radicosum* was found here to belong to “*Non-Coprinus Type*” sensu Gorovoj et al. (1987) (for type name, see Kaneko and Sagara 2001b), we also compared this species with other “*Non-Coprinus Type*” species studied previously (Kaneko and Sagara 2001a,b) with respect to their sensitivity to light and gravity and their fruit-body development in light from below.

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Materials and methods

Fungal species

Stock cultures of *H. radicosum* were obtained as follows. Strain NAO 676 (IFO 32939) was isolated on 24 October 1992 from the tissue of a fruit-body collected in Kutsukimura, Shiga Prefecture, Japan; strain KHR 981 (IFO 33212) was isolated on 20 October 1998 from the tissue of a fruit-body collected in Kutsuki-mura; and strain KHR 982 (IFO 33213) was isolated on 29 October 1998 from the tissue of a fruit-body collected in Miyama-cho, Kyoto Prefecture, Japan.

Culture media

The isolates were subcultured and maintained at 10°C on malt-yeast-soytone agar (MYP agar; Bandoni and Johri 1972) containing 7 g malt extract (Difco, Detroit, MI, USA), 1 g bacto soytone (Difco), 0.5 g yeast extract (Difco), and 15 g agar in 1000 ml distilled water. To obtain fruiting of the fungus, we used the medium employed by Ohta (1998) with the vitamin mixture excluded; it consisted of 90% (w/w) barley grain, 10% (w/w) sawdust of broad-leaved trees, water, and additional nutrients. This medium was prepared as follows: 10 g of the mixture of barley grain and sawdust was put in 30-mm (internal diameter) test tubes, nutrient solution (20 ml) was added, and the tubes were capped with cotton stoppers; similarly, 35–90 g of the mixture of barley grain and sawdust was put in glass bottles or polypropylene bottles (200–500 ml), nutrient solution (60–150 ml) was added, and the bottles were covered with aluminum sheets (Sun-Sheet 12–12; Asahi Techno Glass, Funabashi, Japan) (see Fig. 1 in Kaneko and Sagara 2001b). The medium was allowed to stand for 4 h, stirred well, and then autoclaved at 120°C for 40 min.

Incubation

The foregoing media were inoculated with 5–15 ml of spawn that had been grown on the MYP agar.

In experiments 1–4, the tubes and bottles with the inoculated media were incubated at $22^{\circ} \pm 1^{\circ}\text{C}$ in dim light, 10–30 lux (lx). About 6 weeks after inoculation, when the whole surface of the medium was covered with a mycelium, they were shifted to low-temperature conditions ($15^{\circ} \pm 1^{\circ}\text{C}$, 60% relative humidity) with a photoperiod of 12 h light: 12 h dark under fluorescent tubes. The intensity of light from above, measured at the upper surface of the culture medium, was 800–1500 lx ($\sim 1.2\text{--}2.2\text{ W/m}^2$; $1\text{ lx} = 1.46\text{ mW/m}^2$) depending on the position in the incubator. About 1–3 weeks after the shift to low temperature, primordia began to appear, sometimes fasciculately. These cultures were then used in the following experiments.

Experiment 1. The culture was turned surface-down to allow fruit-bodies to emerge from its undersurface, and rotated on the rotator (Fig. 1) to expose the fruit-bodies

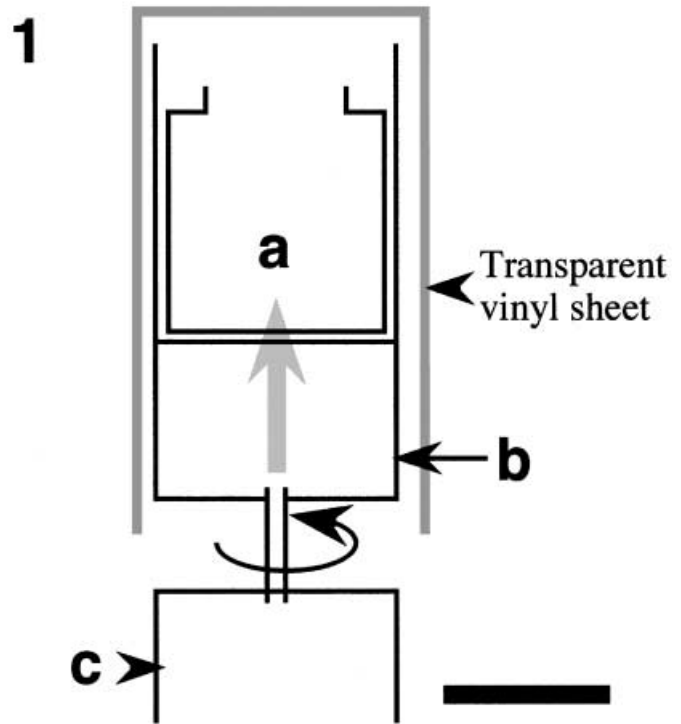


Fig. 1. Apparatus used in experiments 1–3. *a* Glass bottle containing a culture (e.g., Fig. 3), *b* transparent plastic cylinder, *c* rotator. (For details, see Kaneko and Sagara 2001a,b.) In experiment 1, the culture bottle (*a*) and the transparent plastic cylinder (*b*) were rotated with a rotator (*c*) in the direction of the arrow, and hence unilateral light was made to be uniform, i.e., vertically upward (half-tone arrow); in experiments 2 and 3, *a* and *b* were not rotated, and hence unilateral light was provided. Bar 10 cm

to light from below. (For the term “light from below,” see Kaneko and Sagara 2001a.) The fluorescent tubes and the apparatus fitted with the rotator were the same as those used in the previous studies (Kaneko and Sagara 2001a,b). The intensity of light provided from below, measured at the undersurface of the culture, was 1000–1600 lx ($\sim 1.5\text{--}2.3\text{ W/m}^2$) depending on the position in the incubator.

Experiment 2. The culture was turned surface-down and placed on the rotator without rotating to expose fruit-bodies to light from obliquely below. (When the rotator was not rotated, light was provided from the side, but, owing to shading by the culture itself, the fruit-bodies were actually exposed to light from obliquely below.)

Experiment 3. The culture was left surface-up to allow fruit-bodies to emerge from its upper surface, and placed on the rotator without rotating to expose the fruit-bodies to light from obliquely above.

Experiment 4. The culture was left surface-up in the incubator without being placed on the rotator, to expose fruit-bodies to light from above.

In experiments 5 and 6, the culture was placed in a cardboard box, which was then sealed, and incubated in the dark at $22^{\circ} \pm 1^{\circ}\text{C}$ and 60% relative humidity to examine the effect of light on the fruit-body development. About

6 weeks after inoculation, the culture was shifted to a lower temperature ($15^{\circ} \pm 1^{\circ}\text{C}$, 60% relative humidity).

Experiment 5: the culture was turned surface-down.

Experiment 6: the culture was held surface-up.

In experiments 7 and 8, some of the cultures used for experiments 1–6 were shifted from dark to light conditions or vice versa 11–12 weeks after inoculation to study the effect of the shift.

Experiment 7. Some of the cultures used for experiments 5 and 6 were shifted from the dark to the light. By the time of this shift, fruit-bodies had already appeared and grown upward without cap and gill formation.

Experiment 8. In contrast to experiment 7, some of the cultures used for experiments 1–4 were shifted from the light to the dark. By the time of this shift, fruit-bodies had already appeared and differentiated into cap and stipe, but their gills and basidiospores were still immature.

Results

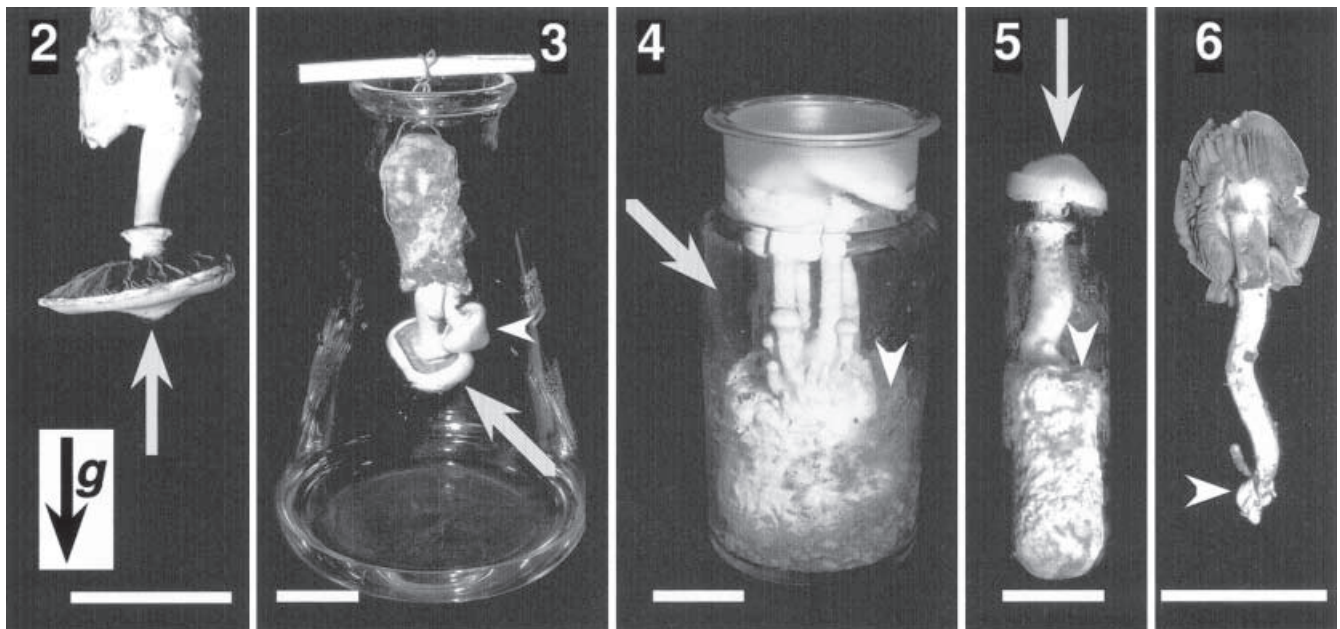
Fruit-bodies emerged from the upper surface of the culture, i.e., from the surface onto which the spawn was inoculated. Even after the culture was turned upside-down, primordia continued to emerge from its previously upper (now under) surface.

Development of the fruit-bodies in the light (experiments 1–4)

In the light from below (experiment 1), most of the fruit-bodies grew straight downward throughout all stages of their development (Fig. 2), even after the onset of basidiospore formation. Gills faced upward. Basidiospores formed normally; they left basidia, but mostly accumulated between gills. Thus, the light from below did not disturb normal formation of primordia, normal differentiation of caps, stipes, and gills, but disturbed spore dispersal because of the inverted posturing of the fruit-bodies.

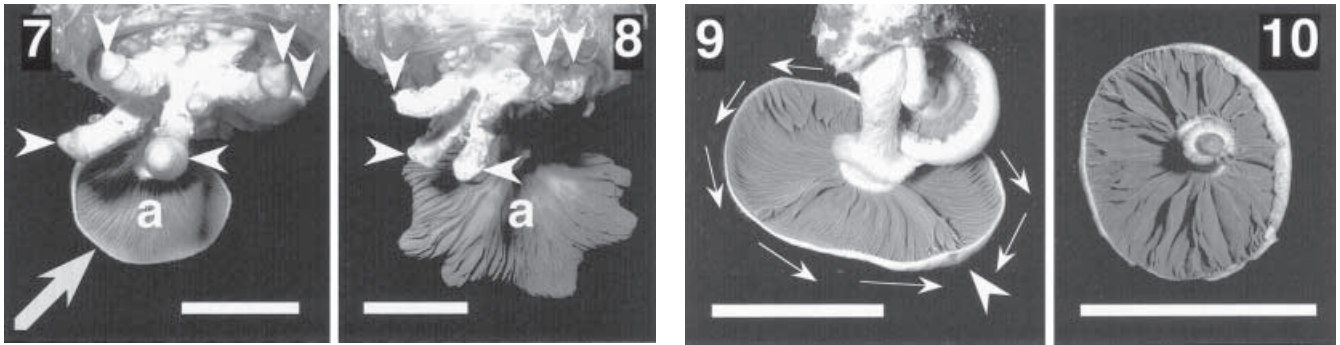
In the light from obliquely below (experiment 2), most of the fruit-bodies grew straight downward throughout all stages of their development (Fig. 3), in the same manner as in experiment 1. In the light from obliquely above (experiment 3), most of the fruit-bodies grew straight upward and matured normally (Fig. 4). In the light from above (experiment 4), most of the fruit-bodies grew upward and matured normally (Fig. 5). Under these light conditions (experiments 1–4), none of the fruit-bodies formed pseudorhizas (Fig. 6).

The fruit-bodies sometimes appeared fasciculately (for experiment 3, see Fig. 4). When the cultures were held upside down (experiments 1, 2), most of those fasciculate fruit-bodies emerged obliquely downward but quickly bent upward, either before the onset of basidiospore formation



Figs. 2–6. Development of *Hebeloma radicosum* fruit-bodies in the light (experiments 1–4). Half-tone arrows, directions of light. Bars 3 cm. **2** Fruit-body growing straight downward in the light from below (experiment 1) (NAO 676). *g*, gravity vector. After appearance of the primordia on the upper surface of the medium, the medium was taken out of the test tube and hung upside down in a glass flask as shown in Fig. 3. **3** Fruit-bodies growing straight downward in the light from obliquely below (experiment 2) (NAO 676). They did not grow toward

light; the one on the right (arrowhead) was forced to bend, pressed by one on the left, after ceasing elongation. **4** Fruit-bodies growing straight upward in the light from obliquely above (experiment 3) (NAO 676). Basidiospores dispersed from the fruit-bodies (arrowhead). **5** Fruit-body growing straight upward in the light from above (experiment 4) (NAO 676). Basidiospores dispersed from the fruit-body (arrowhead). **6** Fruit-body taken out of the test tube in Fig. 5, showing the absence of pseudorhizas at its base (arrowhead)



Figs. 7, 8. Fruit-bodies emerged fasciculately and bent upward from the beginning of development, independent of the direction of light (here, it is from obliquely below) (experiment 2) (KHR 981). *Halfpne arrow*, direction of light. Bars 3 cm. **7** Fruit-bodies bent upward without basidiospore formation (*arrowheads*) or before its onset (*a*). **8** Fruit-bodies of Fig. 7, 1 month later. Vertical sections of the caps show the absence of mature gills (*arrowheads*). Fruit-body (*a*) did not bend upward completely, and showed swelling and waving of the cap and gills

(see *a* in Figs. 7, 8) or without proceeding to basidiospore formation (*arrowheads* in Figs. 7, 8).

Responses of the cap and gills to the light (experiments 1–4)

When exposed to light from below (experiment 1) or from obliquely below (experiment 2), the cap often tilted (Fig. 2; see also Fig. 15) or swelled entirely with a wavy margin (Figs. 3, 9); gills tilted toward the lower parts of the tilted or waved cap (Fig. 9), or was swollen and wavy (Figs. 2, 10). Such behavior also occurred in the fruit-bodies that appeared fasciculately and bent upward incompletely (Fig. 8, *a*).

When exposed to light from above (experiment 4) or from obliquely above (experiment 3), the cap was first convex (Fig. 4) and then flat without swelling, and the gills grew downward.

Development of the fruit-bodies in the dark (experiments 5, 6)

In the upside-down cultures incubated in the dark (experiment 5), the fruit-bodies (pseudorhizas; see Discussion) appeared fasciculately from the undersurface of the medium (Fig. 11). Most of them emerged obliquely downward, and then bent upward like those in the light (experiments 1, 2; Figs. 7, 8). However, unlike those in the light, they did not develop a cap and continued growing upward. They often grew through the gaps between the medium and the bottle wall (Fig. 11A) but not into the medium itself (Fig. 11B).

In the cultures left surface-up and incubated in the dark (experiment 6), the fruit-bodies emerged fasciculately from the surface of the medium and grew upward from the beginning (Fig. 12); they remained immature (Figs. 12, 13). In one of these cultures, the fruit-bodies branched at the mouth of the bottle (Fig. 14).

Figs. 9, 10. Behavior of caps and gills in the fruit-bodies growing downward in the light from below (experiment 1) and in the light from obliquely below (experiment 2). Bars 3 cm. **9** Fruit-bodies of Fig. 3, 6 days later; gills of the left fruit-body tilted (*arrows*) toward the lowest part (*arrowhead*) of the wavy cap. **10** Gills of the fruit-body in Fig. 2, 13 days later, have swelled and become wavy

Under dark conditions (experiments 5, 6), the fruit-bodies appeared more abundant than in the light, being thinner and longer, but did not mature. Thus, the darkness did not disturb the formation of primordia and the upward growth of stipes but disturbed the maturation of fruit-bodies.

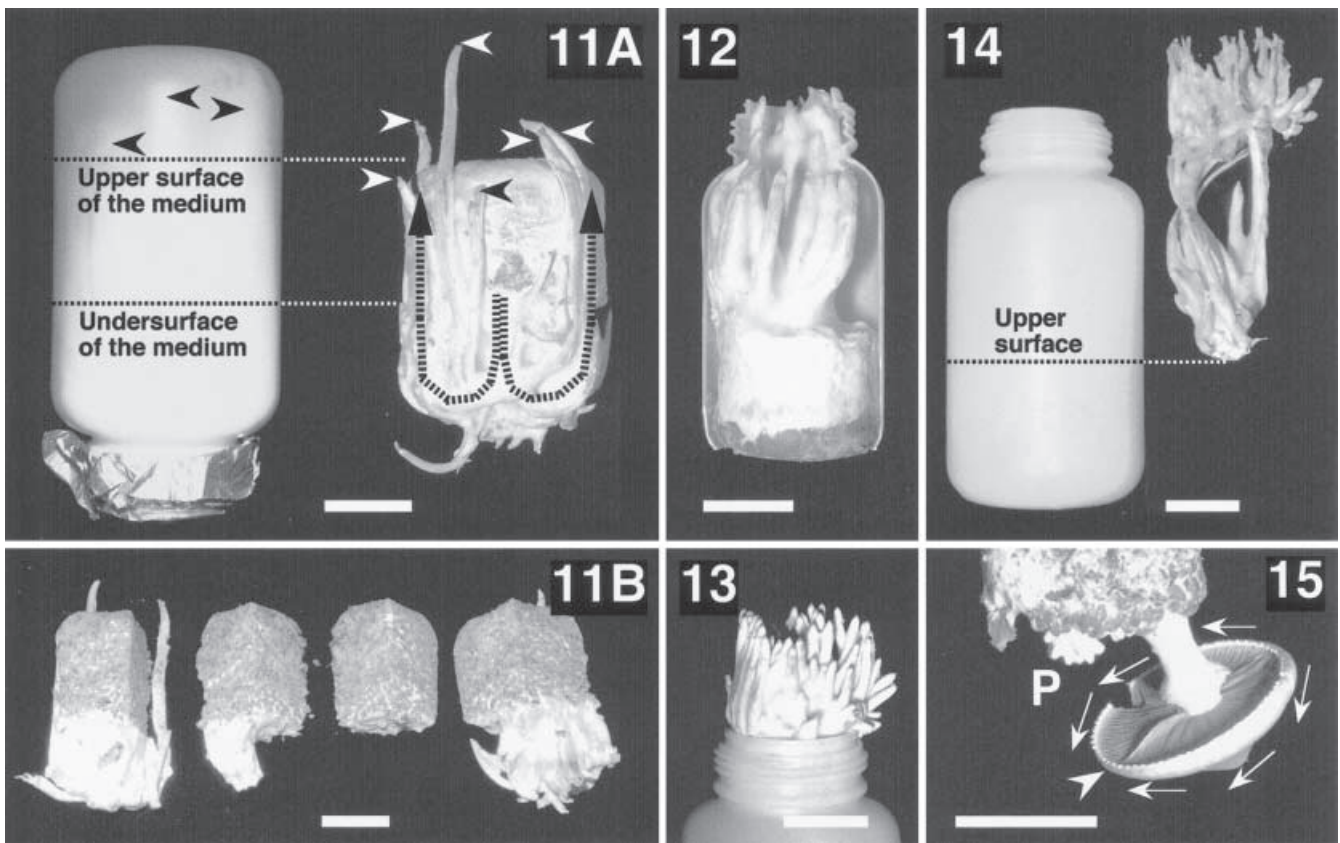
Development after shift in the light condition (experiments 7, 8)

When the incubation condition was shifted from dark to light at the developmental stage, as shown in Fig. 13 (experiment 7), none of the fruit-bodies grew further (Fig. 14). When the culture was shifted from the light to the dark immediately after the differentiation of caps and stipes and before the maturation of gills and basidiospores (experiment 8), the fruit-bodies mostly grew further and matured (Fig. 15), in the same manner as in the light.

Discussion

Negatively gravitropic and nonphototropic growth

The *H. radicosum* fruit-bodies did not grow toward the unilateral light from obliquely below (experiment 2; see Fig. 3) or from obliquely above (experiment 3; see Fig. 4), which indicates that they do not possess phototropism. When they emerged obliquely downward in a fascicle, they grew upward even before basidiospore formation or without basidiospore formation (experiments 1, 2, 5; see Figs. 7, 8, 11), suggesting that they exert only negative gravitropism from the beginning of development. Thus, it may be concluded that the *H. radicosum* fruit-body is not phototropic but only negatively gravitropic. In this respect it differs from the fruit-bodies of all other species studied previously (Kaneko and Sagara 2001a,b), which showed phototropic growth and, after the onset of spore formation, negatively gravitropic growth. Thus, the straight upward growth in the light from above (experiment 4; see Fig. 5) as well as that in



Figs. 11–13. Development of *Hebeloma radicosum* fruit-bodies in the dark (experiments 5, 6). Bars 3 cm. **11** Fruit-bodies emerged from the undersurface of the upside-down culture medium and grew upward after bending their stipes (experiment 5) (KHR 981). **A** The upside-down culture bottle (*left*) and the culture taken out of the bottle, showing the growth of fruit-bodies in it (*composite photograph*). The fruit-bodies emerged from the undersurface of the medium, bent upward, grew through the gaps between the shrunken medium and the bottle wall (*arrows*), and remained immature (*arrowheads*). The medium slipped down in the bottle during incubation as it shrank and accordingly some space occurred above the upper surface (originally the bottom) of the medium. **B** The medium vertically cut into fourths, showing the absence of penetration by the fruit-bodies. **12** Fruit-bodies growing upward after emerging from the upper surface of the culture medium (experiment 6) (KHR 981). The bottle was cut vertically. **13** Fruit-bodies growing upward after emerging from the upper surface of the culture medium (experiment 6) (KHR 982)

Figs. 14, 15. Further development of the fruit-bodies, 1 month after the shift from the dark to the light (Fig. 14) or 18 days after the shift from the light to the dark (Fig. 15). Bars 3 cm. **14** Fruit-bodies of Fig. 13, taken out of the bottle (at *left*), showing branching at the mouth of the bottle and failure of further growth. Light was provided from upper left as in Fig. 4 (experiment 3). **15** Fruit-body growing obliquely downward and showing tilting of the cap and gills (KHR 981). *P*, primordia. Gills were tilted (*arrows*) toward the lowest part (*arrowhead*) of the cap. At the time of the shift to darkness, the medium bearing the growing fruit-body was taken out of the culture bottle and hung upside down in a glass bottle as in Fig. 3

the dark, which did not proceed to basidiospore formation (experiment 6; see Figs. 12, 13), is not a phototropic but only negative-gravitropic response. Also, the straight downward growth in the light from below (experiment 1; see Fig. 2) is not a phototropic but latently negative-gravitropic response, as is discussed in the next section.

Straight downward growth in the light from below and straight upward growth in the light from above were observed in all species used in the previous studies (Kaneko and Sagara 2001a,b). Thus, these responses may be common to all hymenomycete fruit-bodies, whether or not they possess phototropism. Furthermore, the “two null-positions for gravitational curvature” proposed by Plunkett (1961) (see Kaneko and Sagara 2001a) seem to exist in all phototropic and nonphototropic hymenomycete fruit-bodies.

Downward growth in the light

The *H. radicosum* fruit-body, although negative-gravitropic from the beginning, did not grow upward in the light from below (experiment 1; see Fig. 2) or from obliquely below (experiment 2; see Fig. 3). It grew straight downward, which may be explained in the same way as previously (Kaneko and Sagara 2001a): the downward growth, although apparently phototropic or positively gravitropic, occurs under conditions of latent negative gravitropism; the fruit-body growing vertically downward is unable to bend upward because of obstruction by its own body and is forced to grow downward. The fruit-body may gain a chance to bend only when its stipe is tilted away from the vertical (experiments 1, 2; Figs. 7, 8). The straight downward growth occurred in the upside-down cultures in the light (experiments 1, 2; see Figs. 2, 3) but not in the dark (experiment 5; see Fig. 11). Why it did not occur in the dark is discussed later.

Taking this nonphototropic *H. radicosum* into consideration, we again assume that all the hymenomycete fruit-bodies grow straight downward throughout all stages of their development, if they are exposed to light (from below, in the case of phototropic ones) and if they emerge vertically downward and grow straight without twisting (see Kaneko and Sagara 2001a,b). Thus, the straight downward growth in the light from below under conditions of latent negative gravitropism is common to all phototropic and nonphototropic hymenomycetes, common to “*Coprinus Type*,” “*Non-Coprinus Type*,” and “*Polyporus Type*” (Gorovoj et al. 1987), i.e., three different types of hymenomycetes as for gravitropic mechanism, and also common to epigeous agarics, lignicolous agarics, and deep-colonizing mycorrhizal agarics (Kaneko and Sagara 2001a,b).

Sensitivity to light

Hebeloma radicosum differs from *Tephroclybe tesquorum* and *Coprinus* spp. in that the former formed primordia in the dark but the latter two did not (Kaneko and Sagara 2001a).

Although the *H. radicosum* fruit-body does not possess phototropism and does not require light for primordia formation, it seems to require light for differentiation and maturation, as shown by the results in the light (Figs. 2–10) and in the dark (Figs. 11–13). These characteristics of *H. radicosum* may correspond to the two aspects of its growth habits in the field: it colonizes and forms primordia deep in the ground (in the dark), and its fruit-body matures and disperses basidiospores on the ground (in the light). Thus, we argue that the *H. radicosum* fruit-body requires negative gravitropism but not phototropism, and that it has to be initiated in the dark and to mature in the light. Thus, this fungus seems to possess necessary and sufficient competence to respond to light and gravity for dispersing basidiospores successfully.

The conclusion that the *H. radicosum* fruit-body requires light for maturation appears to conflict with the result of experiment 7, in which the fruit-body grown in the dark remained immature even after the shift to the light (see Fig. 14). We suppose that, by the time of this shift, the necessary materials for further development might have been exhausted. Higher temperature and lower humidity, which are inevitably generated under light, might have also affected the fruit-body development.

Contrary to the foregoing results, in some cases the fruit-body matured after the shift from the light to the dark (experiment 8; see Fig. 15), which indicates that application of the light stimulus at a particular developmental stage may be sufficient for fruit-body maturation; continuous light may not be necessary.

Pseudorhiza formation

We consider the long stipe with immature cap observed in the dark (experiments 5, 6; see Figs. 11–13) to be a pseudorhiza, even though it was aerial. This consideration

follows Buller (1931), who regarded an elongated stipe-base of *Coprinus sterquilinus* (Fr.) Fr. grown in the dark as a pseudorhiza. The upward growth of the “pseudorhiza” indicates that it possesses negative gravitropism. This fact was verified for the first time in a laboratory experiment.

The formation of pseudorhizas in the dark (Figs. 11–13) but not in the light (Figs. 2–8) may be consistent with the observation by Buller (1934) that, if the fruit-body of *Xerula radicata* (Relhan : Fr.) Dörfelt developed on a stump, the pseudorhiza scarcely formed. Thus, the formation of pseudorhiza is simply one step in fruit-body development in the dark; the pseudorhiza is not a morphologically indispensable structure. The pseudorhiza can be regarded as a stage previous to the ordinary stipe and therefore may better be termed “prostipe,” as proposed by Sagara (1999).

The pseudorhizas grew upward through the gaps between the medium and the bottle wall (see Fig. 11), and not through the medium, perhaps because the medium was too dense for them to penetrate. This phenomenon is consistent with the observation by Buller (1934) that, during upward growth through the substratum, the pseudorhiza of “*Coprinus macrorhizus*” directly penetrated the softer parts of the substratum and bypassed the harder parts by oblique growth.

The pseudorhiza branched only when its upward growth was obstructed by contact with the mouth of the culture bottle (see Fig. 14). In “*Coprinus macrorhizus*,” Buller (1934) observed that such branching occurred when the pseudorhiza had been injured.

An overview of the responses of Non-*Coprinus* Type agarics to light and gravity

Straight downward growth in the light from below and straight upward growth in the light from above were observed not only in *H. radicosum*, but in all Non-*Coprinus* Type agarics studied previously (Kaneko and Sagara 2001a,b), i.e., *T. tesquorum*, *Flammulina velutipes*, and *Hypsizygus marmoreus*.

The tilting of gills as shown in Fig. 9 was also observed in *T. tesquorum*, *F. velutipes*, and *H. marmoreus* (Kaneko and Sagara 2001a,b). This behavior of gills, which is common to Non-*Coprinus* Type agarics, may represent their positive gravitropism. The swelling and waving of the cap and gills (see Figs. 9, 10) might have resulted from excessive transfer by gravity of water or other liquids from the culture medium to the fruit-body, as discussed previously (Kaneko and Sagara 2001a). *Hebeloma radicosum* stipes did not twist, like *Hypsizygus marmoreus* stipes and unlike the other Non-*Coprinus* Type species (*T. tesquorum* and *F. velutipes*) stipes (Kaneko and Sagara 2001a,b). The absence of twisting in *H. radicosum* and *H. marmoreus* stipes may be due to the thickness of their stipes.

Thus, when exposed to light from below, the fruit-bodies of all four Non-*Coprinus* Type species we studied, including *H. radicosum*, commonly showed straight downward growth, gill gravitropism, and cap swelling (see Table 2 in Kaneko and Sagara 2001b). However, *H. radicosum* differs

from the other species studied previously (Kaneko and Sagara 2001a,b) in the following respects: *H. radicosum* formed fruit-bodies in the dark, although they matured only in the light; they did not show phototropism; they showed only negative gravitropism from the beginning of development. Such a characteristic sensitivity to light and gravity may be related to the growth habit of this species, which forms primordia deep in the ground.

Atypical upward bending

We consider that the atypical upward bending observed in *H. radicosum* (see Figs. 7, 8) was caused simply by its obliquely downward emergence. In our previous study (Kaneko and Sagara 2001a), some fruit-bodies exposed to light from below bent upward after growing downward. As discussed subsequently (Kaneko 2001), this bending of the fruit-bodies is thought to have been caused by (1) their obliquely downward growth toward the light, which actually came from obliquely below because of shading by neighboring fruit-bodies; (2) their obliquely downward emergence for physical reasons; or (3) their asymmetrical growth such as stipe twisting. *Hebeloma radicosum* does not grow toward light and rarely shows stipe twisting. Therefore, its upward bending must have been caused by its obliquely downward emergence, as observed in the fasciculate fruit-bodies in the light (experiments 1, 2; see Figs. 7, 8) and also in the dark (experiment 5; Fig. 11).

The observation that fruit-bodies of *A. campestris* emerged from the upside-down culture and bent upward (Buller 1909) may be similarly interpreted: the upward bending of the fruit-body must have been caused by its obliquely downward emergence, because the fruit-body showed nonphototropic growth and rarely twisted, as in *H. radicosum*.

The higher frequency of bending of the fruit-body in the dark (experiment 5) than in the light may be a consequence of the thinner and longer stipe, because such a stipe may gain, during elongation, a chance for bending more frequently, as previously discussed (Kaneko and Sagara 2001a).

Application of the equilibrium concept

All fruit-bodies of *H. radicosum* eventually grew upward or downward. The equilibrium concept of Kaneko (2001) should be applicable here and to any agaric fruit-bodies having no phototropic behavior. However, as already discussed, the final growth direction (θ_g in Kaneko 2001) of the *H. radicosum* fruit-body seems to be determined only by the initial growth direction (θ_i in Kaneko 2001), not by light direction (see θ_p in Kaneko 2001) or by fluctuation of the growth direction.

The obliquely downward growth of the fruit-body with cap tilting (Fig. 15) suggests the existence of catastrophe and neutral equilibrium around the downward growth, as discussed previously (Kaneko 2001; Fig. 16). Kaneko (2001) considered that θ_r , the critical range of θ (growth direction of the fruit-body) in which the downward growth can occur, may be determined by the light intensity, i.e., θ_r may be smaller the lower the light intensity. This principle may also be applicable to the nonphototropic *H. radicosum* fruit-body, as we frequently see it bending negatively gravitropically in the dark (see Fig. 11).

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