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PSILOCYBE MUTANS SP. NOV.: MORPHOLOGY AND VARIATION

by

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PSILOCYBE MUTANS SP. NOV.: MORPHOLOGY AND VARIATION

by Kent H. McKnight

P. mutans is described and placed in the Strophariaceae beside *P. coprophila*. Its natural substrate is cow dung, but it grows and fruits readily on artificial media. Great morphological variation was observed on fruiting bodies produced in culture. A study of this variation, along with the general life history of the fungus, was undertaken because it promised to shed new light on the relationship of the gasteromycetes and agaricales.

The life cycle of *Psilocybe mutans* conforms with that commonly found among heterothallic hymenomycetes. Matings of twenty-two single spores in all combinations showed that the sexual behavior was tetrapolar. The percentage of spore germination was so low that is was necessary to postpone certain investigations, such as those involving detailed analysis of the genetics of the organism. Freezing the spores, and treatment with an aqueous solution of hemoglobin were found to increase the germination rate appreciably but not sufficiently for all phases of the work. No significant increase was obtained by treating the spores with various chemicals.

Gymnocarpous, hemiangiocarpous and angiocarpous fructifications are all produced in culture. Cultures grown in the presence of light produced more agaricoid fructifications and fewer gasteroid fructifications than those grown in darkness. Cultures grown in total darkness produced gasteroid and other

anomalous forms but no agaricoid fructifications. Both agaricoid and gasteroid fructifications were produced in cultures grown in 25 and 50 foot candles of light, but no gasteroid fruiting bodies were produced in cultures which received more than 50 foot candles. It is concluded that light provides a morphogenic stimulus necessary for the production of agaricoid carpophores.

The hemiangiocarpous fruitbodies, which are considered as "normal," place the fungus in the genus Psilocybe. The angiocarpous (gasteroid) fructifications are significant because they are similar to those found in the genus Secotium (sensu lato) of the Gasteromycetes, and thus a "bridge" between the Strophariaceae and the gasteromycetes, such as those known for other groups (Thaxterogaster-Cortinarius, for instance) is demonstrated. Matings of single spore isolates from both types have consistently shown that both are interfertile, and that from any successful mating various morphological types of fruitbody may result. Inbreeding of the progeny of agaricoid fruiting bodies for 14 generations failed to produce a pure strain for this character. The development of fruitbodies presumed to be gasteroid show "lacunar", "coralloid" and "unipileate" patterns.

This is the first time a connection to the gasteromycetes has been shown to occur within a single species. Since Psilocybe mutans produces carpophores characteristic of a well-defined group of hymenomycetes, and since no related species are known among the gasteromycetes, it is concluded that the manner of fruiting of P. mutans supports the theory that certain

gasteromycetes probably evolved by reduction from the Agaricales. It is possible also, that certain species of the Secotiaceae in the gasteromycetes represent teratological forms of species of the Agaricaceae.

A true albino mutant was isolated from cultures of otherwise typical P. mutans. It possessed hyaline spores as well as colorless fruitbodies. Both show the same range of morphological variation, the same pattern of sexuality and are mutually compatible. Both colored and albino fructifications were obtained in matings of the offspring from crosses.

PREFACE

Psilocybe mutans n. sp. was first discovered by Dr. Alexander H. Smith. It appeared on cow dung collected in Montana during the summer of 1951. The original collection was obtained after incubating the substrate in the laboratory. Dr. Smith succeeded in isolating the organism and getting it to grow and reproduce in pure culture on nutrient agar medium. The cultures thus obtained were given to me for further study. The only information previously published on this species is contained in a brief note which appeared in Mycologia (McKnight, 1953).

It is my pleasure to acknowledge a debt of gratitude to Dr. Smith for his generosity in giving me these cultures and for his constant inspirational guidance throughout the course of the ensuing research. In addition to the members of the Special Committee, I should like to express my gratitude to Dr. R. J. Lowry and Dr. E. E. Steiner, who have advised me on the cytological and genetic aspects of this problem, and to Dr. A. S. Sussman, who has contributed most generously of his time and energies to advise me during the studies on spore germination and other aspects of physiology. Dr. L. E. Weymeyer has counseled me on many phases of the research and has helped me constantly with many incidental details. Miss M. E. Barr has contributed an invaluable service in assisting with the editing and typing of the manuscript.

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CHAPTER I

INTRODUCTION

Definition and Limitation of Problem

Mycologists have long recognized the close relationship between certain members of the Agaricales and some gasteromycetes. DeBary postulated as early as 1884 that the gasteromycetes are a reduced group which may have evolved from the Agaricales. The possibility of evolution between these two major taxa was recognized also by Brefeld (1889), who contended, however, that the hemiangiocarpous agarics may have developed from the gasteromycetes. Thus two opposing views on the relationships of these two groups were established very early. Each view has gained a considerable amount of support from later investigators, and an abundance of evidence has been amassed purporting to show the direction of evolution between various members of the gasteromycetes and agarics. A number of species have been described[1] which are considered as representing intermediate stages in the series; i.e., showing some characteristics which are typical of the gasteromycetes and others typical of the Agaricales. In all of these the fructifications are clearly intermediate; that is to say, they are usually regarded as distinct

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[1] Since this report considers so many diverse aspects of the biology of this organism the review of the literature, and consequently an enumeration of the various connections between the agarics and the gasteromycetes, are presented in Chapter IV, Discussion.

species with both agaricoid and gasteroid characteristics but it has not been possible in any case to relate them positively to any known species of agaric or gasteromycete. The present study concerns a fungus which produces carpophores characteristic of the genus Psilocybe in the Agaricales. At the same time it produces fructifications whose characteristics permit them to be classified as gasteromycetes. The problem of this study is to determine the factors responsible for production of these two fruiting forms and if possible to evaluate the significance of the organism as it relates to phylogeny of the gasteromycetes and the Agaricales.

It was considered necessary, therefore, to obtain as much information as possible on various aspects of the biology of the species in question. Its life cycle has been studied, as well as certain aspects of cytology and genetics. Since the species has not been reported previously in botanical literature, its taxonomy also had to be investigated. It was found necessary to investigate certain aspects of physiology before other phases of the problem could be carried out. The organism proves to be exceptionally well adapted for purposes of research. So much so, in fact, that it has been necessary to terminate certain studies short of completion in order to deal more extensively with the central problem. This thesis, then, deals with a very broad range of studies on the biology of the species, some of which are given only a general treatment, whereas the problem of the relationship of the gasteroid and agaricoid forms is accorded more extensive coverage.

CHAPTER II

MATERIALS AND METHODS

General

The origin of the cultures studied was explained in the preface. The organism is known only from these cultures which originated from a single clone. Sterile technique was used in maintaining it in pure culture.

The species studied was found to grow and fruit readily on a variety of nutrient media. Most of the investigations reported here, however, utilized some sort of malt extract agar. The basic formula is as follows:

Malt extract agar:

Malt extract	20.0	grams
Maltose	5.0	grams
$MgSO_4$	0.5	gram
$CaNO_3$	0.5	gram
KH_2PO_4	0.25	gram
Agar	30.0	grams
Distilled water	1	liter

A variation of this formula which was frequently used was designated as malt extract agar number 2. This medium contained the same ingredients as the basic formula except that 1.5 grams of peptone was added and horse dung decoction was substituted for the distilled water. A second variation, malt extract agar number 3, followed the same basic formula with the following exceptions: 1.5 grams of peptone was added, dung decoction was substituted for distilled water, and

the malt extract was reduced to 10 grams, and 5 grams of dried brewer's yeast was added.

The anatomical details of the carpophores were analyzed for taxonomic purposes from both fresh and dried material. The dried specimens were moistened with 95 percent ethyl alcohol and soaked in distilled water. Freehand sections were studied microscopically both in Melzer's reagent[1] and in 5 percent KOH.

Isolating Single Spores

Haploid strains of the fungus studied were obtained by isolating spores which had been germinated on a very firm, clear agar medium. Three percent water agar was poured in a very thin film on a petri dish and inoculated with a dilute suspension of basidiospores. To obtain the spore suspension a mature fruiting body of the fungus was transferred by means of sterile forceps to a tube of sterile distilled water. The spore suspension was sprayed over the surface of the agar plate or a few drops placed on one side of the plate and dispersed over the surface by tilting it, the excess water then being drained off. After incubating at 25° C. for 24 hours, germinating spores were located under the high power of the microscope and isolated by means of a sterile, slender scalpel or a wire loop. The latter instrument was made by pounding the platinum wire of a transfer needle until it was flattened, then bending it to form a loop. The loop was then bent at an angle of about 65° from the axis

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[1] Formula for Melzer's reagent:

Potassium iodide	1.5 grams
Iodine	0.5 gram
Chloral hydrate	22.0 grams
Water	20.0 milliliters

of the needle. Once a germinating spore was located under the high power of the microscope, the tube of the microscope was raised up out of position with the coarse adjustment and, with the microscope condenser closed to allow the minimum amount of light, the loop was lowered over the refracted spot of light on the agar plate. The block of agar thus isolated could then be examined under the microscope to be sure that it contained only a single germinating spore before being transferred. The isolates were grown first on petri dishes of nutrient agar at 25° C. and later transferred to agar slants and stored in the cold room.

Matings

Malt extract agar number 2, poured in thick plates, was used as a medium for the matings. The haplonts to be mated were previously grown on this same medium. Small squares of the haploid mycelium being tested were placed about an inch apart. The cultures were incubated in a constant-temperature oven at 25° C. and examined for the presence of fruiting bodies at about five-day intervals. At the end of three weeks' time the cultures which did not produce fructifications were examined microscopically to see if clamp connections had been formed.

Morphology

The developmental morphology of the fructifications was studied from material embedded in paraffin and sectioned on a rotary microtome. Fructifications of various ages were fixed in weak chrome-acetic fixative and dehydrated in an ethyl-butyl alcohol series. Usually a small block of the agar substrate was cut out and processed with

the carpophores on it. Sections were cut at 15 microns and stained either with Heidenhain's hematoxylin or with crystal violet and orange G.

Cytology

The nuclear behavior of the basidium was determined from aceto-carmine smears prepared according to the following schedule:

1. Fix for 15 to 20 minutes in acetic alcohol (3 parts absolute ethyl alcohol to 1 part glacial acetic acid).
2. Replace fixative with acid-alcohol (equal parts of 95 percent ethyl alcohol and concentrated HCl) for about 4 minutes.
3. Return to acetic-alcohol solution for about 10 to 15 minutes.
4. Transfer to a small vial containing a few drops of aceto-carmine dye and a few grains of powdered iron filings.

Since the fructifications are small and the basidia are exposed on the hymenium, the pileus was prepared whole. A small fragment of lamella was mounted in a drop of stain after about 15 minutes and the elements of the hymenium were squeezed out by applying pressure to the cover slip. Details of nuclear behavior were followed in both the colored and albino forms of the species in carmine smears and in the colored form with paraffin preparations. None of the techniques used provided a sufficiently clear view of the chromosomes for counts or detailed analysis of reduction division.

Spore Germination

Spores for germination tests were obtained by crushing several fructifications in a small beaker containing about 20 milliliters of sterile distilled water. This was then strained through a fine wire screen and the liquid centrifuged to concentrate the spores. The heat treatments utilized a hot-water bath with a serum bottle suspended to a depth greater than the level of the spore suspension which it contained. Twenty milliliters of sterile distilled water was introduced into the bottle and the system allowed to equilibrate to the desired temperature. About 1 milliliter of a very concentrated spore suspension was added by means of a hypodermic syringe and timing of the treatments was begun immediately. One-milliliter samples of the treated spore suspension were removed by means of a hypodermic syringe and introduced into 35-milliliter flasks which contained 2 milliliters of sterile distilled water. Sterile cork stoppers were then replaced in the flasks and the cultures incubated at 25° C. on the shake machine. For those experiments in which nutrients and growth factors were used to treat the spores 0.1 milliliter of inoculum was introduced by means of a graduated pipette into flasks containing 2 milliliters of the solution. All equipment and solutions which came into contact with the spores were sterilized.

CHAPTER III

EXPERIMENTAL DATA AND OBSERVATIONS

Description of Species

Psilocybe mutans sp. nov.

Pileus 5-9 mm. in diameter; subglobose to convex with incurved margin at first, becoming broadly convex to plane when fully expanded, often with upturned margin at maturity; surface smooth, moist, hygrophanous, glabrous to fibrillose; "deep olive buff"[1] to "cream buff" in color; margin acute, often faintly striate at maturity; flesh thin, odor and taste not distinctive. Lamellae subdistant and rather thick; narrow; adnate to adnexed, sometimes emarginate; sordid watery gray when young, very dark purple brown at maturity. Stipe 20-24 X 1-2 mm.; equal; solid; surface fibrillose; "cream buff" to "isabella color"; tissues of stipe turn light cinnamon color in iodine.

Spores 8.4-11.7 (12.6) X 5.6-7.7 μ, ovoid to ellipsoid in face view, somewhat flattened on one side in side view, truncate, smooth; purplish fuscous in mass, dull ochraceous brown in KOH. Basidia generally 4-spored, but occasionally 2-spored ones are found, 23-35 μ long. Pleurocystidia lacking. Cheilocystidia abundant, often causing the gill margin to apear whitish, fusoid ventricose, with long necks which are frequently contorted, 24-35 μ long, 6.7-7.2 μ broad in

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[1] Names of colors within quotation marks are those of Ridgeway (1912).

ventricose portion, apices acute. Gill trama made of loosely compacted hyphae which are subparallel to loosely interwoven, 6.0-10.5 μ in diameter, faintly ochraceous in KOH; subhymenium distinct, composed of a 2-3 celled layer of pseudoparenchymatous hyphae. Pileus trama consisting of thin-walled hyphae, light ochraceous brown in iodine; surface of pileus covered by a thin layer of very narrow hyphae, 3.0-4.5 μ in diameter, beneath which is a thick layer of larger (4.0-10.5 μ in diameter), loosely interwoven hyphae. The outermost layers are covered with a heavy coating of yellowish incrustations which often form a more or less reticulate pattern. Clamp connections of frequent occurrence. Type is deposited in the University of Michigan Herbarium.

Habit, Habitat, and Distribution

Very little is known about the natural habitat and distribution of this fungus. All that can be said regarding its ecology at the present time is that cow dung serves as a natural substrate.

Observations

No attempts were made to transfer the fungus from agar back to cow dung. Attempts to culture it on autoclaved horse dung in the laboratory met with little success. Mycelium was produced and in one culture many fruiting primordia appeared and a few very anomalous fruiting bodies developed. The fungus does grow readily and fruits abundantly on a variety of nutrient media. One culture produced repeated crops of fruiting bodies in a standing liquid medium consisting of a 0.6 percent solution of maltose in distilled water.

Morphology

Germination of Basidiospores

The spores of Psilocybe mutans are apparently capable of germination at the time they are shed from the basidium or shortly thereafter. The first evidence of spore germination is the production of a globose vesicle having a volume about twice that of the spore (Plate I, Fig. 25). This usually appears at the germ pore, but occasional spores are seen which germinate through the apiculus (Plate I, Fig. 27). One or more hyphal branches grow outward from the vesicle and by continued growth and repeated irregular branching the haploid mycelium is produced (Plate I, Fig. 29). The cytology of the germinating spore was not studied.

Haploid Mycelium

Oidia are produced very early on the young haploid mycelium. The oidiophores originate in clusters at hyphal tips. They consist of helical branches which twist together forming a compact knot. Later they become septate and the individual segments fall apart as oidia (Plate I, Figs. 6-12). When these are mature they are enclosed in a large drop of liquid. The hyphal tips which bear the oidiophores grow upward from the substratum and their vast numbers help to impart a fluffy appearance to the haploid culture. The oidia are cylindric to allantoid, measuring 3.5-4.7 X 1.0-2.2 μ.

The cultures grown from single spore isolations exhibit a considerable amount of variation in macroscopic appearance. Although each culture displays its own individual characteristics, three rather poorly defined categories may be identified. These are not

wholly distinct since all shades of intergrading forms are found. Equally significant is the fact that no culture has been observed to maintain its separate characteristics without variation. Sectoring of some sort has been observed in almost all of the isolates examined for the characteristic. It has been impossible to identify a consistent pattern in the sectoring or to predict which of the three general mycelial types will be produced by sectoring in any given culture. With these limitations in mind, the three categories described below have been established:

Type I, Fluffy

Mycelium forms a more or less uniform, dense, feltlike mat. There is an extensive aerial mycelium and copious production of oidia. Examples: cultures 1 (Plate V, Fig. 1), 6, 17, II.

Type II, Granular

Mycelium less fluffy than Type I and characterized by the presence of sclerotia. Examples: cultures 16, IV, V (Plate V, Fig. 2), VIII.

Type III, Powdery

Mycelium grows closer to the substrate, giving it a somewhat powdery appearance. The rate of growth is markedly slower than that of other cultures. Examples: cultures 5 (Plate V, Fig. 3), 8.

It has been impossible to establish a correlation between these mycelial types and the mating types or any other genetic entities.

Diploid Mycelium

The diploid mycelium is fairly readily distinguished from the haploid by its macroscopic appearance. It is coarser in texture and

apparently less branched, assuming a radially striate appearance on the agar plate. Clamp connections are frequently found, although many septa lack them. Oidia are rarely produced and aerial branches are less frequent. Thus the mycelium lacks the "fluffy" appearance characteristic of haploid cultures. The growth rate appears not to be appreciably different from that of the haploid mycelium.

Fruiting Bodies

Origin and development of agaricoid fruiting bodies.--Primordia of the fructifications appear first as small nodular bodies about 0.5 millimeter in diameter. At first they are indistinguishable from the sclerotia found on the haploid mycelium. Later they become globose to ellipsoid in shape and finally the stipe and pileus primordia may become differentiated.

At maturity the fruiting bodies are extremely variable in gross morphology. Two extreme forms, agaricoid and gasteroid, are readily identified. The agaricoid carpophores have a general structure characteristic of species of Psilocybe (Plate II, Fig. 1). Their development is hemiangiocarpous. The hymenium is completely enclosed in the early stages of development and is later exposed. The partial veil ruptures very early and is most frequently entirely lacking at maturity, although rarely remnants may be seen on the stipe or on the edge of the pileus of some mature fructifications.

Gasteroid Fruiting Bodies

Primordia of the gasteroid fruiting bodies are indistinguishable from those of agaricoid forms up to the time when the stipe of the

latter becomes differentiated and the pileus begins to expand. The gasteroid forms, however, are angiocarpous in development. The hymenium is not exposed at maturity. This is the one characteristic which the gasteroid fructifications have in common and which distinguishes them from agaricoid carpophores. This extreme condition is quite rare, although many fructifications approaching it have been found (Plate II, Fig. 2).

Intergradation Between Agaricoid and Gasteroid Forms

A complete intergrading series of fruiting bodies, from agaricoid to gasteroid, is produced in culture under average laboratory conditions. Some essentially globes fruiting bodies are found with unexpanded pilei but which have the edge of the pileus separated slightly from the stipe. From this point on, all degrees of expansion of the pileus are to be seen.

A similar intergradation is found with regard to characters other than expansion of the pileus. The stipe varies greatly in length. In some forms with either angiocarpous or expanded pilei, the stipe is lacking or nearly so. Others are found with an elongated stipe and partially to completely expanded pileus. Varying degrees of anastomosis of the lamellae are observed. The characteristic agaricoid form has very little or no anastomosing of its lamellae. Others are seen with occasional ridges between the gills and still others with frequently anastomosed gills (Plate III, Figs. 3 and 4). The extreme forms have a poroid hymenophore (Plate III, Figs. 1 and 2; Plate IV, Fig. 1). Another anomalous type of fructification has a general shape varying from subglobose to inverted pyramidal. These are sessile and have the hymenium exposed over the smooth

or wrinkled upper surface of the basidiocarp (Plate IV, Fig. 1). Some show a striking similarity to a cup fungus. A similar type of carpophore which appeared occasionally in many cultures has the upper surface of the pileus modified in such a way that it consists of a series of depressions, or lacunae, and ridges. It may be stipitate or sessile (Plate III, Figs. 1 and 2). Some cultures produced clavate or cylindrical outgrowths of tissue which resemble stipes lacking pilei. Occasionally these were found to have patches of smooth, wrinkled or costate hymenial areas on their upper surfaces (Plate IV, Fig. 2). They form an intergrading series with the type described immediately preceding.

Variation in Internal Structure of Gasteroid Carpophores

A study of serial longitudinal sections of gasteroid carpophores revealed a great diversity of internal structure. The hymenium lines the walls of cavities formed by the anastomosing tramal plates. In the extreme gasteroid forms the cavities are merely irregularly shaped lacunae with little or no identifiable organization. In those forms which approach the agaricoid condition, the tramal plates are definitely gill-like, and as a cross section of the carpophore will show they may form a radial pattern. Three general types of structure may be recognized with regard to development of the stipe. Each is illustrated by serial longitudinal sections in Plates VI to VIII. Again, intergrading forms are found in very high frequency. Some specimens have a percurrent stipe (Plate VIII) to which the tramal plates are attached. Others have only a partially percurrent stipe (Plate VI), and in still others the stipe does not penetrate the

hymenial tissues at all (Plate VII). Some specimens are sessile; that is, the stipe is completely lacking.

Cytology of the Basidium

The basidia are predominantly four-spored, although occasionally one-spored, two-spored, and three-spored ones are found. Spores of both agaricoid and gasteroid forms are borne obliquely on the sterigmata and they are probably discharged forcibly. The nuclear behavior of the basidium follows a pattern common in the agarics. The smallest basidia observed were about 4.0 X 7.0 μ. At this stage they are binucleate and clamps are usually seen with no difficulty (Plate I, Fig. 13). The penultimate cell characteristically gives rise to another clavate cell from a point near the base of the basidiole. The ultimate fate of this cell was not determined. It is considered that it may become either a basidium or a paraphysis. The two original nuclei of a basidiole enlarge as the basidiole grows in size (Plate I, Figs. 14 and 15), and fuse when the basidiole is about 18.5 μ in length. The large fusion nucleus remains near the center of the basidiole as this organ continues to enlarge. An extended prophase of the reduction division occurs during this time (Plate I, Fig. 16). The metaphase and later stages of reduction division are seen near the distal end of the basidium when its size is near that attained at maturity. The four nuclei which are produced as a result of the second division soon migrate down to a point near the center of the basidium where they remain while the sterigmata and spores form (Plate I, Figs. 18-21). The time at which the sterigmata begin to form seems to be quite variable, as some basidia were seen which had very small sterigmata when the fusion nucleus was still in the prophase of reduction division.

One nucleus migrates into each spore. The actual migration of the nuclei into the spores was not observed but the above conclusion was deduced from the following facts: (1) uninucleate spores which have been shed from the sterigmata are seen (Plate I, Fig. 24); (2) not more than four nuclei are produced in the basidium; (3) no nuclei were seen in old basidia which had shed their spores. The spores apparently attain a considerable size before the nuclei enter. On one basidium the spores were 6.5 μ in length before the nuclei left the basidium. Within a short time after the spores are shed from the basidium they become binucleate (Plate I, Fig. 23). Since basidia bearing fewer than four spores are so uncommon, their nuclear behavior was not studied.

A somewhat anomalous situation found in a number of fruiting bodies is the production of giant spores. These were found infrequently and in very small numbers when they did occur. In a fructification produced by mating strains 15 and 16 the normal spores were found to measure 6-9 X 10.2-12.0 μ, while the giant spores were 9.0-10.2 X 15-16 μ (Plate I, Fig. 5). The giant spores are not greatly different in shape from the normal spores except that the germ pore is noticeably smaller in proportion to the size of the spore and it appears to be produced on an extended neck. They are, however, much darker in color than the normal spores. No giant spores were seen attached to basidia.

Sexuality

Figure 1, page 17, shows the results of experiments which were carried out in an effort to determine the mating characteristics of this fungus. Matings were attempted in all possible combinations between twenty-two single spore isolates. These single spore isolates

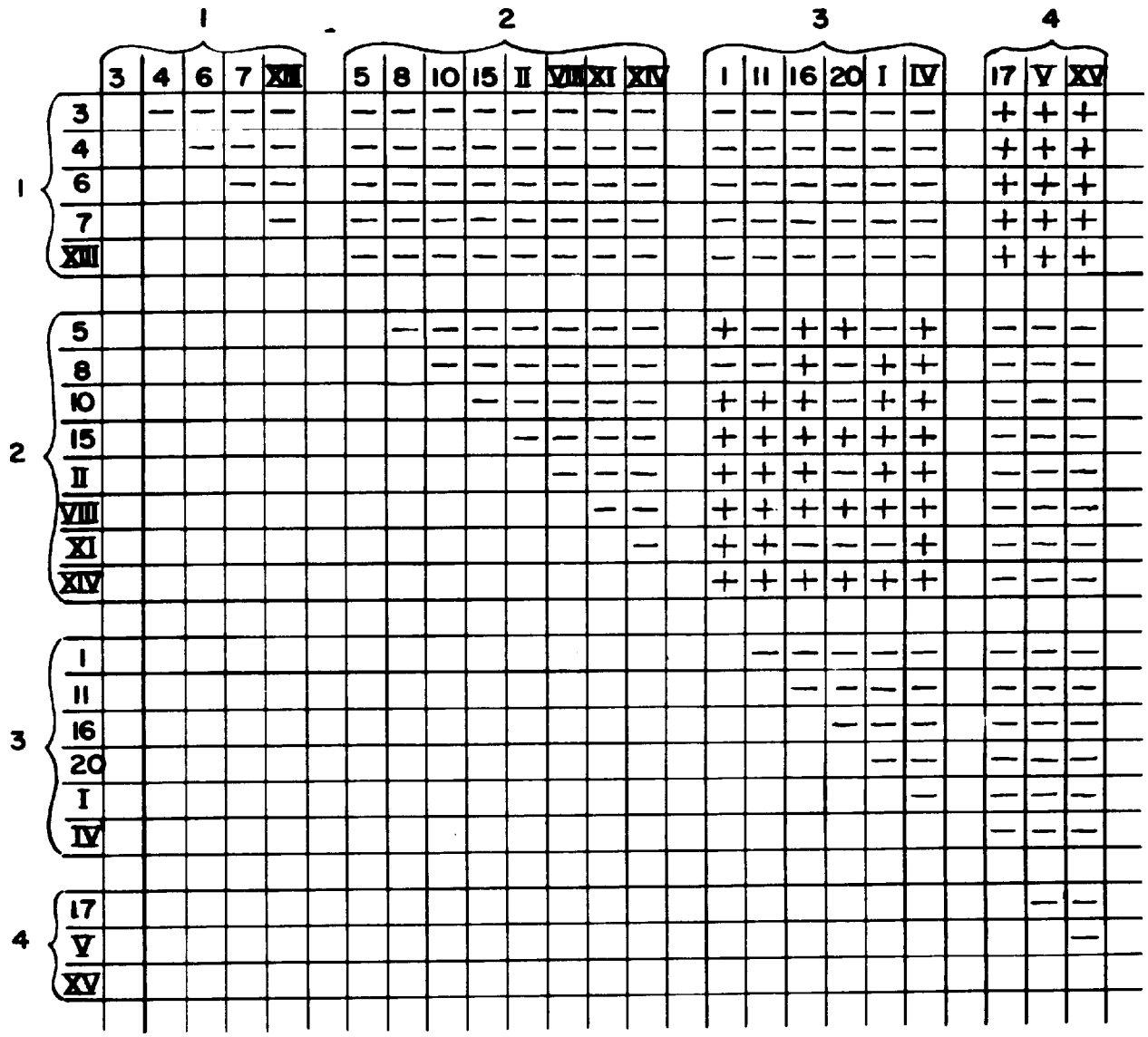

Figure 1. Pairing reactions of twenty-two single spore isolates of Psilocybe mutans.

which have been assigned arabic numerals were obtained from agaricoid carpophores, whereas those designated by roman numerals were from gasteroid fructifications. A + sign in the chart indicates that mating occurred between the strains tested, and a - sign indicates that they failed to mate. The decisive criterion for determining when mating had occurred was the production of fertile diploid carpophores by the strains being tested. Although the haploid and diploid mycelium differ somewhat in appearance, it was rarely

possible to be positive that mating had occurred until fructifications were produced on the mycelium. In a few cases carpophores did not mature but the presence of primordia and clamp connections was considered to be adequate evidence that mating had taken place. The single spore isolates were not all obtained at the same time and the matings were carried out over a period of two years' time.

The time required for completion of a life cycle was found to be very short. Carpophore primordia were often found within a week after mating and mature fruiting bodies were frequently produced within ten days. The first fruiting bodies frequently appeared at the point of contact of the two mycelia or in the general area where the first contact occurred. This was not always the case, however. Frequently the first fructifications would appear on the hyphae of one of the haplonts, sometimes even on the opposite side of the colony from the point of contact of the two strains. In no case were fruiting bodies seen to develop on hyphae which were produced before the time of fusion of the haplonts. It seems, however, that they may appear on any part of the colony which develops after the two mycelia come into contact.

The species is heterothallic and, as shown by Figure 1 (p. 17), tetrapolar. However, it does not conform absolutely to the expected pattern for a tetrapolar fungus. It will be seen from Figure 1 (p. 17) that mating fails to occur between certain combinations in mating types two and three. Out of 172 matings attempted, there are 10 cases wherein they fail to meet the prediction.

Several other anomalies in the fruiting habits of this fungus have been studied. Two single spore isolates, numbers 18 and III, fruited very shortly after the spores were isolated and before they had been mated with any other strain. The fructifications were in no way different from those obtained in matings of other strains.

Clamp connections were found on the mycelium and carpophores and repeated subcultures of these two strains continued to fruit. This behavior suggests that these two strains were initiated from basidiospores which were diploid.

A number of haploid strains produced fruiting bodies when the cultures became quite old. The fruiting bodies produced are often quite different from those produced as a result of mating two strains. Clamp connections are not seen on the "haploid" fructifications which are often semisterile. Many of the basidia are abnormal in appearance, being highly vacuolate and lacking sterigmata. Fruiting bodies are produced in small numbers and are frequently anomalous in appearance (Plate V, Fig. 4). A number of viable spores were obtained from one such carpophore which appeared in culture II. These were mated with the following strains of known mating type: 7, IV, V, XIV. The results are shown in Figure 2.

	II-5	II-8		II-9	II-12		II-3	II-6	II-13
7	−	−		−	−		−	−	−
IV	+	+		−	−		−	−	−
V	−	−		+	+		−	−	−
XIV	−	−		−	−		+	+	+

Figure 2. Pairing reactions of seven single spore isolates of "haploid" fructification of P. mutans with four strains of known mating type.

Here it will be seen that in the seven spores isolated from a single "haploid" fruiting body, three of the four mating types were found.

Certain diploid cultures exhibit a distinct concentric zonation in production of fruiting bodies. This was not true of all cultures, but was particularly noticeable in cultures OG, OK, ON, OR, and 4A. In culture OK a zone of nonfruiting mycelium was produced immediately

surrounding the inoculum. This was in turn surrounded by a zone of sterile gasteroid fructifications and then a zone of fertile gasteroid fructifications. Culture OR had a similar organization except that the position of the fertile and sterile fructifications was reversed. A similar effect was observed in cultures OG, ON, and 4A except that the central zone of nonfruiting diploid mycelium was lacking. In cultures OG and 4A the innermost zone of fructifications was sterile and these were surrounded by a zone of fertile fructifications. Culture OG had a second zone of sterile fructifications and a second zone of fertile fructifications in addition. Culture ON also had two zones of fertile fructifications but only one zone of sterile ones which, of course, was between the two fertile zones. Culture 4A was unusual in that some sort of yeast-like organism had contaminated the culture in the zone of fertile fructifications. Three spots of contamination were evident. In the areas immediately surrounding the contaminant the fruiting bodies were stipitate with expanded pilei, whereas the other fertile fructifications were predominantly gasteroid.

An unusual type of sectoring was observed in diploid cultures OH and OQ. These cultures produced rather typical gasteroid fruiting bodies except in certain sharply delimited sectors. In these areas the mycelium had the characteristics of haploid mycelium; that is, it was rather fluffy in texture (powdery in OQ), produced abundant oidia, and very few clamp connections. The hyphae were smaller in diameter and lacked the characteristic radial orientation of the diploid hyphae. In these sectors an occasional sterile gasteroid fructification was produced, but the prolific fruiting of ordinary diploid mycelium was noticeably absent. Two successive subcultures of the characteristic diploid sectors of culture OH were

made. In both cases the new cultures showed a conspicuous tendency to produce similar haploid sectors.

Effect of Light on Fruiting

Three experiments were conducted to test the effect of light on fruiting. The cultures for all three experiments were grown on malt extract agar number 3. In the first experiment two cultures grown at room temperature on a table near a north window were compared with two cultures incubated at 25° C. in a constant-temperature oven. The oven is practically light-tight, although brief exposures were admitted as the cultures were inspected daily. All of the test cultures were initiated from basidiospores produced on agaricoid fructifications. The cultures grown in the dark fruited less prolifically than those grown in the light. The most conspicuous difference, however, was in the morphology of the carpophores. The light-grown cultures produced a much higher percentage of agaricoid fructifications than the dark-grown cultures. Moreover, the light-grown cultures produced predominantly agaricoid carpophores, whereas the dark-grown cultures produced fruiting bodies which were mostly gasteroid or otherwise aberrant, but not agaricoid.

The second light experiment differed from the first only in that a third series of cultures was grown in the darkness and alternated between the 25° oven and the 15° cold room. These cultures were enclosed in a can with a tight-fitting cover and given about 8 hours in the cold room and 16 hours in the incubating oven each day. This refinement of the experiment was undertaken to isolate the effect of light from that of fluctuating temperature. The results of this experiment were similar to those of the previous one, only more striking. The light-grown cultures produced a very high

proportion of agaricoid fructifications (estimated at over 85 percent) (Plate IX, Fig. 1). The cultures grown at constant temperature in the darkness produced all gasteroid and other aberrant fructifications. None were agaricoid (Plate IX, Fig. 3). The cultures grown in darkness at fluctuating temperatures likewise produced no agaricoid fructifications (Plate IX, Fig. 2). Although very little difference in morphology is seen between these and dark-grown cultures maintained at constant temperature, the former matured several days sooner, as did the cultures grown in light.

The third experiment in this series was designed to test the effect of various light intensities upon fruiting. Five cultures were incubated at 20° C. for 13 days at each of the following light intensities:

"Dark control." Cultures enclosed in light-tight can.
25 foot candles
50 foot candles
100 foot candles
200 foot candles
400 foot candles

The following observations were made:

1. No agaricoid fructifications were produced in the dark control. A few aberrant carpophores were produced, but none were truly agaricoid.
2. Fructifications in the dark control matured more slowly than any of the others.
3. Fructifications in the dark control were mostly sterile. Very few were colored by spores.
4. Gasteroid fructifications are common in samples grown at 25 and 50 foot candles but lacking in those grown at higher light intensities.

5. Cultures grown at 25 foot candles tend to be generally more robust than any of the others. There were a few extra large fructifications in each plate grown at this light intensity.
6. The cultures grown at 400 foot candles appear to be somewhat less robust than any of the others.

Albino Mutant

An albino mutant appeared after the fungus had been grown in culture for several months. The fruiting bodies of the albino are readily distinguishable by their pure white color (Plate X). They lack all of the pigments of the colored forms including the yellowish incrusting pigments of the pileus trama. The first albinos appeared in a culture which was originated by a mass inoculation of spores from fructifications of cultures OR and OG. Two plates were inoculated with a water suspension of spores. Both plates produced agaricoid and gasteroid fruiting bodies. Albinos appeared only on plate 1. The fruiting population on this plate was composed as follows:

3 clumps of colorless gasteroid fructifications,

4 clumps of colored gasteroid fructifications,

2 clumps of colored agaricoid fructifications,

3 clumps of gasteroid fructifications which were mostly colored but contained a few colorless fructifications.

One albino fruiting body was transferred to a new plate of agar and the mycelium which originated from it was designated culture OS. All of the fruiting bodies which were produced by this mycelium and subcultures of it are colorless. Spores of one of the original albino fruiting bodies were sown on agar and produced a large population of carpophores in the F_1 generation, 402 of which were albinos and

18 colored. Ten albino fructifications were selected at random from the F_1 and the following spore counts were obtained:

Sample No.	White Spores	Colored Spores
1	537	0
2	531	0
3	569	0
4	519	0
5	534	0
6	563	0
7	583	0
8	536	0
9	504	0
10	517	0

Four colored fructifications from the same culture gave the following spore counts:

Sample No.	White Spores	Colored Spores
1	522	606
2	117	550
3	3	571
4	3	587

A careful search of the albino fructifications revealed some which had colored spores. A few had more colored than hyaline spores but were still perfectly white even under the dissecting microscope. One agaricoid fructification with both hyaline and colored spores was used as the source of inoculum for three cultures which produced all albino fruiting bodies. These constitute the F_1 generation of a series of inbreeding experiments which followed. A number of these albino fructifications were examined. Most of them had all hyaline spores but a few had a very small number of purple brown spores (less than 0.1 percent).

An albino fruiting body from the F_1 generation which had all hyaline spores was used as a source of inoculum for four cultures which constituted the F_2. These produced all albino fruiting bodies, five of which were examined and no colored spores were seen. In

the F_3 generation occasional colored spores were seen and in succeeding generations only albino spores have been seen. At the present time the albino cultures have been propagated through thirty-three generations from basidiospores. For the last fourteen generations only agaricoid fructifications were used for inoculum although aberrant fructifications were still produced in the thirty-third generation. No colored fruiting bodies have been seen in any generation subsequent to the F_1, although a few colored spores were seen in the F_2 and F_3.

The albino appears to differ from the colored forms only in its lack of pigmentation. It is heterothallic and tetrapolar and the four mating types have been isolated and found to be interfertile with those of the colored forms. When an albino mycelium is mated with that from a colored spore the progeny (F_1) are all colored. Furthermore, the spores produced on the F_1 are all colored. When these spores are inoculated in mass, however, the progeny (F_2) consist of both albino and colored fructifications.

Albino fructifications exhibit the same range of morphological variation as the colored ones. Agaricoid, gasteroid, and all intergrading forms are found. Although they were not subjected to carefully controlled experiments it appears that they respond to light in a manner similar to the colored forms.

Spore Germination

Several attempts to increase the percentage of spore germination by heating at temperatures of 40°, 50°, and 60° C. in a hot-water bath yielded uniformly negative results. Only an occasional germinating spore was seen both in the samples which were heated and in the controls. A slightly better response was obtained by freezing and by treating the spores with certain chemical compounds.

The results of the latter-named tests are summarized in Figures 3 and 4, page 27.

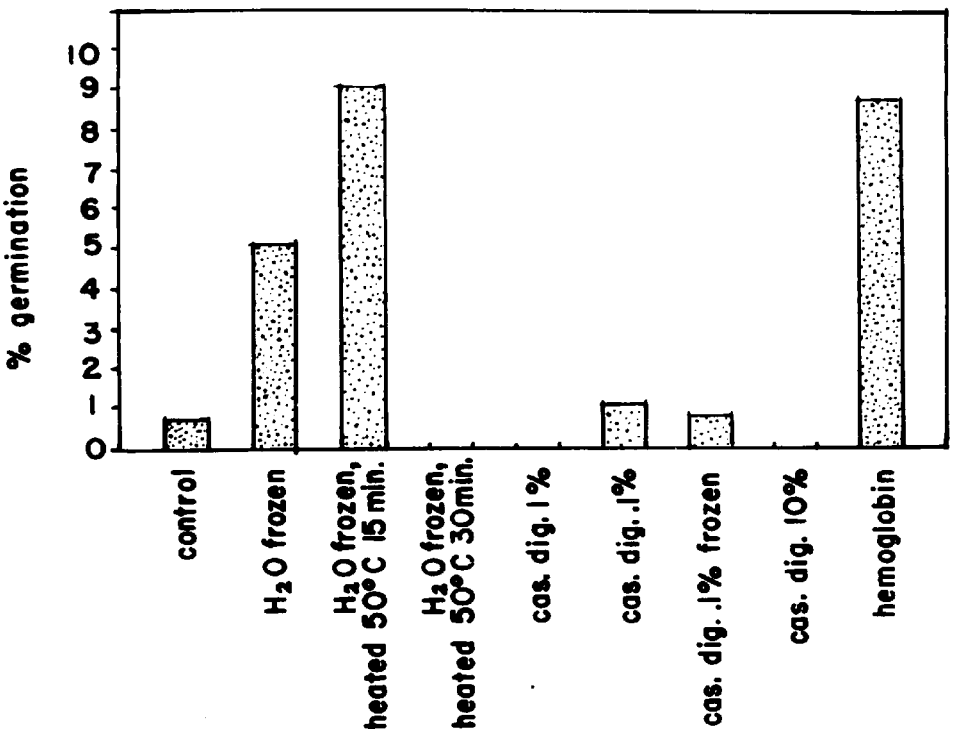

Fig.3 Per cent spore germination after incubation for 21 hours at 25°C in shake cultures.

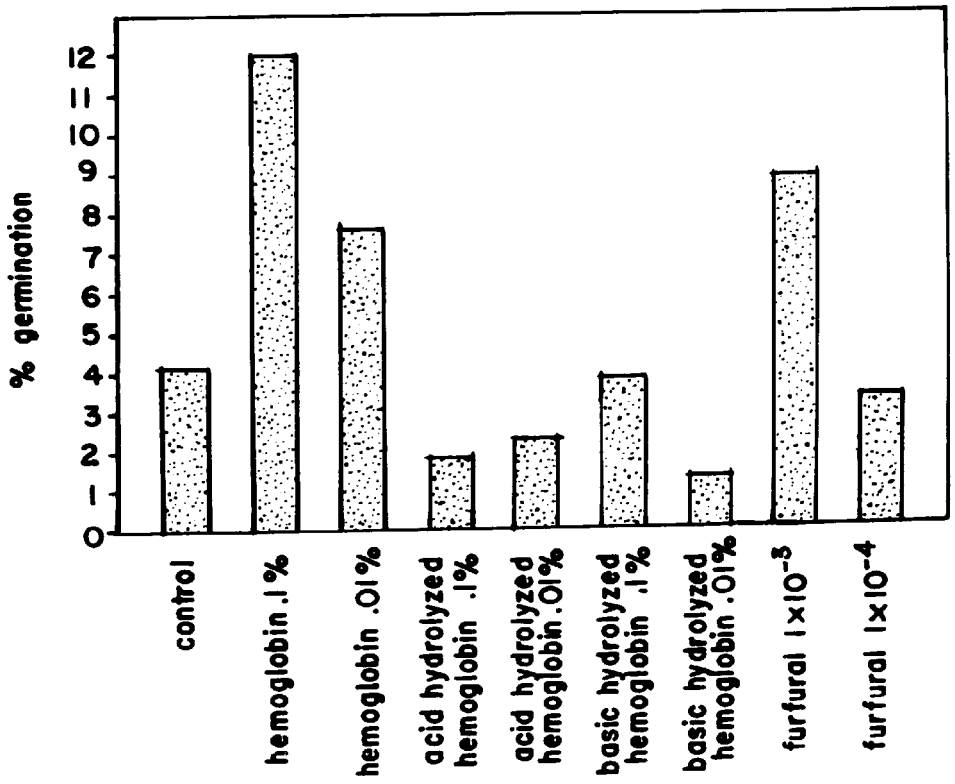

Fig.4 Per cent spore germination after incubation for 7½ hours at 25° C in shake cultures.

CHAPTER IV

DISCUSSION AND SUMMARY

Taxonomy of the Species

The fungus studied in this investigation was found to belong in the genus Psilocybe (Fr) Kummer in the sense of most authors, Singer (1949) excepted. It was found to be a previously undescribed species and is named Psilocybe mutans.

The classification proposed by Singler (1949) is one of the most extensive recent works treating the Agaricales. It is, however, particularly difficult to place P. mutans in a genus following Singer's system. He recognizes the genus Deconica and distinguishes it from Psilocybe largely on the basis of spore size (p. 506):

> It appears to the author that these genera are separable on the basis of a character which is very easy to observe, viz. the shape and size of the spores. This one character is always correlated with at least one alternative character, i. e. in Psilocybe, the spores are either larger than 11 μ or non lentiform, and at the same time, the pileus is non-hygrophanous, or the veil is strongly annuliform, or else pleurocystidia are present.

It is interesting to note that in his key on p. 498, Singer sets 10 μ as the arbitrary line of distinction between the two genera and in his discussion of Deconica on page 508 he says its spores are ". . . usually smaller than 9 μ, more rarely 11 μ in length." Thus it will be seen that the spores of P. mutans (8.4-11.7 [12.6] X 5.6-7.7 μ) extend through the range supposedly separating these two genera and beyond the upper limit set by Singer for Deconica. At the same time, since the pileus is hygrophanous, the veil nonannuliform, and

pleurocystidia are lacking, it lacks all three accessory criteria--one of which is always present, according to Singer, in Psilocybe. It becomes difficult, then, to determine which of the genera recognized by Singer is the logical place for this species. In my opinion P. mutans is most closely related to Psilocybe coprophila (Bull. ex Fries) Kummer and P. merdaria (Fr.) Ricken. Singer (1949) places these two species in his section Merdariae along with P. blattariopsis (Speg.) Singer. This group of species has been accorded various treatments by recent authors. Kuhner and Romagnesi (1953) include coprophila and merdaria in the preoccupied genus Geophila of Quelet, but in separate subgenera with coprophila being lodged in the subgenus Psilocybe and merdaria going to Stropharia. Smith (1949) recognizes Psilocybe and Stropharia as genera by the traditional character of an annulus being present in the latter and not in the former, and thus considers that coprophila should be placed in Psilocybe while merdaria should be included in Stropharia (Smith, A. H.: unpublished manuscript). Lange (1939) includes both coprophila and merdaria in the genus Stropharia. None but Singer makes mention of P. blattariopsis.

Psilocybe mutans is easily distinguished from Psilocybe blattariopsis by the size and shape of the spres. Those of the latter species are described by Spegazzini (1889) as being sphaeroid ovate and 9-10 X 8-9 μ whereas the spores of P. mutans are flattened, being ovoid to ellipsoid in face view and attaining a length of 11 μ while the width is frequently as small as 5.6 μ. In addition, the lighter colors, considerably smaller size, and more delicate habit should serve to make P. mutans very readily distinguishable from Spegazzini's species. An additional difference may be found in the habitats of the two species. Psilocybe blattariopsis is reported from soil in a rich meadow, while mutans is coprophilous.

Similar characters distinguish P. mutans from P. merdaria. Again the smaller size of carpophores and lighter colors are characteristic of mutans. The spores of merdaria are generally larger. Lange (1939) characterizes the stipe of P. merdaria as being brownish inside from the base up, a condition lacking in Psilocybe mutans. The veil which attains a considerable development in merdaria is lacking or at most rudimentary in mutans.

Psilocybe coprophila appears to be more closely related to P. mutans than any of the other species of this section. Probably the most convincing evidence that the two are distinct taxa at the species level concerns their sexuality. Gilmore (1926) and Brodie (1935b) describe P. coprophila as being heterothallic and bipolar, whereas mutans has been shown in this study to exhibit tetrapolar sexuality. Although this criterion can probably be considered sufficient for recognizing the two as distinct species, it is of little practical value to the taxonomist. Gilmore (1926) showed that P. coprophila occasionally produces abnormal fructifications in culture. While none of these are gasteroid, they do resemble certain anomalous fructifications produced by Psilocybe mutans (compare with Plate III). Thus the ability to produce gasteroid carpophores in culture may be of taxonomic significance in distinguishing Psilocybe mutans from closely related species, but it shares with P. coprophila the ability to produce nongasteroid teratological forms. The spores of P. mutans appear to be consistently smaller than those of P. coprophila and, once again, mutans is smaller and more delicate of stature as well as paler in color. Although the latter two differences appear to be rather distinctive it may be well to keep in mind the fact that mutans has been studied entirely from specimens which were grown in culture. It has not yet been collected in the field. A comparison of these characteristics in the two species grown under more similar

conditions is highly desirable. The spore print and the color of the mature lamellae of the two species offers a usable point of distinction. Psilocybe mutans has a dark purplish fuscous spore print while that of P. coprophila is a lighter purplish brown. The pileus trama is much thinner in mutans than in coprophila and in the latter species the pileus trama turns reddish brown in iodine, while that of P. mutans remains ochraceous brown. It must be noted, however, that tissues of the stipe of both species turn reddish brown in iodine. The viscid cuticle is more difficult to demonstrate in P. mutans than in P. coprophila and it is possible that the cuticle itself never becomes as well developed in the former species. However, this, as well as characteristics of the pileus trama, are characters which may result from conditions of culture, especially in view of the many anomalies which have been shown to develop with respect to the morphology of P. mutans grown under suboptimal light conditions.

A comparative study must be made of Psilocybe coprophila and its related species in order to bring into clearer focus the importance of the production of these abnormal fructifications in relation to the phylogeny of the group. Although I have dealt here only with the differences between P. coprophila and P. mutans, in order to establish them as distinct species, they have so many characteristics in common that they must be accorded the closest systematic relationship. Since P. coprophila is placed in the genus Psilocybe by most authors, it is logical that P. mutans should be placed there also, especially since it lacks an annulus.

Morphology

Germination of Basidiospores

Morphologically the germination of the basidiospores of Psilocybe mutans appears to be no different from that commonly described for species of the hymenomycetes having spores with a thick wall and apical pore. The production of a large, globose vesicle which exceeds the size of the spore appears to be a constant characteristic. Brefeld described and illustrated the same sort of structure on the germinating spores of Coprinus stercorarius in 1877. He also illustrated the germination of basidiospores in several species which did not produce an enlarged germinal vesicle. This minor morphological distinction in the early stage of the primary mycelium has been recorded for many species of hymenomycetes by numerous workers since Brefeld. Ferguson (1902) and Baden (1915) give good historical sketches of the early studies on spore germination.

One of the most extensive recent studies on germination of the spores of hymenomycetes was that of Kneebone (1950), who compiled data on the germination of 564 species of basidiomycetes. Although he was primarily interested merely in inducing spores to germinate rather than in increasing the percentage of spore germination, there are certain striking similarities in his results and mine. Kneebone reported negative results in response to heat treatments, which corresponds with what I have found for Psilocybe mutans. On the other hand, he found that storing the spores at -7° C. gave promising results. A number of species were germinated for the first time following this treatment. Figure 3 (p. 27) shows the results of an experiment with the spores of P. mutans in which 0.7 percent germination occurred in the control while storage at -1° C. for 24

hours increased germination to 4.8 percent, and freezing followed by heating at 50° C. for 15 minutes increased germination to 9.1 percent. In the same experiment spores treated with 0.1 percent hemoglobin attained 8.9 percent germination. The results of a second test with hemoglobin and with furfural are shown in Figure 4 (p. 27). Again, treatment with 0.1 percent hemoglobin produced a significant increase (12.1 percent) over the control (4.1 percent) while hydrolyzed hemoglobin gave no increase. In this test, furfural in a concentration of 1×10^{-3} increased germination to 8.9 percent. Attempts to duplicate the results obtained here with furfural failed, while a similar stimulation was found in repeated experiments by the addition of hemoglobin. Various other compounds used which failed to increase spore germination included malt extract, yeast extract, casamino digest,[1] hematin, ribose nucleic acid, and a filtrate of the solution in which a yeast-like organism had grown. Repeated experimentation with these compounds singly and in combination gave no significant results.

The results obtained with hemoglobin and with freezing (possibly with furfural also) suggest that additional experimentation in this regard may be of value. In order to carry out genetic studies which are needed particularly in connection with the albino, it is imperative that a higher rate of spore germination be obtained. Attempts to increase the percentage of spore germination in the Agaricales have, in the past, met with varied success. Ferguson (1902) found that the spores of Agaricus campestris germinated more readily in the presence of the mycelium of that species than in distilled water or in a number of chemical compounds and

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[1] Tryptic digest of casein, manufactured by Sheffield Farms Co., Inc., New York.

decoctions. A similar effect was noted in Psilocybe mutans when, during the process of isolating single spores, it was noted that there seemed to be a higher rate of spore germination in the area immediately surrounding the growing hyphae than in areas distant from growing mycelium. Kneebone (1950) found that the filtrate of a culture of Agaricus campestris showed some stimulation to certain strains of this species as well as to Volvaria volvaceae Fr. Other authors have reported a similar type of stimulation in response to the by-products of other organisms such as yeasts (Fries, 1941) and bacteria (Baden, 1915). Duggar (1901) reports "perfect" germination of the spores of Coprinus comatus in bean and dung decoction, while Kauffman (1934) found that beef broth, 4 percent sucrose, and 0.5 percent glycerin gave increased germination in the same species. Findlay (1933) reported that spores of Merulius lacrymans (Wulf) Fr. were induced to germinate in response to treatment by malic acid and phosphoric acid as well as a medium made up of an old autoclaved culture of Coniophora cerebella! From these and other reports in the voluminous literature on spore germination one gets the idea that the substances capable of inducing spore germination in a given species are extremely diverse in nature and may have their origins in a variety of (sometimes exotic) sources. However, due to the urgent need for obtaining a higher rate of spore germination in Psilocybe mutans, it is suggested that the search for a practical activator continue. The data obtained thus far suggest that both chemical and physical factors used individually or in combination may be effective.

Haploid Mycelium

Oidia.--The oidia of Psilocybe mutans conform to the definition given by Brodie (1936). Their formation is similar to that reported for Coprinus lagopus by Brodie (1931) in that they are borne in a droplet of liquid and are produced only on the haploid hyphae. The ontogeny of the oidia of P. mutans differs from that of the latter species, however, in that the oidiophores are not so distinct and the oidial branches become tightly coiled before septation and subsequent breaking up (Plate I, Figs. 6-12). This manner of oidial development was described and illustrated for Psilocybe coprophila by Falck (1902). Production of oidia on the haploid hyphae only conforms with the situation reported for various tetrapolar hymenomycetes and differs from the situation in Collybia velutipes (Brodie, 1936), Pholiota aurivella (Brodie, 1936), and Pleurotus pinsitus (Vandendries, 1934) where oidia are reported to occur on both haploid and diploid mycelium. Brodie (1936, p. 315) reports that, "For about a week after the germination of a basidiospore of C. velutipes the young mycelium spreads radially over the nutrient medium but produces no oidia during this time." This delay in production of oidia by the young haplont is contrary to the situation found in P. mutans, where it was observed that oidial formation may begin on the young hyphae within a few hours after the basidiospore germinates.

Variation in haploid culture.--Variation of the mycelium of fungi grown in culture (particularly wood-destroying fungi, both parasitic and saprophytic) has been described at length, so that the variation described for Psilocybe mutans is not to be considered as unusual, particularly when dealing with single spore isolates. Davidson, Campbell, and Vaughn (1942) emphasize the fact that isolation of cultures

from single spores or masses of spores tend to show greater variability than do tissue isolations. This is, of course, what one would expect for heterothallic species. In view of the effects of light described here on the fructifications of Psilocybe mutans it is interesting to note the following conclusion by Long and Harsch (1918, p. 39) regarding the mycelium of wood-rotting fungi of various species: "The sunlight was found to accentuate the colors and tone down the mycelial growth of the fungus, thereby making it more characteristic and uniform for a given species than when placed under similar conditions in the darkness or in weak diffused light." No effect of this sort was observed on either haploid or diploid mycelium of P. mutans. The instability of the different haploid strains of P. mutans is directly contrary to the results reported by Dickson (1934, 1935, 1936), who made extensive cultural studies on Coprinus sphaerosporus. Dickson found that the mycelium produced by single spore isolates of C. sphaerosporus possessed distinctive morphological characteristics which remained stable in culture. When grown on horse-dung agar he was able to divide thirty single spore isolates into two categories on the basis of morphological characters which corresponded fairly well with the two "sexual" strains of his bipolar fungus. On other media these two groups were indistinguishable, but he was able to identify twenty-eight individual types of mycelium which remained stable in culture. Only three distinct morphological types of haploid mycelium have been recognized in P. mutans and these are found to be very unstable, sectoring being common in most of the cultures. It has not been possible to correlate them with mating types as Dickson did for Coprinus sphaerosporus, but further studies on different media may yield results of this nature. While the emphasis in this study has been on variation in the fructifications, the work of Dickson suggests that additional interesting facts may be elucidated

by a concentrated study of the haplonts. One of the most interesting features of the haploid mycelium of P. mutans is the production of sclerotia by certain strains. Since the sclerotia lack internal differentiation and an outer zone of thickened cells, they resemble the type described for Rhizoctonia solani by Townsend and Willetts (1954). In spite of their many similarities, Psilocybe coprophila and P. mutans appear to differ in that sclerotia are not produced on the haploid mycelia of the former species; at least they were not reported either by Brodie (1935b) or Gilmore (1926). The sclerotia described by Brodie (1935a) for Panaeolus subbalteatus Berk. are quite different morphologically from those of P. mutans in that the former are described as having greenish-blue primordia which later become darker and harder. In P. mutans the sclerotia may become yellowish or ochraceous in color and they remain soft in texture. It appears that some of them develop into carpophores after diploidization of the haplont, although this possibility should be investigated more thoroughly.

Development of the Fruiting Bodies

The ontogeny of the fructification of the hymenomycetes has occupied the attention of many of the leading mycologists of the last century. According to Reijnders (1948), Hoffman reported on a number of species in 1856 and in two subsequent papers. Since that time innumerable reports on the development of various species of agarics and of gasteromycetes have appeared and considerable controversy has attended certain conflicting reports. Reijnders (1948, 1952) has recently reviewed the more significant contributions in this field, and has added a voluminous fund of knowledge on the

subject as a result of his own investigations covering over fifty species of the agaricales.

Studies on the ontogeny of the agarics have been directed largely to determining two things: (1) the order of development of the different parts of the fructification; and (2) the manner of origin of the hymenium. Arguments on the first of these two questions are treated in considerable detail by Atkinson (1906), Corner (1934), and Hein (1930). Singer (1949) (see also Josserand, 1952) summarizes the view of most present-day authors regarding the origin of the hymenium by describing four types of development: the gymnocarpous, pseudoangiocarpous, hemiangiocarpous, and angiocarpous. (As a result of his extensive studies, however, Reijnders [1948] recognizes eight types of development.) Although the agaricoid forms of Psilocybe mutans are characteristically hemiangiocarpous--using Singer's terminology--it is interesting to observe that, in the teratological forms, both gymnocarpous (Plates III and IV) and angiocarpous development is found (Plate II, Fig. 2).

Singer (1949) states that gymnocarpous development is "the common type of development in Russulaceae, most of the Boletaceae, in numerous genera of the Tricholomataceae (Rhodopaxillus, Leucopaxillus, Mycena, etc.)." The mature gymnocarpous fructifications of P. mutans, however, bear little resemblance to agaric sporophores. Their appearance is more like that of the fruiting bodies of various members of the Aphyllophorales, whose development is also gymnocarpous. Whether or not these teratologies of P. mutans can be attributed to atavism is certainly highly questionable, but the striking similarity in morphology brings this suggestion immediately to mind. The fact that these abnormalities have been observed only in plants grown under suboptimal light conditions and the fact that they seem to be progressively more abundant in successive generations

grown in the dark, indicate a disturbance in the morphogenic mechanism of the organism rather than a heritable character. This will be considered in greater detail at a later point in the discussion.

The occurrence of angiocarpous development in Psilocybe mutans is noteworthy by reason of its rarity in the agarics. Singer (1949) cites Galeropsis and the gasteroid forms of Boletinus decipiens as examples of this type of development in members of the Agaricales. Thom and Lathrop (1925) have reported similar gasteroid fructifications for an agaric which they identified tentatively as Psilocybe atomatoides Pk. These, along with the extreme gasteroid forms of P. mutans must be considered as having angiocarpous development since their spores are not discharged outside of the fructification. The hymenial tissue remains enclosed throughout its entire development. The manner of development of the agaricoid (or boletoid) form of Psilocybe atomatoides and Boletinus decipiens has not, to my knowledge, been reported, and gymnocarpous forms have not been reported for either of them. It appears, then, that Psilocybe mutans occupies a singular position in this respect At the present time it seems to be the only species reported which exhibits variation in morphological development ranging from that characteristic of the Aphyllophorales to that of the gasteromycetes.

Almost all modern authors attribute a great importance to ontogeny in defining the taxonomic relationships of the agarics. With this in mind, it was considered essential to compare the details of development in the agaricoid and gasteroid forms. However, it soon became apparent that this could not be accomplished until more was known regarding the conditions which effect production of the different types of carpophores. At the present time it is impossible to predict with complete accuracy which type of carpophore will mature from a given primordium. Although cultures grown in the light

generally produce agaricoid carpophores almost exclusively, occasional teratological fruiting bodies are found. These are, however, in a minority small enough that a reasonably accurate picture of the general ontogeny of the fructifications as regards the position of origin of the lamellae has been obtained. The production of gasteroid carpophores is less predictable. Cultures grown in darkness do consistently produce a high percentage of gasteroid fructifications. Some cultures grown in the complete absence of light produced no agaricoid fructifications. For the most part, however, dark-grown cultures contain all sorts of anomalous carpophores. In addition to those which are entirely gasteroid, there are some in which the pileus expanded slightly and thus are not truly angiocarpous. The response to light has been so striking that it seems likely that further experimentation will yield results which will make it possible to grow cultures under conditions which will consistently produce all gasteroid fructifications. If this can be achieved, a more detailed comparison of the developmental morphology of the agaricoid and gasteroid fruiting forms would be highly valuable. However, it hardly seems worth while to undertake such a comparison until the conditions affecting production of the various types of carpophores are more fully understood.

Aberrant carpophores of various types are of very common occurrence in the Agaricales, particularly when grown in culture. Singer (1949) has described a number of anomalous fructifications to which he applies the term "carpophoroids" as being "sterile bodies that have no visible purpose but are formed the same way and under similar conditions as the normal basidia-bearing carpophores. . . ." He includes here such things as the well-known sterile fructifications of Rhodophyllus abortivus and Panus tigrinus, to which we might add the abortive form of Psathyrella hirta Pk.

sensu Smith (1934). Singer refers to the fact that carpophoroid forms may bear occasional spores and sometimes form hymenophore-like tissue, which leads him to the conclusion that "the only explanation of these strange, apparently functionless bodies is that of an atavistic aberration whereby a gastroid form is occasionally maintained in normally gymnocarpous or hemiangiocarpous or pseudo-angiocarpous species, which often leads to sterility." He compares carpophoroids to the gasteroid forms of Boletinus decipiens. It seems possible that this interpretation might well be influenced by that author's frequently expressed opinion that the Agaricales have evolved from gasteromycetous ancestors (Singer, 1936, 1945, 1949, 1951). The gasteroid forms of Psilocybe mutans do not fit the definition of carpophoroids cited above since they are fertile, and since their occurrence is favored by environmental conditions dissimilar to those which influence the formation of the agaricoid carpophores. The only other cultural study of gasteroid fructifications to come to my attention is that previously referred to of Thom and Lathrop (1925), who found fertile gasteroid fructifications of a species of Psilocybe which were produced under cultural conditions different from that which produced the agaricoid fructifications:

> All sorts of monstrous forms occur upon these wet media. Globose or subglobose masses which never opened up as pilei were observed in wet agar cultures. When broken open, these often showed fully formed gills and ripe spores. On dry, closely packed fiber, the fruits tend to be solitary and resemble fairly closely those gathered in the field.

Thus, since the gasteroid fructifications of the only two species which have been studied in culture are fertile and since they appear to be produced under conditions different from those which favor the agaricoid condition, it seems logical to exclude them from the definition of carpophoroids.

Although fertile gasteroid fructifications appear to be rather uncommon among the Hymenomycetes, the other anomalous carpophores produced by Psilocybe mutans resemble aberrant forms reported for various other species by many authors. In addition to the many forms described by Singer (1949), the reports of Boudier (1890), Zattler (1924), Buller (1909), Dickson (1934), Bille-Hansen (1953), and Aschan (1954) represent rather characteristic examples of the sort of accounts which appear from time to time in the mycological literature. Peck (1903) has described a malformation of the pileus and lamellae of Clitocybe dealbata which is commonly recorded for various agarics and which resembles the "poroid" forms of Psilocybe mutans illustrated in Plate III, Figures 3 and 4. The closely related species, P. coprophila, has been shown to produce anomalous forms similar to those illustrated in Plate III, Figures 1 and 2. These stipitate fructifications with a costate hymenophore were studied by Gilmore (1926), who claims to have shown that they are inherited abnormalities. Long and Harsch (1918) have described modified hymenophores for Polystictus hirsutus some of which resemble the sessile, smooth or wrinkled hymenophores found occasionally on Psilocybe mutans. Thus, of the various anomalies exhibited by this species, the gasteroid forms appear to be the most unique, and possibly the most interesting from the point of view of phylogeny.

Cytology of the Basidium

The details of nuclear behavior in the basidium of Psilocybe mutans were found, as far as they were followed, to conform to the characteristic pattern for 4-spored agarics as reported for Agaricus campestris by Colson (1935), and as reported by various authors for

many other species of the hymenomycetes (Olive, 1953). Although my preparations did not permit an accurate count of the chromosomes, a small number (haploid probably less than 6) is indicated.

The production of giant spores in Psilocybe mutans is of particular interest. Smith (1934) has shown that in certain species of Mycena an increase in spore size was correlated with the 2-spored condition. Although basidia bearing less than 4 spores were found occasionally in P. mutans, no attempt was made to correlate them with spore size, and no 2-spored strain or variety of the species has been obtained. The fact that certain single spore isolates were found to be diploid presents another possible correlation with the giant spores. A cytological study of giant spores in this species would be particularly interesting, but since they were never seen attached to basidia and were seen only very rarely this has been impossible to date.

Sexuality

The results of 172 pairings which were made to determine the type of sexual behavior of Psilocybe mutans indicate the well-known tetrapolar pattern although certain deviations occur. This type of sexuality was first described by Kniep (1920), and is now generally recognized as being the most common type of sexual pattern among the hymenomycetes. An excellent recent review of tetrapolar sexuality is given by Raper (1953).

The "barrage" phenomenon of Vandendries and Brodie (1933) and the "flat" heterocaryosis reported first by Papazian (1950) for Schizophyllum and later by Raper and San Antonio (1952) were not observed during the matings performed in this study. Raper (1953) suggests that these phenomena may not be readily observed in

cultures grown on a rich medium similar to that used here. Since I was interested also in studying the fruiting bodies which were produced following mating, the cultures were allowed to grow well beyond the period where heterocaryosis could be observed best before they were examined.

Single spore isolates 18 and III (also two isolates not reported in this thesis) show an interesting deviation from the typical tetrapolar pattern. These cultures fruited very soon after the spores had germinated and before they had been mated with any other strain. It is assumed that they represent diploid spores although the possibility of contamination is admitted. The latter is considered unlikely, however, in view of the fact that there has been no evidence of contamination between noncompatible strains which would have shown up in subsequent matings. Since the probability of contamination by noncompatible strains is twice as great as the probability of contamination by compatible strains, it seems that contamination can safely be eliminated as an explanation of these diploid cultures. Gilmore (1926) reports a similar event in Psilocybe coprophila. The occurrence of anomalous behavior such as production of diploid spores can be rationalized quite easily, but the frequency with which it occurs in P. mutans is harder to explain. The 4 diploid single spore isolates represent approximately 4 percent of all the isolates made. (A total of 102 single spore isolates were observed in culture, although only 22 are reported in the mating chart.) This phenomenon apparently is quite uncommon and merits further study.

According to Raper (1953) the production by haploid strains of fruiting bodies having progeny of only the same mating type as the parent has been reported for a large number of tetrapolar species of hymenomycetes. What were thought to be fructifications of this type were found in a number of haplonts of P. mutans. For the most

part the "haploid" fructifications were produced only when the cultures were quite old. They appeared to be mostly sterile and usually had aborted basidia. Analysis of 7 viable spores isolated from one such carpophore showed that three of the four mating types were found among the 7 spores (Fig. 2, p. 19). This suggests that the fructifications were not the results of "haploidy" as originally assumed. No explanation can be offered for this anomaly at the present time. Further study is needed.

Phylogenetic Considerations

In discussing the gasteroid form of Boletinus decipiens, Singer (1949, p. 652) writes:

> It would be very interesting to show experimentally or otherwise what precisely causes the aberrant carpophores to remain gastroid. This may throw some more light on the phylogenetic implications of this subject. Could it be that these carpophores were subjected to dry conditions while still in the primordial stage?

At this time no one has--to my knowledge--studied Boletinus decipiens in culture. Hence Singer's query remains unanswered. However, recognizing the similarity between the gasteroid forms of Boletinus decipiens and Psilocybe mutans, we may derive some considerations of phylogenetic significance from the study of gasteroid forms of the latter species. It was with this thought in mind that the present study was undertaken.

The various theories regarding the phylogenetic relationships of the Agaricales and the gasteromycetes have been reviewed recently by Singer (1949), Bessey (1950), and Holm (1949). Briefly stated, the essential points of the three most acceptable theories and the more prominent adherants to each are: (1) the gasteromycetes are

derived from the Agaricales by reduction, DeBary (1884), Heim (1948), Rogers (1934); (2) the agarics are derived from the gasteromycetes, Brefeld (1877), Lohwag (1924, Singer (1936); (3) gasteromycetes and agarics are derived independently of each other from primitive hymenomycetes, Fischer (1900), Gäumann (1926), Bessey (1935), Greis (1938, according to Holm, 1949), Dodge (1931). In order to understand the bearing of Psilocybe mutans on these theories it may be well first to define the criteria which distinguish the Agaricales from the Gasteromycetae.

The work of Persoon (1801) has been established as the starting point of nomenclature in the gasteromycetes. His class, Angiocarpi, included most of the plants now placed in the gasteromycetes as well as many myxomycetes, ascomycetes, and rusts. The one characteristic which they shared was their angiocarpous development. The history of Persoon's original class has been one of attrition. As more natural relationships have been recognized among them the majority of the species which he included in the Angiocarpi have been removed to other taxa by more recent systematists. Cunningham (1942), who is probably the most widely known living authority on the gasteromycetes, has given a good historical account of the many taxonomic arrangements dealing with this group since Persoon. In defining the limits of the gasteromycetes, Cunningham says (p. 1):

> The Gasteromycetes is a traditional sub-class of fungi which was established by Fries to contain those species in which the spore-bearing hymenium is enclosed until maturity within the peridium. This feature alone separates members from the other great group of Basidiomycetes--the Hymenomycetes--in which the hymenium is exposed throughout the life of the plant.

Later, in speaking of spore dispersal in the gasteromycetes, Cunningham (1942, p. 6) says: "Spores are not violently discharged from the basidia, as is the case with hymenomycetes; instead, they become

detached by pressure or collapse of the basidia, and lie in the glebal chambers--often completely filling them--until set free." These appear to be the two basic criteria which distinguish the gasteromycetes from the agarics in the minds of most taxonomists today. If, as Cunningham states, the primary criterion distinguishing the gasteromycetes is the angiocarpous development of the sporophore, it is apparent that although many genera and species have been removed from the gasteromycetes on the basis of other characters, the differentiation between these two major taxa is still largely on the same basis as that employed by Persoon (1801) and Fries (1821-1832). It must be apparent, also, that the gasteroid form of Psilocybe mutans possesses characteristics of both major taxa, but since it is angiocarpous, it must necessarily be classified as a gasteromycete. This would be in accordance with the conclusion of Singer (1949, p. 65) regarding the gasteroid form of Boletinus decipiens: ". . . the gastroid form of Boletinus decipiens which, biologically speaking, is a Gasteromycete rather than a bolete. . . ." Thus Psilocybe mutans may be said to belong to a very select group of fungi, being one of three species known which have been shown to produce fruiting bodies of both the gasteromycete type and the agaric type. The other two species are, of course, Boletinus decipiens and the Psilocybe reported by Thom and Lathrop (1925). Among these, however, P. mutans occupies a singular position in that it is the only species in which gasteromycete fruiting bodies have been grown in culture from spores which are positively known to have been borne on agaric fruiting bodies. Hence it is the first species known in which an agaric has given rise to progeny which are gasteromycetes. It seems likely that similar results might be obtained with both of the other two species should they be studied in culture.

Although it is relatively easy to determine that the gasteroid form of Psilocybe mutans may be classified as a gasteromycete according to modern concepts, it is rather difficult to place it in an order or genus within the gasteromycetes. Most authors (Coker and Couch, 1928; Cunningham, 1942; Smith, 1951; Zeller, 1949) attempt to distinguish those gasteromycetes which have a percurrent columella from those which lack of columella or possess a partially percurrent one. Lohwag (1924) used the developmental morphology as a basis for determining the relationships within the gasteromycetes. Those with a lacunar type of development were considered as most primitive, and the coralloid, unipileate, and multipileate types were regarded as progressively more advanced. These concepts have been generally followed by most authors since Lohwag. In this connection Dodge (1931, p. 457) has outlined a detailed series of steps whereby the stipe and columella may have originated from an ancestral form which lacked them:

> The primitive Gasteromycetes consisted of spherical or somewhat irregular fructifications with no differentiated sterile tissues and no stipe, the rhizomorphs on which the fruit-bodies were borne ending at the peridium. Gradually a cushion was formed at the point of attachment from which the larger tramal plates originated. This increased in size, penetrating farther into the fructification until it fused with the peridium at its tip, forming a percurrent columella. Along with this development, the end of the rhizomorph developed a stipe to raise the fertile portion of the fructification above the substrate for better dispersal of spores.

Cunningham (1942, p. 12), however, maintains that the developmental types "have little if any value as a basis for taxonomic treatment of genera and higher ranks." But he does distinguish the two families of his order, Hymenogastrales, on the basis of development of the stipe and columella. The gasteroid form of Psilocybe mutans presents a difficult problem in this respect, since some specimens can

be interpreted as having a lacunar type of development (Plate VII), while others have coralloid (Plate VI), and still others unipileate (Plate VIII) development in accordance with Lohwag's definitions (1924). Thus, all of the stages in Dodge's (1931) theoretical series are represented in this one species. The different morphological forms of gasteroid fructifications would have to be placed in different genera and perhaps in different orders of the gasteromycetes: those with percurrent stipe going into Secotium, the "coralloid" forms in Truncocolumella, and the "lacunar" forms in some related genus of the Rhizopogonaceae. Furthermore, as previously explained, these types pass by imperceptible intergradations into the agaricoid form. This may be sufficient evidence to cast serious doubt upon the value of these developmental types as a primary basis for determining the relationships within the gasteromycetes as Lohwag and later authors have done. Perhaps other criteria should be given more emphasis in this regard. In view of the highly artificial nature of the criteria which distinguish the gasteromycetes from other major taxa of the higher fungi it is most surprising to me that many taxonomists still try to arrange them in a single phylogenetic series (Gaumann, 1952; Bessey, 1950; Cunningham, 1942). It seem that we are coming more and more to the realization that the gasteromycetes are indeed a very highly polyphyletic group. According to Holm (1949), Greis (1943) suggests a connection between the Tuberales and certain gasteromycetes, and Holm (1949) expands this view to state that some of the Hymenogastrineae may have evolved from members of the Tuberales while others could have descended by reduction from the Agaricales. In any case, the clear demonstration of the production of gasteromycete fructifications by an agaric adds to the evidence supporting the concept that the Hymenogastrales are a very artificial assemblage of species whose origin probably was not completely

independent of the Agaricales as proposed by theory number (3) of our previous scheme (p. 46).

The facts obtained from this study of the morphology of Psilocybe mutans seem to focus attention again on the close relationship of the agarics and the gasteromycetes and the possibility that one group may have originated--in part at least--from the other. The various connecting series between the two groups have been discussed at length in recent works by Singer (1936, 1949, 1951), Heim (1948, 1951, 1952), and Reijnders (1952). The numerous "bridges" between the gasteromycetes and the Agaricales which have been recognized as a result of the assimilation of the investigations of many workers may be summarized as follows:

Coprinus--Montagnea: Lloyd (1904a), Underwood (1899), Patouillard (1900), Lohwag (1924), Morse (1933)
Amanita--Torrendia: Singer (1949)
Rhodophyllus--Richoniella: Romagnesi (1933)
Conocybe--Galeropsis--Cyttophyllopsis: Heim (1950), Singer (1936)
Paxillus--Boletus--Truncocolumella--Rhizopogon: Malençon (1938), Heim (1952), Singer (1936)
Agaricus--Longula: Barnett (1943), Zeller (1943)
Russula--Elasmomyces: Bucholtz (1902, 1903), Heim (1948, 1952)
Cortinarius--Thaxterogaster: Singer (1951), Heim (1951)
Boletinus decipiens--Truncocolumella: Singer (1945, 1949)

As a result of the close relationship exhibited by these various connecting series, these authors are generally in accord that one group most probably evolved from the other. They do not agree on the direction of evolution between the agarics and the gasteromycetes. Thus, theories (1) and (2) (pp. 45-46) are still hotly debated. It seems doubtful that either will gain general acceptance over the other in the near future. However, the evidence obtained from

Psilocybe mutans may be applied to certain points of argument in the controversy.

Singer (1945, p. 255) refers to the gasteroid form of Boletinus decipiens as being "atavistic aberrations whose formation may be favored by climatic factors, e.g. sudden decrease in humidity during the fruiting period." In a more rencent paper (Singer, 1951, p. 226), he says that the tendency of certain Cortinarii to form anomalous hymenophores again represents an atavistic tendency "brought about by conditions favoring local detrimental mutations" and he concludes that these so-called "atavistic tendencies" suggest that the Cortinarii may have evolved from Thaxterogaster. If we assume, as Singer does for Boletinus decipiens and Cortinareus, that the teratological forms of Psilocybe mutans represent atavism we are faced with a most paradoxical situation. The gasteroid forms of P. mutans are wholly angiocarpous in development whereas certain poroid and other anomalous forms are gymnocarpous throughout their entire development. If, then, as Singer suggests, we interpret anomalous fructifications as representing atavism, Psilocybe mutans would be in the highly improbable position of having evolved at the same time from both angiocarpous and gymnocarpous ancestors! That is to say, this species would have originated from both the Gasteromyceteae and the Aphyllophorales. The only other alternative would be to consider that either the gymnocarpous or the angiocarpous teratological forms represent atavism while the other represents a condition more advanced than the intermediate hemiangiocarpous (agaricoid) condition. This, of course, is inconsistent with the original rationale and in addition presents us with the impossible task of deciding which should be considered as atavistic and which as advanced.

It is my opinion that the aberrant fructifications of P. mutans should not be interpreted as atavism, but are more probably expressions

of morphological variation on the part of a very labile species. This conclusion is supported by the fact that it has been impossible to date to obtain "pure strains" of any of the aberrant forms in culture. I have not been able to demonstrate that these are heritable characteristics, but have shown that morphological variation does occur in response to different light intensities. Further genetic studies in this regard are desirable, but it is not considered advisable to carry them out at this time because of the very low percentage of spore germination which has been obtained in this species. Another argument opposing the consideration of the aberrant carpophores of P. mutans as atavisms derives from the apparent lack of any species within the gasteromycetes with which the gasteroid forms might be connected. The gasteroid forms of P. mutans may be accepted as gasteromycetes due to their angiocarpy, but no other species of gasteromycetes have spores of this type; e.g., thick walled with apical germ pore, purple brown in color fading to dull yellowish brown in KOH. If we are to consider these forms as atavistic, we must assume that all ancestral forms with similar spores have lost out in the struggle for survival or remain undiscovered; a most unlikely premise in view of the fact that in all of the other series linking the agarics and gasteromycetes spore structure is a very constant criterion. Indeed, in most cases it is the most important characteristic linking the two taxa. Since no possible ancestral relative can be found among the gasteromycetes for Psilocybe mutans, and since its morphological variations proceed in so many directions, it seems more logical that they represent the manner in which evolution is now progressing, rather than the direction from which the species has come.

Of all the anomalous forms presented by this species the gasteroid form is the most common, and, for the present argument,

the most significant. Conard (1915, p. 101) concluded from his study on the developmental morphology of Secotium agaricoides that "It is to be regarded as a primitive or arrested agaric,--perhaps a paedogenic form, reaching its reproductive maturity in the 'button' stage." Following this same line of reasoning, Buller (1922) postulated a series of simple mutations which would result in the derivation of Secotium agaricoides from an ancestor in the genus Agaricus. It is suggested that these ideas might be applied in interpreting the gasteroid form of Psilocybe mutans. This sort of reasoning leads ultimately to the interesting speculation that some of the species of Secotium may actually be nothing more than teratological forms of certain species of agarics.

Regardless of the assumed direction of evolution between them, the discovery of a new connecting link between the Agaricales and the Gasteromycetae is a significant contribution. The gasteroid forms of P. mutans and of the Psilocybe atomatoides (?) of Thom and Lathrop (1925) represent the first connections between the Strophariaceae of the Agaricales and the gasteromycetes.

Although variations of the sort described here for P. mutans are not entirely unusual for the hymenomycetes, the range of variation exhibited by this species is indeed unique. This extreme lability within a single species supports the contention that the major taxa of the higher fungi are truly heterogeneous assemblages of physiognomically similar yet phylogenetically diverse entities.

Both Heim (1951) and Singer (1951) have expressed the belief that fruiting anomalies of certain hymenomycetes may represent adaptations in response to adverse conditions of a xerophytic environment with the implication that this may have some significance with regard to evolution. Thaxterogaster, Boletinus decipiens, and certain species of Secotium are singled out as evidence for this hypothesis.

In this connection, also, Psilocybe mutans offers a distinctive contribution, as does the Psilocybe atomatoides (?) of Thom and Lathrop (1925), for here we have a clear demonstration of a similar sort of variation in response to stimuli of the environment other than those imposed by a xerophytic habitat. The anomalies of P. mutans are shown to occur under suboptimal light conditions. The effective stimuli in the case of Psilocybe atomatoides (?) are more difficult to assess with certainty but they are quite obviously unrelated to elements of a xerophytic environment.

Effect of Light on Fruiting

One of the most significant aspects of this study is the demonstration of the effect of light on formation of the carpophores. Light has long been thought to affect the fruiting of agarics, but few controlled tests have been made of the role of light in this regard. Although the present study is a mere beginning in this line of investigation, a few significant facts have been discovered concerning the role of light in fruiting of P. mutans.

Lange (1939) reports the collection of etiolated specimens of the closely related species, Psilocybe coprophila, which are very similar to certain aberrant fructifications produced in cultures of P. mutans grown in dim light. Gilmore (1926), who studied P. coprophila in culture, reports that fruiting practically ceased during the months from December to February and began again early in March. Although she does not describe the light conditions under which her cultures were grown, her results are very similar to the situation found here with cultures of P. mutans grown on a laboratory table near a west window. For two years I have observed a diminution in the number of carpophores and the perfection of

their development during the winter months. Cronin (1951) and Long and Harsch (1918) have demonstrated that light affects the color of hymenomycetes in culture, a fact which may be of considerable importance because of the great significance attributed to color in the taxonomy of this group. From a study of Collybia velutipes in culture Aschan (1954, p. 574) reports: "Light was found to be necessary for complete development of fruit bodies. Without light only mycelial growth or fruit body rudiments with stipes but no pilei were produced." Although this is reminiscent of certain anomalies found in Psilocybe mutans, it is just the reverse of the situation found by Levine (1922), who reports that Coprinus ephemerus and C. stercorarius grown on horse dung decoction agar produced fruiting bodies which were more normal in appearance when grown in semidarkness. His statement that they produced carpophores more abundantly during the winter and spring months than during the summer is the opposite of what Gilmore (1926) found for Psilocybe coprophila and the situation reported here for P. mutans. It is interesting to note that these four species occupy essentially the same substrate, and all except P. mutans are known to be quite widely distributed throughout temperate regions. The ecological significance of this response to light is not entirely clear. Since the two Coprini do not fruit in nature during the winter months in northern climates we may speculate that there is potentially, at least, a competition for substrate among the four species. Could it be that the Coprini are favored on a substrate deposited in shaded areas, while the two species of Psilocybe are more successful on dung in sunny areas? The problem may be worthy of more intensive ecological study.

One other aspect of the researches of Gilmore (1926) on abnormalities in fructification of Psilocybe coprophila invites

comparison with P. mutans. She claims to have shown that the ability to produce abnormal basidiocarps in P. coprophila is governed by inheritance (although the evidence from her data appears to me to be far from conclusive). Her conclusion (1926, p. 428) is that:

> It is possible that the tendency to fruit abnormally is present in the mycelium of these crosses but is able to express itself only under certain environmental conditions. The amount of moisture present is possibly the critical factor. . . .

In Psilocybe mutans I have been unable to isolate any genetic stock in which the ability to fruit abnormally is not present, given the proper environmental conditions. In one series of tests fourteen generations were grown from mass inoculations of basidiospores from agaricoid fructifications. In the fourteenth generation both agaricoid and gasteroid fruiting bodies were formed. Since these two species are very closely related it would be interesting to study their abnormalities in cultures grown simultaneously under identical conditions.

The researches of Buller (1909) on Polyporus squamosus represent one of the most extensive studies on the effect of light on a hymenomycete. He showed that plants grown entirely in the dark produce highly abnormal fructifications and that an exposure to light for as short a time as one hour was sufficient to cause the production of normal carpophores. He also determined that fruiting bodies which were exposed to light in very early stages of development and then grown entirely in the dark would develop normally. A similar situation is suggested in Psilocybe mutans since very few cultures were produced which contained all gasteroid carpophores and only the "dark controls" of light experiment number 3 were grown in absolute darkness until they were harvested. Almost always a few fruiting bodies with partially or fully expanded pilei were produced.

Light experiment number 3 gives the most significant data of any of the experiments on the effect of light and it must be considered as somewhat tentative since the experiment has not yet been repeated. From this test we can conclude that light appears to be necessary for the production of agaricoid fructifications in this species. This is indicated by the data tabulated on page 22, in which it was shown that no agaricoid fructifications were produced on the cultures grown in a light-tight can. It must be observed, however, that the can was also very nearly air-tight, and the relative humidity was visibly greater inside the can than outside, as evidenced by the fact that agar in the "dark" cultures was dried out less than those which were grown on the open shelf. Very likely the oxygen and carbon dioxide contents of this restricted environment were considerably different from that in light-grown cultures also. However, in view of the similar results of the two previous experiments in which humidity and gas exchange were not variables, it must be concluded that light offers a significant morphogenic stimulus in formation of fruiting bodies of this fungus. This is further indicated by the fact that in the same experiment no gasteroid fructifications were produced in cultures grown in light intensities greater than 50 foot candles, but were present in cultures grown in 25 and 50 foot candles. Further investigations on the morphogenic effect of light on the developing carpophores of P. mutans offer most promising prospects for future research. Before this is undertaken, however, it would be advisable to investigate the nutritional requirements of the organism.

The significance of the light effect in regard to taxonomy and phylogeny of the species, which is the primary concern of this investigation, has been discussed in a previous section.

Albino Mutant

Albinism in the hymenomycetes has received very little attention in the literature. This may be due partly to the fact that it is often difficult to recognize an albino agaric as such. Some idea of the confusion which prevails as a result of a rather loose application of the term "albino" by some authors may be obtained by examining some of the early reports of albinos among the higher fungi such as those of Schiffner (1923), Klee (1924), and Schaeffer (1952). It is not exactly clear whether all of the fungi reported by Schiffner and Klee are true albinos, for they refer to some of them as being pale or faded and as having a few pale spores on an abortive hymenium. Klee (1924) does refer to a white-spored Psalliota arvensis which apparently is a true albino (although his description is not detailed enough to preclude the possibility of it being a Lepiota), but in general it seems that these authors failed to distinguish between albinos, semisterile fructifications of colored species, and pallid forms of colored species. A good example of this confusion is indicated by Schaeffer's illustration (1952, Tafel XVIII, Fig. 63a) of an "albino von Russula rubra" which has an essentially white stipe and pileus with cream-colored gills of the same tint as those illustrated for the typical red-colored form of the species. Such fungi are clearly not albinos since they do produce some color. The Psalliota arvensis reported by Klee (1924), and the geasters reported by Lloyd (1904b) and by Smith (1951), on the other hand, are in all probability good examples of true albinos, for they represent white, hyaline-spored specimens of fungi which in the typical forms of the species are colored.

Tricholoma cystidiosum Smith illustrates the difficulty in recognizing an albino in the agarics. This is a pure white fungus with

hyaline spores. When Smith (1941) described the species he noted its resemblance to Inocybe geophylla (Fries) Quélet in habitat, stature, and microscopic characteristics, but placed it in the genus Tricholoma on the basis of its "spores (which) differ only in their thinner unpigmented walls" (Smith, 1941, p. 15). Singer (1949), obviously considering it to be an albino Inocybe, transferred it to the latter genus, but, interestingly enough, maintained the specific epithet.

The albino form of Psilocybe mutans is a true albino, for it is absolutely colorless, although overmature specimens have a sordid, hygrophanous appearance. Even the incrusting pigments of the hyphae in the pileus trama are lacking. To my knowledge it is the first report of an albino agaric whose ancestry can be definitely traced to colored parents. It appeared to be a relatively unstable mutant in the first few generations but has subsequently become quite stable and successful in competition with the colored forms in artificial color. The albino has been mated with the colored form of the species. The F_1 of such a mating produces only colored fructifications. When these are mated again with the albino or with each other both albino and colored fructifications are produced. A complete genetic analysis of the inheritance of the albino characteristic has not been considered feasible at this time, due to the low rate of spore germination in the species, but the following conclusions have been deduced from its behavior. The fact that albinos do not appear in the F_1 of a colored X albino cross indicates that albinism is recessive in this species. (Since albinos appear in high proportion in F_1 X F_1 matings and in test crosses of the F_1, it is probable that albinism is inherited by one or a few genes.) Since the basidiospores are haploid, and since all of the spores in the F_1 of colored X albino matings are colored, we may conclude that there is a delayed effect of the albino genotype as far as color of the spore is concerned.

This can be explained if we postulate that certain stages in synthesis of the spore pigment actually occur in the basidium where both the genes for color and albinism are present. Then if some intermediate compound or precursor of the pigment is passed into the spore with the cytoplasm, the final synthesis of the pigment may be completed in the presence of the gene for albinism. This would result in the production of colored spores having the albino genotype which the test crosses prove to be the case.

This clear-cut demonstration that albino mutations may occur among dark-spored agarics, and that the mutants may become established in competition with colored forms is highly significant from the point of view of taxonomy and phylogeny. Dr. A. H. Smith has described to me, in a personal communication, a pure white fungus which is in all other respects characteristically a Naematoloma collected by him in the Tahquamenon region of the upper peninsula of Michigan some years ago. He has been reluctant to publish an account of this fungus due to the fact that it would have to be described as a new genus on the basis of its lack of color. The evidence presented here of the origin and establishment of an albino among dark-spored agarics directs the search for the relationships of such colorless anomalies toward the colored species of agarics rather than the white species, and may assist in identifying more accurately the proper taxonomic position for such a plant. In regard to phylogeny, it has been generally recognized that the dark-spored agarics have arisen from ancestors having hyaline spores (and that thick-walled species have been derived from thin-walled forms). The albino of P. mutans gives evidence that some white species with thick-walled spores may have originated from colored species also having thick-walled spores. With this knowledge at hand

it may be possible to delineate the relationships of the agarics more accurately.

Summary

1. Psilocybe mutans is described as a new species of the Strophariceae. Its relationships with other species within the genus and with closely related species of other genera are discussed.

2. The life cycle has been studied and found to conform with that commonly found among heterothallic hymenomycetes. Matings of twenty-two single spore isolates in all possible combinations have shown that the species has tetrapolar sexuality, although occasional deviations from this pattern are reported.

3. The young basidiole is binucleate. Four nuclei are produced as a result of reduction division, one of which migrates into each of the four spores produced on the basidium. The nucleus of the spore divides soon after entering it.

4. Although some of the spores of Psilocybe mutans appear to be capable of germinating at the time they are shed, the percentage germination was found to be very low (usually less than 1 percent). Freezing and an aqueous solution of hemoglobin were found to increase the percentage of germination. No significant increase in germination resulted from heating the spores or treating them with solutions of malt extract, yeast extract, casamino digest, RNA, hematin, and the filtrate of the solution in which a yeastlike organism had grown. Furfural gave inconsistent results.

5. Fructifications of several types are readily produced in culture on a variety of nutrient media within about seven days after the basidiospores germinate. The agaricoid fruiting bodies are hemiangiocarpous in development. Gymnocarpous fructifications

and angiocarpous gasteroid fructifications are also produced in culture. These anomalous carpophores are compared with similar aberrant fruiting bodies which have been reported for other species.

6. Gasteroid fruiting bodies have been grown from mating two single spore isolates of agaricoid carpophores and, conversely, agaricoid fructifications have resulted from the mating of haplonts isolated from gasteroid fructifications. Inbreeding the progeny of agaricoid fruiting bodies for fourteen generations failed to produce a pure strain for this character.

7. "Lacunar," "coralloid," and "unipileate" types of development are all found among the gasteroid fructifications.

8. Since the gasteroid form is angiocarpous in development, it meets the definition of a gasteromycete. Psilocybe mutans, therefore, forms a new connecting link between the Agaricales and the Gasteromycetae. This is the first report of such a "bridge" between the Strophariaceae and the gasteromycetes, and the first case in which such a connection has been established within a single species.

9. Since Psilocybe mutans produces carpophores characteristic of the hymenomycetes and since no related species are known among the gasteromycetes, it is concluded that the manner of fruiting of this fungus supports the theory that certain gasteromycetes may have evolved by reduction from the Agaricales. It is suggested, also, that certain species of the Secotiaceae may represent teratological forms of species of the Agaricaceae, and that further connections between the agarics and the gasteromycetes should be sought on the species level.

10. Cultures grown in the presence of light produced more agaricoid fructifications and fewer gasteroid fructifications than those grown in darkness. Cultures grown in total darkness produced only

gasteroid and other anomalous forms but no agaricoid fructifications. Both agaricoid and gasteroid fructifications were produced in cultures grown in 25 and 50 foot candles of light, but no gasteroid fruiting bodies were produced in cultures which received more than 50 foot candles. It is concluded that light provided a morphogenic stimulus necessary for the production of agaricoid carpophores.

11. An albino mutant of Psilocybe mutans is described and its significance in relation to taxonomy and phylogeny of the agarics is discussed. The albino appears to differ from the colored form of the species only in its lack of color. Both show the same range of morphological variation and the same pattern of sexuality. The albino was mated with the colored form and both colored and albino fructifications were obtained in subsequent matings of the offspring.

APPENDIX

PLATE I

Microscopic Characteristics

Figure

1. Cheilocystidia, X580
2. Basidiospores, X580
3. Lactifers, X580
4. Incrusted hypha from pileus trama, X1580
5. "Giant" spores, X580

6-12. Development of oidia, X1320

13-22. Development of basidium, X1320

23. Binucleate basidiospores, X1320
24. Uninucleate basidiospores, X1320

25-28. Germinating basidiospores, X1320

29. Germinating basidiospore. X365

30-37. Abnormal basidia, X1320

PLATE I

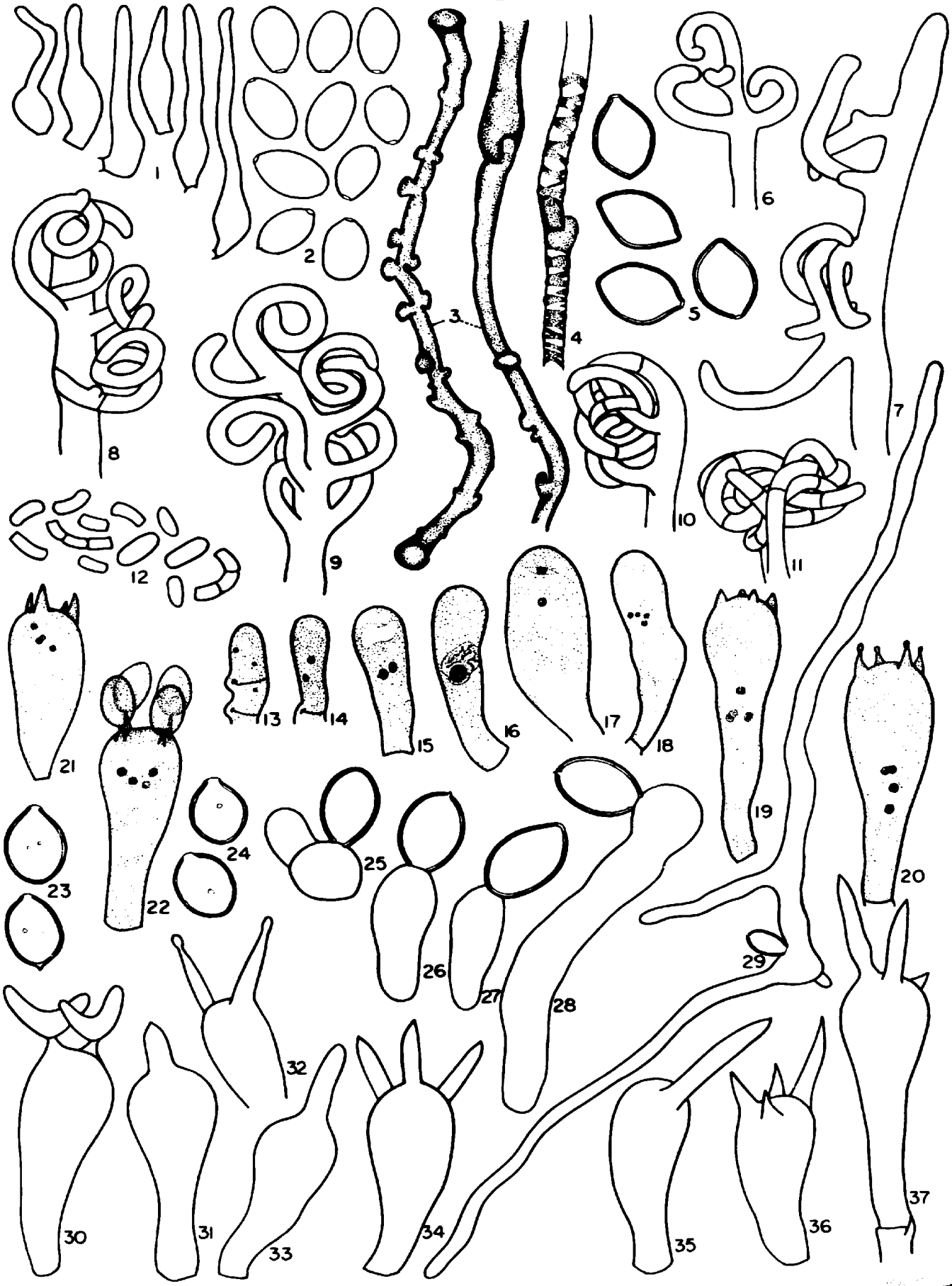
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PLATE II

Agaricoid and Gasteroid Fructifications

Figure

1. Agaricoid carpophores
2. Gasteroid carpophores

Plate II



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PLATE III

Anomalous Fructifications, X14

PLATE III

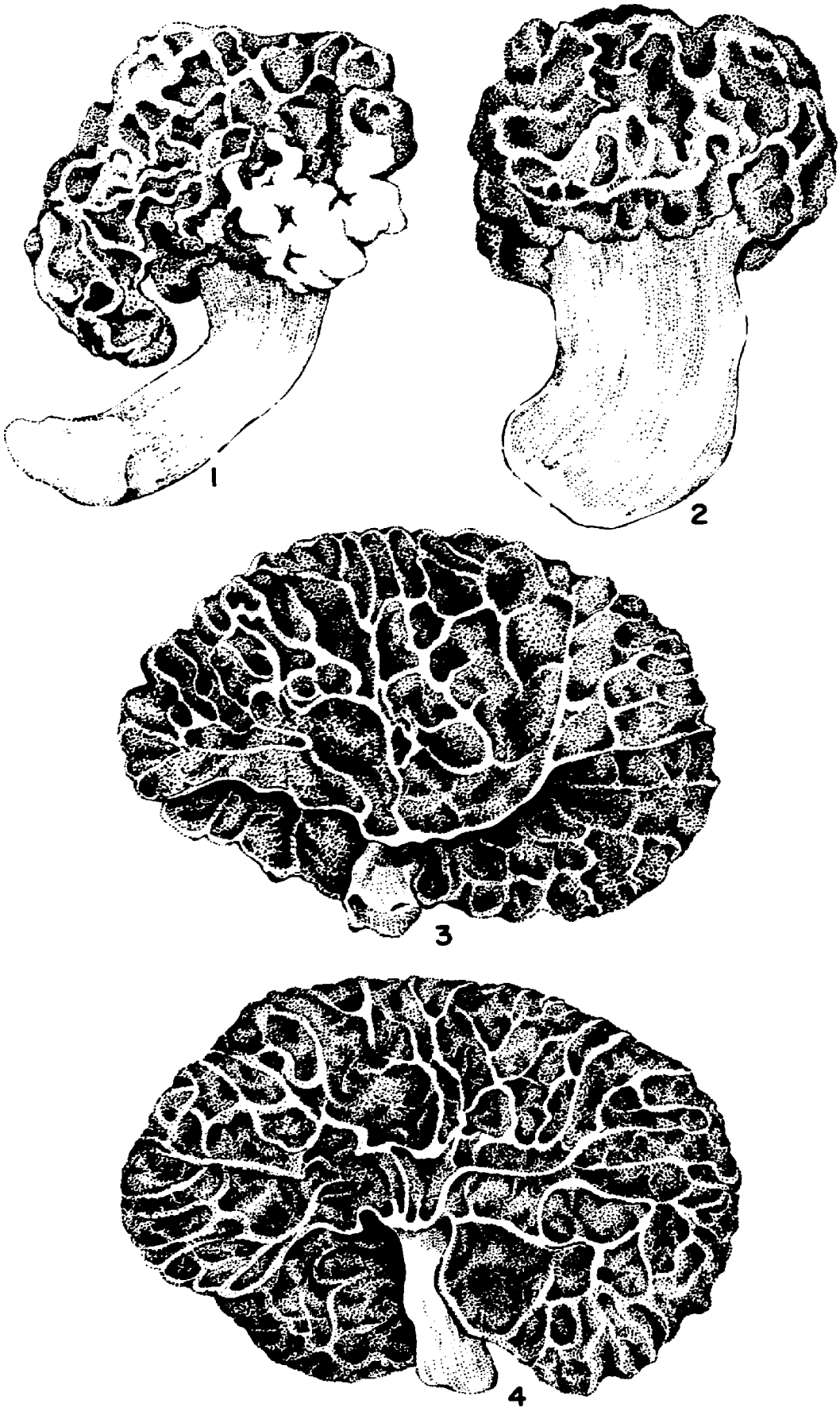

PLATE IV

Anomalous Fructifications

Plate IV



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PLATE V

Single Spore Isolates

Figure

1. Single spore isolate no. 1, "fluffy"
2. Single spore isolate no. V, "granular"
3. Single spore isolate no. 5, "powdery"
4. "Haploid" fructifications produced by single spore isolate no. II

Plate 8

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PLATE VI

Serial Longitudinal Sections of Gasteroid Fructification Showing "Coralloid" Development

PLATE VI

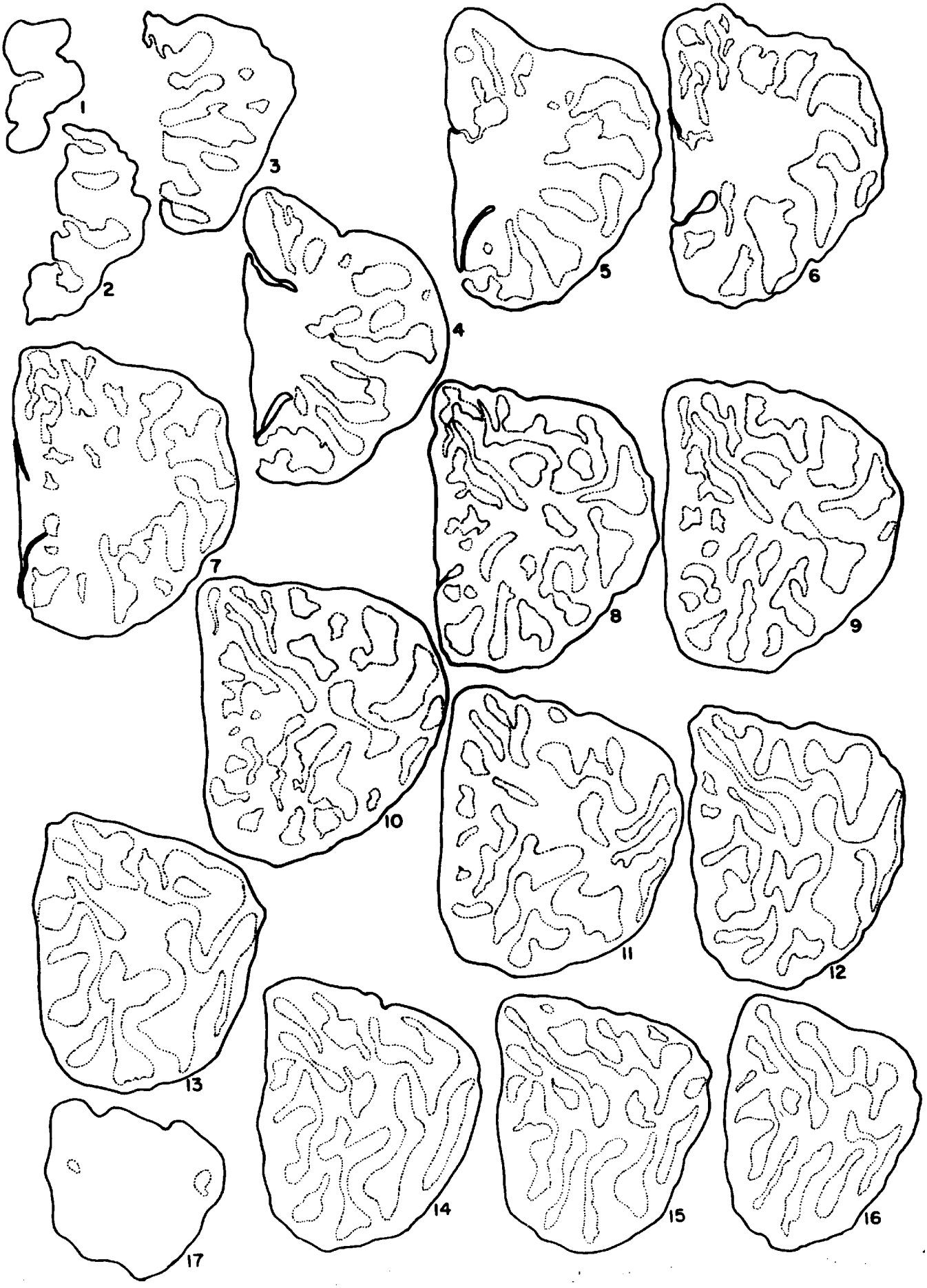

PLATE VII

Serial Longitudinal Sections of Gasteroid Fructification
Showing "Lacunar" Development

PLATE VII



PLATE VIII

Serial Longitudinal Sections of Gasteroid Fructification
Showing "Unipileate" Development

PLATE VIII

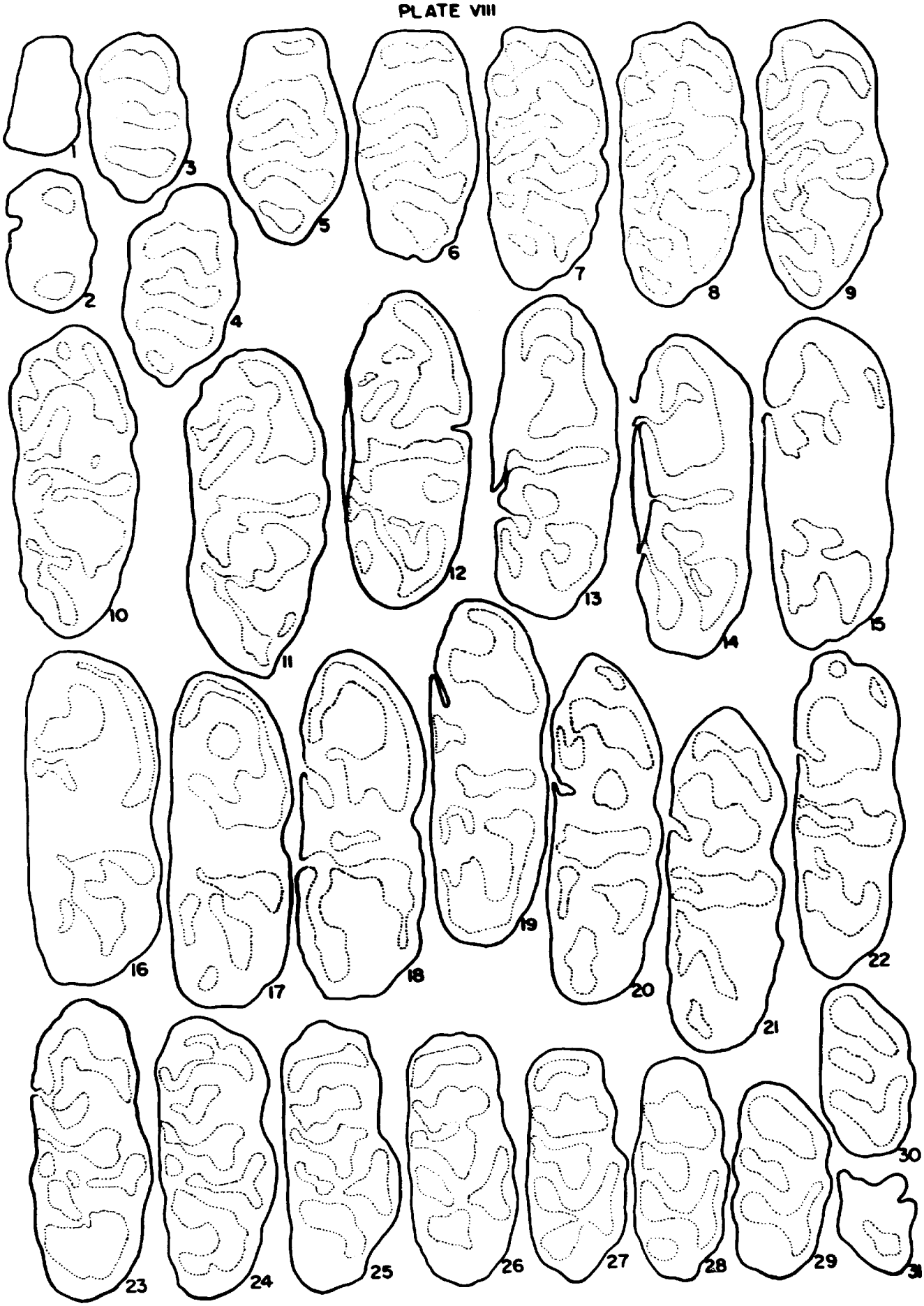
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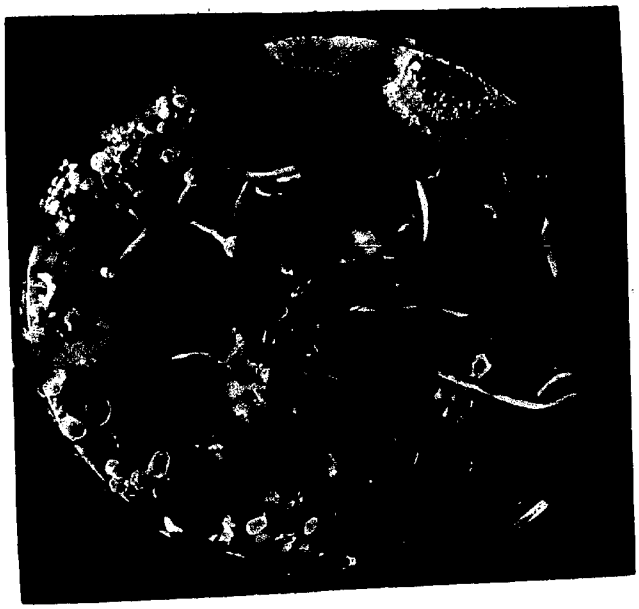
PLATE IX

Fructifications of Psilocybe mutans produced in Response to Different Light Conditions

Figure

1. Culture grown in light
2. Culture grown in dark and alternated between cold room and incubating oven
3. Culture grown in incubating oven

Plate IX



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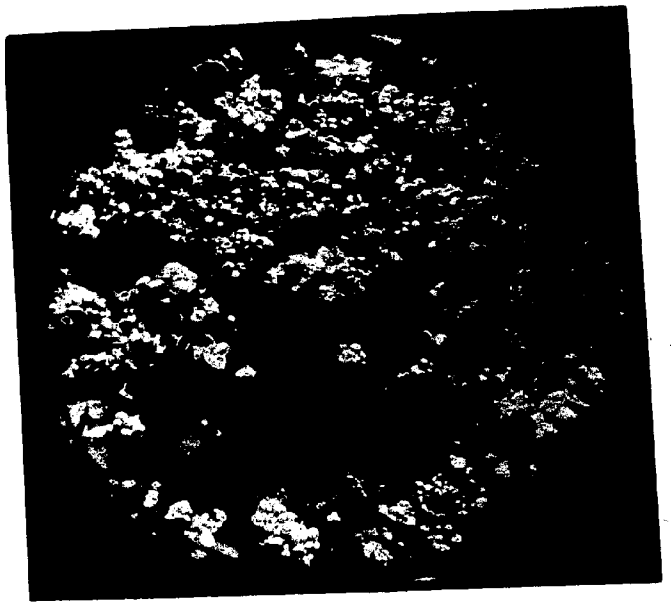

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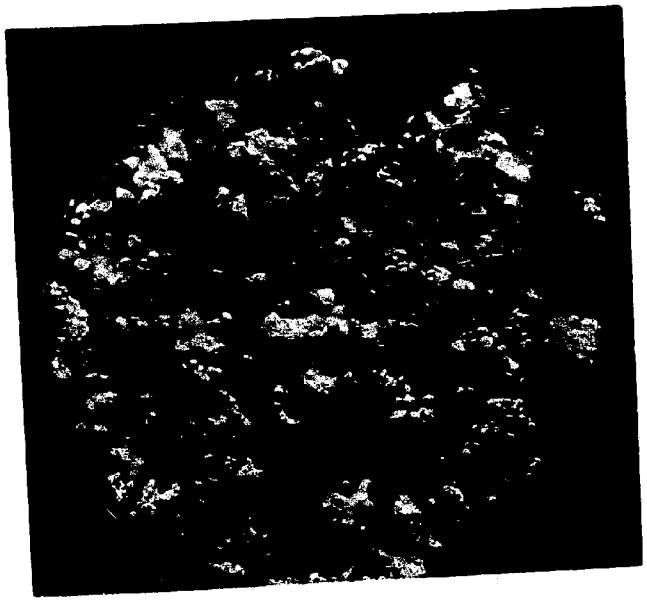

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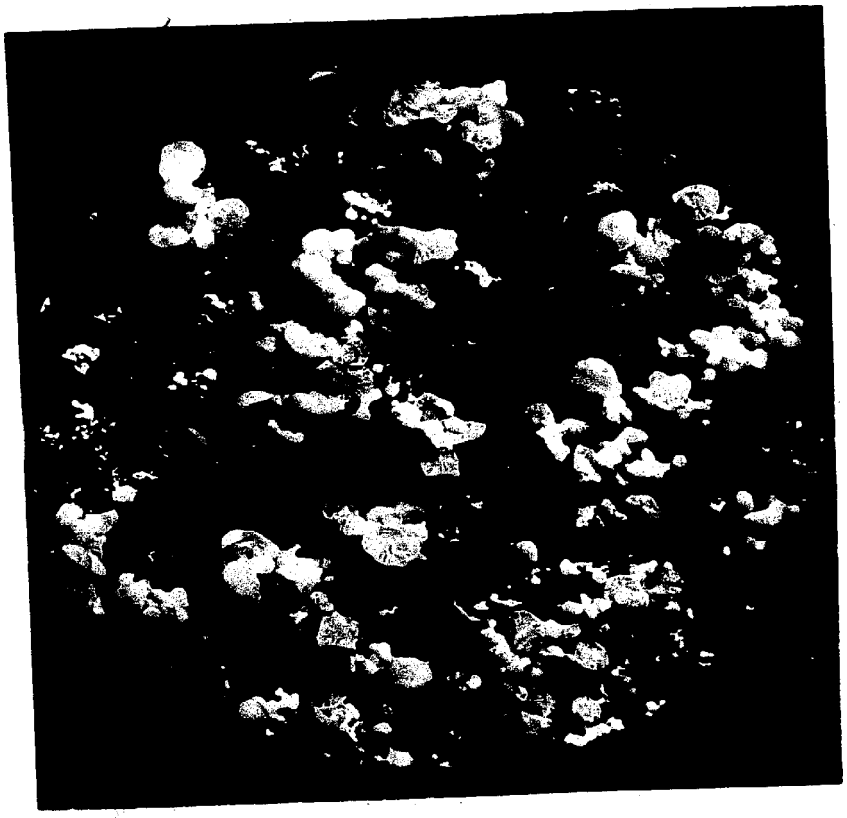
PLATE X

Albino Fructifications



Plate X



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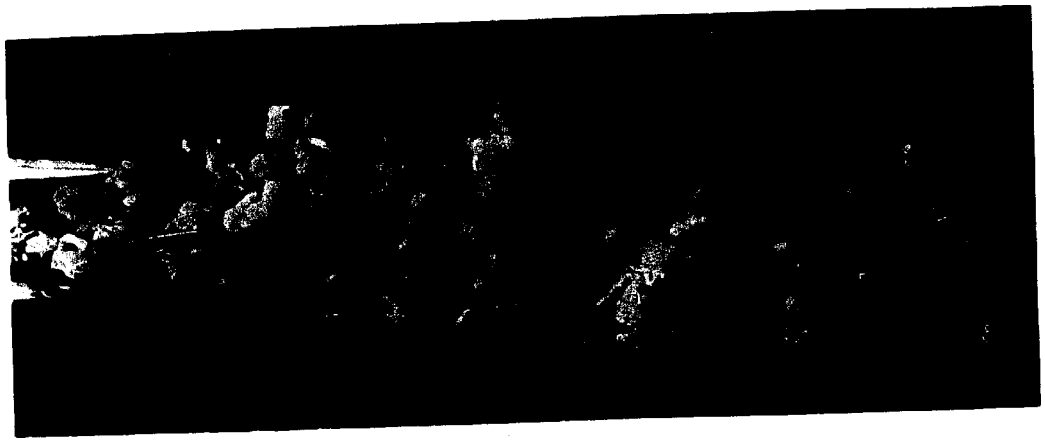

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