Contributions to the Study of the Problem of Sex and Inheritance in Fungi

1. The Problem of Sex in Coprinus Lagopus
2. Sexual Stability in Monosporous Mycelia of Coprinus Lagopus.
3. The Dry-Needle Method of Making Monosporous Cultures of Hymenomycetes and Other Fungi.
4. The Inheritance of Spore Size in Coprinus Sterquilinus.

Being a series of papers submitted as a thesis to the University of Manitoba

BY

William Fielding Hanna
B.A. (Dalhousie); M.Sc. in Agriculture (Alberta)

In partial fulfilment of the requirements for the Degree of Doctor of Philosophy.

WINNIPEG

The Problem of Sex in Coprinus lagopus.

BY

W. F. HANNA, M.Sc. (Alberta).

With fifteen Tables in the Text.

I. Introduction.

THE problem of sex in the Basidiomycetes has occupied the attention of mycologists for more than one hundred and fifty years, and at various times during that period has proved to be a most fruitful subject for speculation and research. The earlier mycologists confined their efforts almost wholly to a search for sexual organs analogous to those present in the Flowering Plants. After much careful study, however, the early theories of sexuality have been abandoned, and the conclusion is now generally accepted that in the Basidiomycetes the last trace of morphological sexual differentiation has disappeared.

With recent advances in cytology, attention has been directed towards the nuclear phenomena which occur during the life-histories of many of the higher fungi. In 1892, Rosen proved that in the aecidiospores and uredospores of *Uromyces pisi* and *Puccinia anserina* there are present two nuclei, while in the mature teleutospores there is present but a single nucleus. He also studied the gill-cells of several species of Autobasidiomycetes, and found that in the young gills of *Armillaria mucida* the nuclei are numerous and disposed in pairs, but that as the gills become more mature, the nuclei increase in size and decrease in number, until finally, each basidium comes to contain but a single nucleus. Rosen's observations were later confirmed and extended by the work of Dangeard, Sappin-Trouffy, Maire, Blackman, and others, with the result that there has been built up a new and promising theory of sexuality in the Basidiomycetes based entirely on nuclear phenomena.

In the Hymenomycetes, that great group of the Basidiomycetes which includes the gilled fungi, it has been shown that, prior to the production of the fruit-body, individual cells of the vegetative mycelium come to possess one or more pairs of nuclei. As the mycelium develops, the pairs of nuclei undergo a series of conjugate divisions, until finally, just before the

[Annals of Botany, Vol. XXXIX. No. CLIV. April, 1925.]

production of the basidium, the number of nuclei in each cell is reduced to a single pair which unite to form the fusion nucleus of the basidium. The fusion of the two nuclei in the basidium has been regarded by Dangeard and others as a reduced sexual act equivalent, nevertheless, to the fertilization which takes place in the higher plants.

The origin of the paired nuclei which first appear in the vegetative mycelium was still to be demonstrated. In 1918 light was thrown on this problem by the researches of Mlle Bensaude, entitled 'Recherches sur le cycle évolutif et la sexualité chez les Basidiomycètes'. Mlle Bensaude (1) showed that *Coprinus fimetarius*, one of the Hymenomycetes, resembles certain Mucorini investigated by Blakeslee in that it is *heterothallic*. Two cultures of *Coprinus fimetarius* were obtained, each of which had originated from a single spore. When sub-cultures from these mycelia were grown separately, they remained for eight months in the *primary* condition, i.e. the mycelium branched irregularly, produced no clamp-connexions, and possessed nuclei disposed singly and not in pairs; furthermore, the mycelia were completely sterile, producing no fruit-bodies whatever. When the two mycelia were brought together and allowed to fuse, they soon developed a *secondary* mycelium characterized by regular branching, the presence of clamp-connexions, the paired condition of the nuclei, and the perfect production of fruit-bodies. Mlle Bensaude (1) was able to show that the paired nuclei of the secondary mycelium divide conjugately, and that each division of the dicaryon is associated with the formation of a septum with a clamp-connexion. Thus, it appeared that the two nuclei which finally fuse in each basidium of *Coprinus fimetarius* are the direct descendants of a single pair of nuclei derived from two different mycelia of opposite sex.

Further research by Kniep, Miss Mounce, and Vandendries has greatly extended the work of Mlle Bensaude. Kniep (5) has shown that *Schizophyllum commune* is a heterothallic species, but that in this species fruit-bodies are sometimes produced from monosporous mycelia. Thus, the phenomenon of heterothallism is not necessarily associated with the sterility of monosporous mycelia. Kniep found, however, that the spores originating from such monosporous fruit-bodies were all of the same sex, and that when sown in polysporous culture the mycelium to which they gave rise never produced clamp-connexions. Therefore, while in a heterothallic species fruit-bodies may develop from monosporous mycelia, such fruit-bodies produce spores which are all of one sex.

Both Kniep (4) and Mlle Bensaude (1) have found that the presence of clamp-connexions is invariably associated with the conjugate division of the nuclei. This fact furnishes a reliable criterion for determining whether a given species of Hymenomycete is homothallic or heterothallic. If clamp-connexions appear regularly on individual mycelia of monosporous origin, the species must be homothallic; while if no clamp-connexions appear on such monosporous mycelia, but only on compound mycelia resulting from the union of two mycelia of opposite sex, the species must be heterothallic.

Miss Mounce (9, 10) has shown that *Coprinus sterquilinus* and *Coprinus stercorarius* are homothallic, and that *Coprinus lagopus* and *Coprinus niveus* are heterothallic. Thus, in a single genus, there exist side by side homothallic and heterothallic species.

The following study was undertaken with the object of further extending our knowledge of the sex of *Coprinus lagopus*. For several reasons this species is particularly well suited to an investigation of this kind. It is a well-known species, and occurs regularly on horse-dung cultures both in North America and in Europe. In the laboratory at Winnipeg, *Coprinus lagopus* appeared on seven out of eight cultures of dung obtained from widely separated points in Canada and England. The culture of dung which failed to produce fruit-bodies of *Coprinus lagopus* had been exposed to the weather for a long time, and produced little fungous growth of any kind. The spores are black, and, therefore, easily seen, and a high percentage of them germinate. Finally, the species requires only from twelve to fourteen days to complete its life-cycle from spore to spore, a circumstance which is of great importance wherever it is necessary to continue the study of sex throughout several successive generations.

II. Methods.

Miss Mounce and Kniep have both studied the sex of *Coprinus stercorarius*, but whereas Miss Mounce (9, 10) found this species to be homothallic, Kniep (5) asserts that it is heterothallic. In view of such contradictory findings, and also as a possible explanation of the varying results which she obtained at different times for *Coprinus lagopus*, Miss Mounce (10) suggested that there may exist both homothallic and heterothallic strains of the same species. In order to investigate this possibility, and, at the same time, to obtain results which will be general and not local in application, fruit-bodies of *Coprinus lagopus* used in the present study have been secured from a number of places widely separated from one another. With the object of securing fruit-bodies, samples of dry horse-dung were obtained from the following places in Canada: (1) Vancouver, British Columbia; (2) Edmonton, Alberta; (3) Shellbrook, Saskatchewan; (4) Winnipeg, Manitoba; and (5) Halifax, Nova Scotia. At a later date, samples were obtained by Professor A. H. R. Buller from three different places at Birmingham, England. Thus, in all, eight different samples of dung were collected at the laboratory in Winnipeg. Each lot of dung

was placed in a crystallizing dish, moistened with tap-water, covered with a closely fitting glass plate, and set upon the laboratory table. In from ten to fourteen days, fruit-bodies of *Coprinus lagopus* made their appearance on all the different lots of dung except the one from Halifax, Nova Scotia. The failure of this culture to produce fruit-bodies of *Coprinus lagopus* was no doubt due to its extremely exhausted condition. The fruit-bodies which came up on the other seven cultures answered to the description of *Coprinus lagopus* given by Lange (8) in his monograph on the genus *Coprinus*, and also resembled in appearance the illustrations of *Coprinus lagopus* drawn by Brefeld (2). If further information regarding this species is required, reference should be made to Buller's 'Researches on Fungi', vol. iii, 1924, where a detailed study of *Coprinus lagopus* will be found, together with numerous photographs and drawings.

Spore-deposits from one or more fruit-bodies of each culture were collected on dry sterilized glass slides, care being taken that spores from only a single fruit-body fell on each slide. The slides were then labelled and kept in a large covered cardboard case so constructed that the slides were well separated from one another. These spore-deposits, originating from different points in England and Canada, provided very suitable material for the study of sex in *Coprinus lagopus*.

All monosporous cultures were made by means of the dry-needle method (3), which has been described in a separate paper. The spores of *Coprinus lagopus* germinate readily in dung-agar, and, while there is some difference in the viability of spores from different fruit-bodies, the average germination throughout the whole of this work was between 80 and 90 per cent. With certain fruit-bodies, it was not uncommon to find that of ten spores isolated and placed in hanging-drops of dung-agar, every one had germinated within twenty-four hours. When the mycelium had grown out into the agar, the drop containing the spore and the mycelium was transferred to a Petri dish into which had been poured a layer of sterile dung-agar about 2 m.m. in thickness. A circular patch of mycelium grew out from the drop of agar and increased rapidly in size until, at the end of four days, it was generally large enough to be used for pairing with other mycelia. If the monosporous mycelia obtained in this way were required to be kept for some time, transfers were made from the Petri dishes to glass tubes 3 inches long and $\frac{9}{10}$ of an inch in diameter, which had previously been about half filled with fresh horse-dung, plugged with cotton-wool, and sterilized in an autoclave for one hour at fifteen pounds pressure.

When the pairings were to be made, a small piece of the dung-agar about 1 c.m. square, bearing some of the monosporous mycelium, was transferred by means of a sterile platinum loop to a Petri dish containing a layer of sterile dung-agar. Another piece of agar was similarly transferred from

a second monosporous mycelium and placed beside the first. At the end of six days, the circular patch of mycelium which had grown outward from the pieces of agar was examined for clamp-connexions. A brief macroscopic examination of the mycelium as a whole was generally sufficient to determine whether or not a secondary mycelium bearing clamp-connexions had developed, but in all cases a final microscopic examination of the cultures was made. While most of the pairings of monosporous mycelia were made in the manner just described, some of those recorded in Table VII were made in tubes of sterile horse-dung. Since the mycelia used in these pairings had been previously transferred to tubes of sterile horse-dung, the operation of pairing was easily effected by removing from the culture tubes small pieces of the dung containing mycelium and placing them together in a third tube of sterile horse-dung. At the end of six days some of the compound mycelium was taken from the tube, mounted in water on a glass slide, and examined under a microscope for clamp-connexions. As the mycelium of *Coprinus lagopus* grows equally well on either dung-agar or sterile dung, the results obtained by the two methods of pairing are perfectly comparable, but the ease with which pairings can be made in Petri dishes gives the former method a distinct advantage.

The dung-agar used for spore germination and for the growing of different mycelia which were about to be paired was made in the following manner: A quantity of fresh horse-dung was placed in a large enamel dish and tap-water was added at the rate of 1,000 c.c. to 300 grm. of dung. The mixture was well stirred, and the resulting liquid was decanted off and strained through cheesecloth to remove the larger particles which it contained. This liquid was then boiled and filtered through cotton-wool. To the decoction thus obtained, agar at the rate of 1·5 per cent. was added and the mixture was boiled until the agar had melted. After filtering again through cotton-wool, the mixture was poured into a series of test-tubes, which were subsequently plugged with cotton-wool and sterilized in the autoclave for one hour at fifteen pounds pressure. On a few occasions, the dung-agar was neutralized with ammonium hydroxide, but this procedure proved to be unnecessary as the mycelium of *Coprinus lagopus* seemed to grow equally well on dung-agar which had not been neutralized.

III. Experimental.

(1) *All possible Pairings of Monosporous Mycelia from Individual Wild Fruit-bodies.*

By pairing together a number of monosporous mycelia of *Coprinus lagopus*, Miss Mounce (10) found that while some unions gave rise to clamp-connexions, others produced no clamp-connexions whatever, but, unlike certain of the Mucorini investigated by Blakeslee, there appeared to be no strict segregation into (+) and (−) strains. In the present study, mono-

sporous mycelia have been obtained from the spores of several wild fruit-bodies which came up on the dung cultures collected from different places.

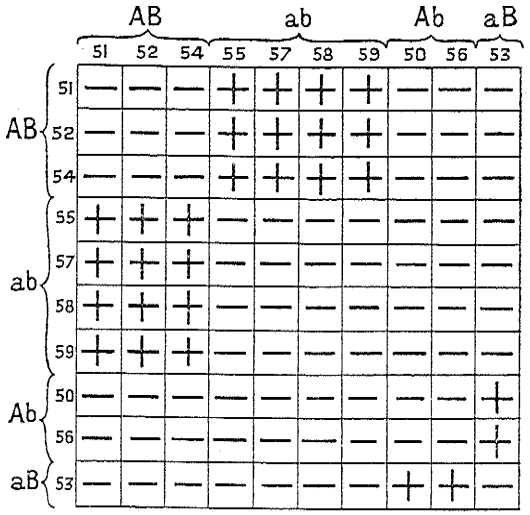


TABLE I. *Coprinus lagopus.* All possible pairings of ten monosporous mycelia from fruit-body No. 1 (Vancouver).

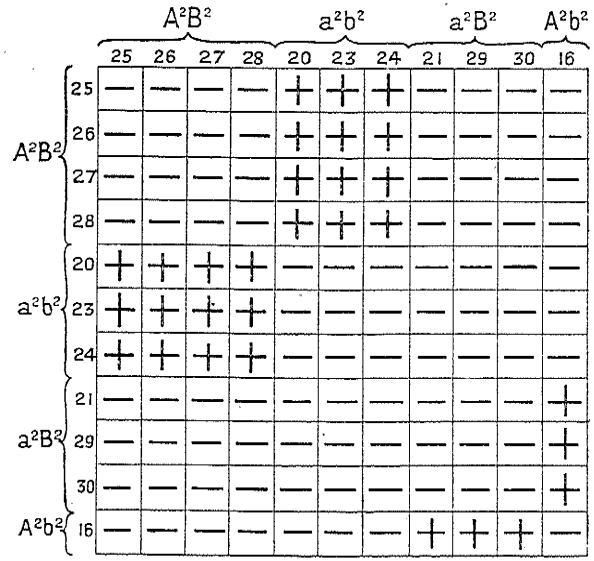


TABLE II. *Coprinus lagopus.* All possible pairings of eleven monosporous mycelia from fruit-body No. 2 (Edmonton).

The results of pairing together monosporous mycelia derived from individual fruit-bodies are given in Tables I–VI. Fruit-body No. 1 (Table I) came from Vancouver; fruit-body No. 2 (Table II) from Edmonton; fruit-body

No. 3 (Table III) from Shellbrook; and fruit-bodies Nos. 4, 5, and 6 (Tables IV, V, and VI) from Winnipeg. Fruit-bodies Nos. 5 and 6 were

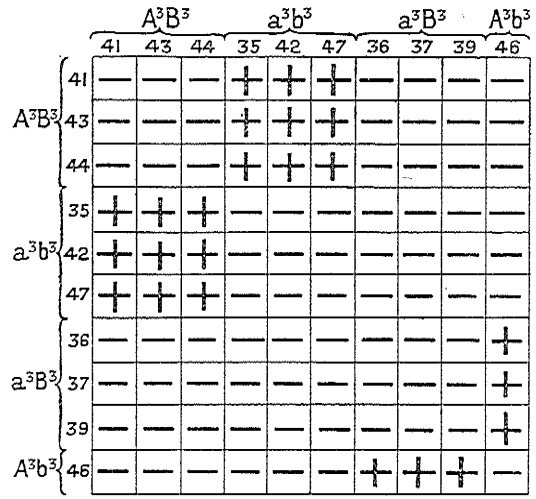


TABLE III. *Coprinus lagopus.* All possible pairings of ten monosporous mycelia from fruit-body No. 3 (Shellbrook).

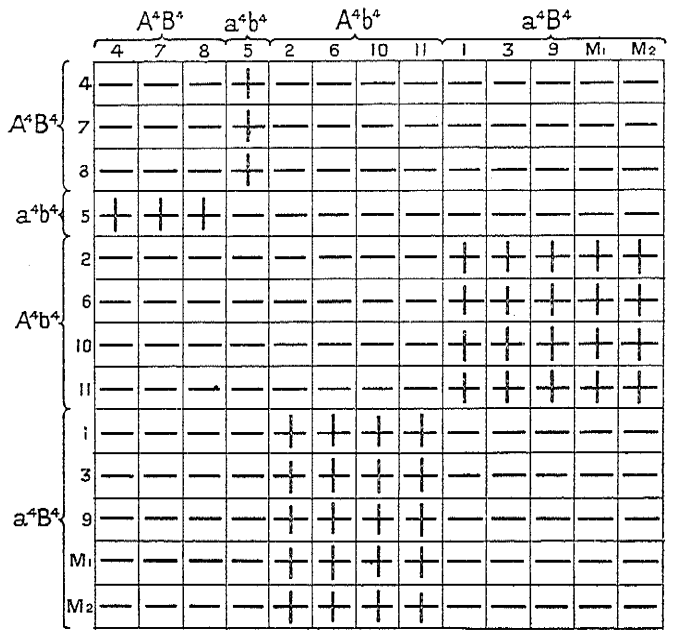


TABLE IV. *Coprinus lagopus.* All possible pairings of eleven monosporous mycelia from fruit-body No. 4 (Winnipeg), and two polysporous mycelia, *M* 1 and *M* 2, from a monosporous fruit-body of mycelium No. 1.

collected at the same time and from the same dish of dung, while fruit-body No. 4 was obtained from a second dish of dung brought from the same stable. The sign (+) in all of the tables indicates that clamp-connexions appeared after the union of the two mycelia ; the sign (−) indicates that no clamp-connexions appeared after the union.

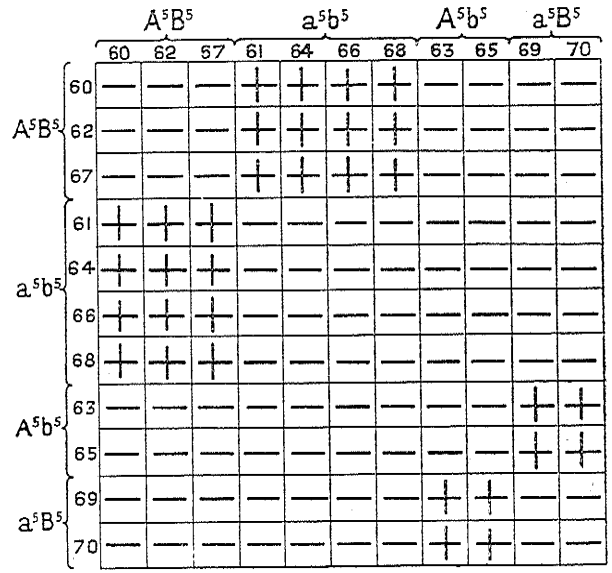


TABLE V. *Coprinus lagopus.* All possible pairings of eleven monosporous mycelia from fruit-body No. 5 (Winnipeg).

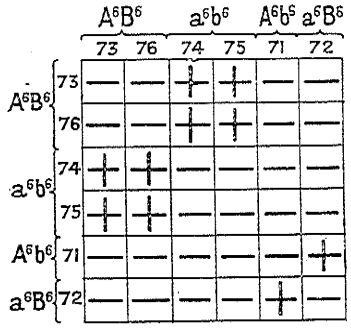


TABLE VI. *Coprinus lagopus.* All possible pairings of six monosporous mycelia from fruit-body No. 6 (Winnipeg).

In each of the above tables, it will be seen that the mycelia fall into our distinct groups. The tables have been rearranged by collecting together the mycelia belonging to each group ; for example, in Table V, mycelia 60, 62, and 67 belong to a first group, 61, 64, 66, and 68 to a second, 63 and 65 to a third, and 69 and 70 to a fourth. Furthermore, it will be seen that the mycelia of the first group react with those of the second, and those of the third react with those of the fourth, but the mycelia of the first and second groups fail to react with those of the third and fourth groups. These results show that the spores of a single fruit-body of *Coprinus lagopus*, while alike morphologically, may be divided sexually into four distinct groups regardless of where the fruit-body producing the spores is obtained. Miss Mounce's suggestion that there may exist both homothallic and heterothallic strains of *Coprinus lagopus* has not been supported by this study, as all strains of *Coprinus lagopus* investigated have proved to be heterothallic.

Kniep (7) obtained tables for *Schizophyllum commune* similar to those above, and explained his results on the theory that sex is determined in this species by two pairs of Mendelian factors (*A a*) and (*B b*). The same theory will explain the sexual reactions of *Coprinus lagopus*. We may suppose that in this fungus, when two haploid mycelia of opposite sex unite and form a diploid mycelium, of each pair of nuclei present in each cell, one has been derived from one haploid mycelium and the other from the other haploid mycelium, i. e. they are of opposite sex. Finally, as a result of conjugate nuclear divisions of the first-formed nuclear pairs, each basidium comes to contain a pair of nuclei of opposite sex. In each basidium the nuclei fuse together, and then the fusion nucleus divides twice with chromosome reduction, with the result that the four nuclei produced are haploid, each of them containing one of the factors (*A a*) and one of the factors (*B b*). The four nuclei pass into the four spores so that, in the end, the spores come to be of the same sex as the nuclei which enter them. From the sexual point of view, therefore, it is possible to have from a single fruit-body but four kinds of spores: (*A B*), (*a b*), (*A b*), and (*a B*).

The mycelia arising from these spores will fall, therefore, into four groups. Those with the genetic constitution (*A B*) will unite with those designated as (*a b*), but with no others, as a union with (*A b*) or (*a B*) would bring together two like factors ; for a similar reason (*A b*) will unite only with (*a B*).

In accordance with the above theory, it has been possible to assign sex factors to each of the groups in Tables I to VI. An examination of these tables will show that, wherever two mycelia possess a common factor, no union has taken place, but where no common factor is present, a union of the two mycelia has occurred resulting in the formation of clamp-connexions.

(2) *The Pairing of Monosporous Mycelia from Different Wild Fruit-bodies.*

Since each of the six fruit-bodies studied was found to possess spores giving rise to four different kinds of mycelia, the following question may now be raised: Are the sexual groups in all of these fruit-bodies identical, or are they different? For example, are mycelia 25, 26, 27, and 28 of Table II the same sexually as mycelia 4, 7, and 8 of Table IV? In order to answer this question, eleven mycelia of fruit-body No. 2 (Table II) were

paired with eleven mycelia of fruit-body No. 4 (Table IV). The results of the pairings, given in Table VII, show that there is *complete fertility* between monosporous mycelia derived from these two wild fruit-bodies. In order to verify this result, a large number of pairings were made between monosporous mycelia of different fruit-bodies. Space does not permit of all these results being presented in tabular form, but a list of the pairings made is given below:

100	pairings	between	fruit-body	No. 1	and	fruit-body	No. 3
100	,,	,,	,,	No. 1	,,	,,	No. 2
100	,,	,,	,,	No. 1	,,	,,	No. 4
100	,,	,,	,,	No. 4	,,	,,	No. 3
100	,,	,,	,,	No. 2	,,	,,	No. 3
36	,,	,,	,,	No. 5	,,	,,	No. 6
12	,,	,,	,,	No. 5	,,	,,	No. 4
12	,,	,,	,,	No. 6	,,	,,	No. 4
8	,,	,,	,,	No. 5	,,	,,	No. 1
8	,,	,,	,,	No. 6	,,	,,	No. 1
14	,,	,,	,,	No. 5	,,	,,	No. 2
14	,,	,,	,,	No. 6	,,	,,	No. 2
10	,,	,,	,,	No. 5	,,	,,	No. 3
10	,,	,,	,,	No. 6	,,	,,	No. 3
8	,,	,,	a Birmingham	fruit-body	and	fruit-body	No. 1
14	,,	,,	,,	,,	,,	,,	No. 2
10	,,	,,	,,	,,	,,	,,	No. 3
14	,,	,,	,,	,,	,,	,,	No. 4
12	,,	,,	,,	,,	,,	,,	No. 5
12	,,	,,	,,	,,	,,	,,	No. 6

In all of the 694 pairings clamp-connexions appeared regularly, thus showing that the sexual groups in all of these fruit-bodies must be different, and that complete fertility results when monosporous mycelia from different wild fruit-bodies are paired together. In the six fruit-bodies studied in Tables I to VI, there have been established therefore, not four, but twenty-four distinct sexual groups. The complete fertility between fruit-bodies 5 and 6 is especially remarkable, since both were found growing side by side in the same dung culture. While it is possible that the spores giving rise to these two fruit-bodies came from widely different districts, it is nevertheless interesting to know that two and possibly many sexual strains of *Coprinus lagopus* exist side by side within a very small area.

In the list of pairings given above, it is also noteworthy that monosporous mycelia from a fruit-body of *Coprinus lagopus* originating in Birmingham, England, have reacted with monosporous mycelia from fruit-bodies of the same species collected at different places in Canada. The fact

that hyphal fusions took place between the English and Canadian strains, resulting in the formation of clamp-connexions, furnishes a conclusive proof that the *Coprinus lagopus* found in England is identical with the species occurring in Canada. In the appearance of the fruit-bodies and spores, as well as in the habit of growth and general appearance of the mycelium, the English strain of *Coprinus lagopus* was in no respect different from the Canadian species. Five monosporous mycelia of the English strain were kept in culture on dung-agar and in tubes of sterile dung for nearly a month, and while they produced an abundance of the characteristic oidia and gave

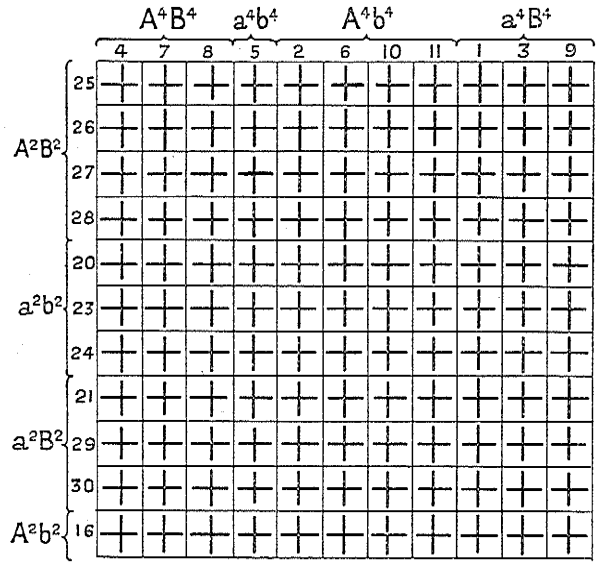


TABLE VII. *Coprinus lagopus*. The pairing of eleven monosporous mycelia from fruit-body No. 2 (Edmonton) with eleven monosporous mycelia from fruit-body No. 4 (Winnipeg).

rise to numerous imperfect fruit-bodies, no clamp-connexions ever appeared. We may, therefore, conclude that the strain of *Coprinus lagopus* found in England is similar to the Canadian species in that it is heterothallic.

Kniep (7) paired monosporous mycelia from different wild fruit-bodies of *Schizophyllum commune* collected at some distance from one another, and found complete fertility between them. Vandendries (12) obtained a similar result with two wild fruit-bodies of *Panaeolus campanulatus*. Further investigations may show that these sexual strains are to be found generally throughout the heterothallic Basidiomycetes, and that each species is made up of a definite number of such strains. On the contrary, the sex-factors for a given species may be undergoing frequent mutations with the result that new sexual strains are continually appearing. Kniep (7) holds the latter view, and has found a considerable amount of evidence to show that

the sex-factors in *Schizophyllum commune* undergo frequent mutations. Thus, for example, a fruit-body possessing the sex-factors A, B, a, and b may give rise to some spores having the mutant factors A', B', a', and b', and in this way a new sexual strain would arise with spores belonging to four new groups which would show complete fertility with the old strain possessing the factors A, B, a, and b.

(3) *The Pairing of Monosporous Mycelia from two Fruit-bodies arising from the same Compound Mycelium.*

(a) *Where the two spores giving rise to the compound mycelium were obtained from the same fruit-body.*

In the preceding experiments, it was found that whenever any two monosporous mycelia from different wild fruit-bodies were brought together, clamp-connexions always appeared. In view of this finding, it becomes of interest to determine whether monosporous mycelia from any two fruit-bodies whatsoever will react in this way.

The simplest case to consider is where the two fruit-bodies have arisen from a compound mycelium produced by the union of two monosporous mycelia of opposite sex which have been obtained from the same fruit-body. If, during the development of the fruit-body and the formation of spores, the various nuclear divisions take place in an orderly manner, it might be expected that, while any number of fruit-bodies might arise from a bisporous mycelium, only four kinds of spores would be formed. If, on the contrary, the nuclear divisions are accompanied by numerous mutations, different kinds of fruit-bodies might arise from the same bisporous mycelium, with the result that the spores from any two fruit-bodies might not fall into the same sexual groups. Separate spore-deposits were, therefore, obtained from two fruit-bodies, A and B, which had arisen on a compound mycelium produced by the union of mycelia 54 and 58 of Table I.

Four monosporous mycelia were isolated from fruit-body A, and ten from fruit-body B. The fourteen mycelia were then paired together in all possible ways as shown in Table VIII. In each fruit-body the spores have proved to be of four kinds, but on a closer examination of the table it will be seen that the four groups of fruit-body A are identical with those of fruit-body B. In other words, the fourteen mycelia have reacted together in every respect as though they had been isolated from a single fruit-body.

(b) *Where the two spores giving rise to the compound mycelium were obtained from different wild fruit-bodies.*

Mycelium 46 of Table III and mycelium 6 of Table IV were paired together, and from this compound mycelium two fruit-bodies, C and D, were produced. Twenty-six monosporous mycelia were then isolated, thirteen

from fruit-body C and thirteen from fruit-body D, and all possible pairings were made between them.

It has already been shown that mycelia 46 and 6 belong to different sexual strains which proved to be completely fertile when paired together; they must, therefore, have no sex-factor in common. Since six groups are possible when four different things are combined two at a time, it might be expected that the spores from fruit-bodies C and D would fall into not four, but six groups.

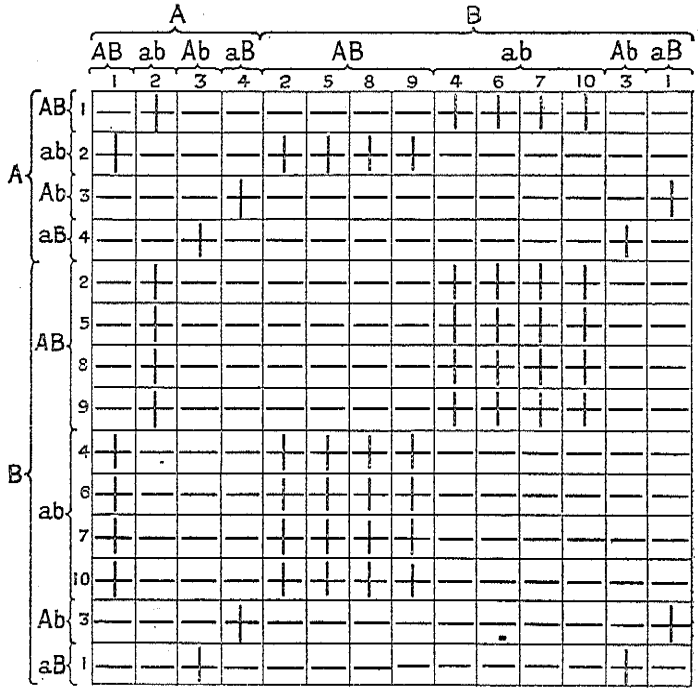


TABLE VIII. *Coprinus lagopus.* All possible pairings of fourteen monosporous mycelia from fruit-bodies A and B of mycelium 54 × 58.

From the results of the pairings given in Table IX, it will be seen that, notwithstanding the hybrid nature of fruit-bodies C and D, only four sexual groups have been found, and that the same groups are present in both fruit-bodies. The two sexual hybrids C and D are, therefore, similar to the two fruit-bodies A and B of the last experiment, and bear the same relationship to each other.

If we assume that the nuclei of mycelium 46 carried the sex-factors ($A^3 b^3$), and those of mycelium 6 the sex-factors ($A^4 b^4$), it should follow that the fusion nuclei of the basidia of fruit-bodies C and D would have the genetic constitution ($A^3 b^3 A^4 b^4$). But, since these fruit-bodies gave rise to only four kinds of spores, we must conclude that, when the reduction

division occurred just before spore formation, no union took place between factors A^3 and A^4 and between factors b^3 and b^4. In other words, although A^3 and A^4 and b^3 and b^4 were sufficiently unlike to permit of the nuclei containing them becoming associated and dividing conjugately with the formation of clamp-connexions, they nevertheless retained a certain likeness

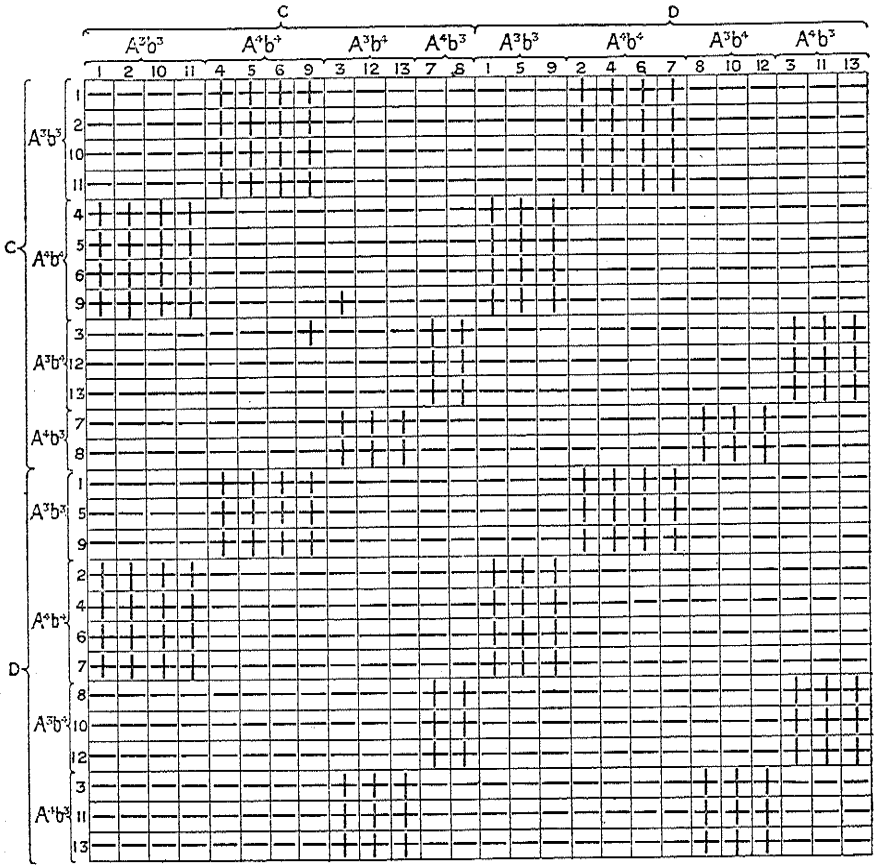


TABLE IX. *Coprinus lagopus.* All possible pairings of twenty-six monosporous mycelia from fruit-bodies *C* and *D* of mycelium 46 × 6.

which exerted a repulsive influence and prevented any unions taking place between them during the segregation of sex-factors.

As a result of the experiments just recorded, we may draw the conclusion that when any two monosporous mycelia of *Coprinus lagopus* are brought together so as to form a secondary compound mycelium, fruit-bodies arising from this mycelium will possess spores belonging to but four sexual groups, regardless of the number of fruit-bodies produced. In Table IX an exception must be noted in the case of the union between

mycelia 9 and 3 of fruit-body *C*. These two mycelia reacted very feebly and produced clamp-connexions only on a single hypha, although the culture grew vigorously and was kept under observation for nine days. This deviation from the normal may be regarded as a mutation, similar to those which Kniep (7) found to occur in *Schizophyllum commune*.

(4) *Sexual Relationship between Parent Mycelia and Spores of a First-generation Fruit-body.*

If, during the development of the fruit-body and the formation of spores, the sex-factors segregate out in a regular manner, it might be

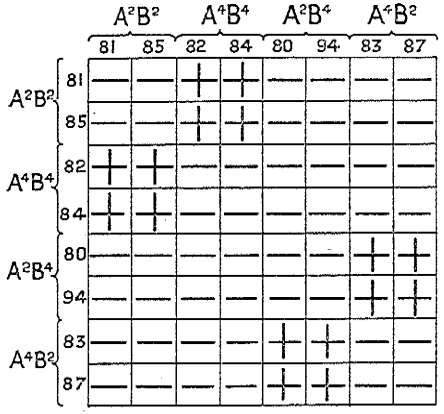


TABLE X. *Coprinus lagopus.* All possible pairings of eight monosporous mycelia from fruit-body *E* of mycelium 25 × 7.

expected that monosporous mycelia from first-generation fruit-bodies would react with the parent mycelia strictly in accordance with the theory of dihybridism. On the contrary, if mutations take place frequently, any abnormalities should appear when the monosporous mycelia from first-generation fruit-bodies are crossed with their parents.

By pairing mycelium 7 of fruit-body 4 (Table IV) with mycelium 25 of fruit-body 2 (Table II), a hybrid fruit-body *E* was obtained. From the spores of this fruit-body eight monosporous mycelia were isolated and all possible pairings were made between them. The results of the pairings are given in Table X. The eight mycelia of this table fall into four groups, reacting with one another in the same manner as did those of fruit-bodies *C* and *D* of Table IX.

Pairings were later made between the eight monosporous mycelia of fruit-body *E* and the two parent mycelia 7 and 25, as well as with other monosporous mycelia representing all the sexual groups in fruit-bodies

4 and 2. In this way, it was possible to make a fairly complete analysis of the sexual constitution of the hybrid fruit-body *E*. The results of the pairings are given in Table XI. Mycelia 81 and 85 have reacted with all of the mycelia from fruit-body 4, but with only mycelia 20 and 23 of fruit-body 2 ; they must, therefore, have the genetic constitution $A^2 B^2$, and are identical with parent mycelium 25. By comparing the reactions of mycelia 82 and 84 it will be seen that they must have the genetic constitution $A^4 B^4$, and are identical with parent mycelium 7. Mycelia 80 and 94 and 83 and 87 will be recognized as sex hybrids, having obtained one-half of their sex-factors from each parent. From the pairings with mycelia 47 and 58 of

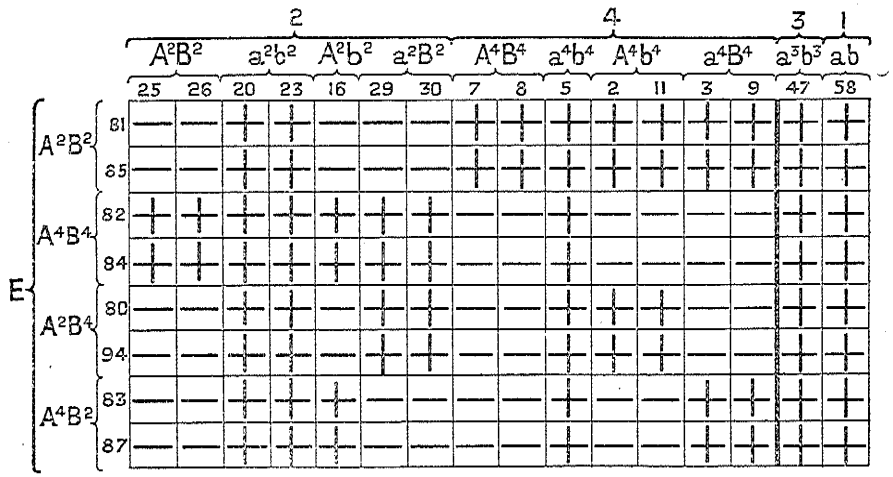


TABLE XI. *Coprinus lagopus*. The pairing of eight monosporous mycelia from a first-generation fruit-body *E* of mycelium 25 × 7 with fourteen monosporous mycelia of the parent fruit-bodies No. 2 and No. 4, and one monosporous mycelium from each of fruit-bodies No. 3 and No. 1.

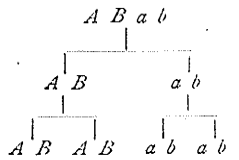
Tables III and I respectively, it is clear that the eight mycelia of fruit-body *E* have reacted like their parents with other sexual strains. During the development of the hybrid fruit-body, the segregation of the sex-factors must have taken place according to the theory of dihybridism, and no evidence has been obtained to show that mutations of sex-factors occurred. We may, therefore, conclude that when two monosporous mycelia of *Coprinus lagopus*, belonging to different sexual strains, unite to form a compound mycelium, a fruit-body arising from that mycelium will produce spores of four sexual groups ; 25 per cent. of the spores belong to group 1, and are of the same sex as one of the parents ; 25 per cent. belong to group 2, and are of the same sex as the other parent ; the remaining 50 per cent. belong to groups 3 and 4, and are hybrids deriving one sex-factor from each parent.

(5) *The Reduction Division and the Segregation of Sex-factors in Coprinus lagopus.*

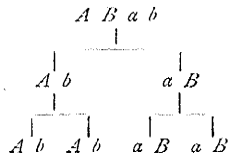
On account of their small size, Kniep (6) was unable to isolate the four spores from individual basidia of fruit-bodies of *Schizophyllum commune*, but using *Aleurodiscus polygonius* he succeeded in obtaining the spores from thirty-five different basidia. When the four spores from each basidium were paired together, he found that in every case the spores from individual basidia were of two kinds only ; i. e. if one pair had the factors (*A B*), the other pair had the factors (*a b*) ; while if one pair had the factors (*A b*), the other pair had the factors (*a B*). From this result, Kniep concluded that the reduction of sex-factors must have taken place with the *first* division of the fusion nucleus.

If, in *Aleurodiscus polygonius*, the reduction of sex-factors takes place with the first division of the fusion nucleus, no other result could have been obtained by Kniep. For example, if the fusion nucleus possesses the factors (*A B a b*), reduction and spore-formation will take place according to one of the following schemes:

I



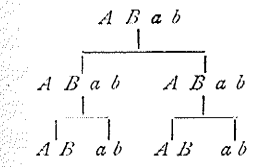
II



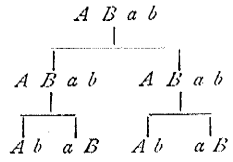
In either case each basidium would come to bear spores in pairs, of two kinds only.

A result similar to that recorded by Kniep might well have been obtained with reduction taking place with the *second* division of the fusion nucleus. Let us suppose, for example, that the fusion nucleus possessed the factors (*A B a b*) ; then reduction and spore-formation will take place in one or more of the following ways:

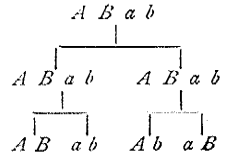
III



IV



V



If the nuclear divisions were to take place as in case III or IV, each basidium would come to bear spores of two kinds only, and the pairing of spores from individual basidia would give results similar to those of

cases I and II, where reduction of the sex-factors takes place in the first division of the fusion nucleus. If, however, the divisions were to take place as indicated in case V, each basidium would come to bear four different kinds of spores. Therefore, although Kniep concludes that in *Aleurodiscus polygonius* reduction of the sex-factors takes place with the first division of the fusion nucleus, he might also have obtained the same result had reduction occurred in the second division. However, since Kniep made a study of thirty-five different basidia, all of which proved to be of the same type, the theory of probability justifies his conclusion that in *Aleurodiscus polygonius* the reduction of sex-factors takes place with the first division of the fusion nucleus.

By adopting the following new method (13) it was possible to secure the four spores from a number of basidia of fruit-bodies of *Coprinus lagopus*. From a young fruit-body which was just beginning to expand its pileus, a gill was removed and placed flat upon a glass slide. A cover-glass held by means of a small pair of forceps was then lowered gently until it touched as lightly as possible the upper surface of the gill; it was then raised quickly. By examining the surface of the cover-glass under the microscope, it was found that in some places the tetrad from a single basidium had adhered to the cover-glass in the form of a minute square. The cover-glass was later fixed to a clean glass-slide by means of a little vaseline or Canada balsam, and the spores from each basidium were removed one at a time by means of the dry-needle method (3) and placed in hanging drops of dung-agar for germination. At the end of twenty-four hours, all viable spores had generally germinated, although spores which were not fully ripe when the gill was removed often germinated after two days. Considerable judgement must be used in selecting the gill at the proper stage of maturity; if the gill is taken when too immature the spores will not germinate, while if left on the fruit-body until deliquescence has set in the securing of well-separated tetrads will be rendered extremely difficult. When germination of all four spores from any one basidium was found to have taken place, and the mycelia had developed sufficiently, transfers were made to plates of sterile dung-agar and, later on, the mycelia were paired together in the manner already described.

Using the above method, tetrads were obtained from eleven basidia, and, in addition, three spores were obtained from each of two other basidia. Six of the tetrads were taken from a fruit-body which had arisen from the union of mycelia 54 and 58 of Table I; the remaining five tetrads, as well as the two groups of three spores, were taken from a fruit-body which had arisen from the union of mycelium 39 of Table III and mycelium 57 of Table I. Thus, the first fruit-body was the product of one sexual strain, while the second was the product of two sexual strains. The results of the pairings brought out the fact that in both fruit-bodies two kinds of

basidia were present; the first type, shown in Table XII, is similar to that shown by Kniep for *Aleurodiscus polygonius*, having only two kinds of spores; the second type, shown in Table XIII, has *spores of four kinds*. Of the 13 basidia studied, 7 were of the first type, and 6 were of the second type. Since a reduction in the second division of the fusion nucleus would result in both types of basidia being present, we are justified in concluding that in *Coprinus lagopus* reduction of the sex-factors takes place in the *second* division of the fusion nucleus.

In the preceding study of the basidia of *Coprinus lagopus* pairings were made only between the four spores of each individual basidium. In order

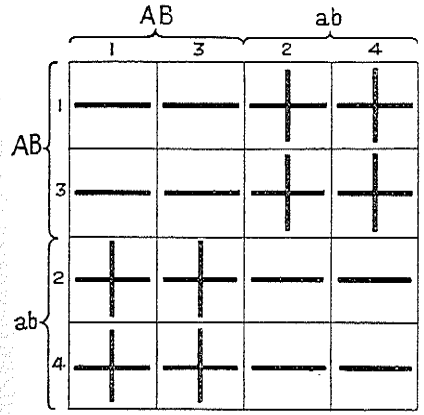


TABLE XII. *Coprinus lagopus*. All possible pairings of four monosporous mycelia from a basidium of fruit-body 54 × 58; spores of two kinds.

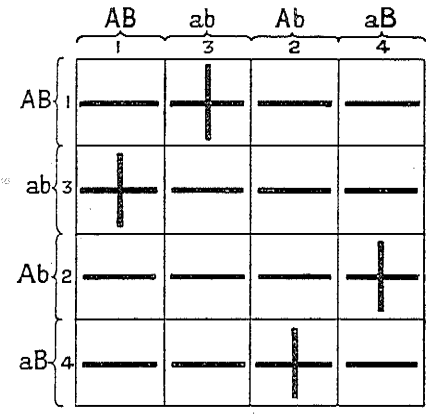


TABLE XIII. *Coprinus lagopus*. All possible pairings of four monosporous mycelia from a basidium of fruit-body 54 × 58; spores of four kinds.

to determine the reaction which the spores from any one basidium will give when paired with the spores from a number of other basidia, all possible pairings were made between the spores from five basidia of fruit-body 39 × 57; the results are embodied in Table XIV. In the whole table only four sexual groups are represented. Basidia 2, 3, 5, and 7 have proved to be alike, each one having spores of four kinds; in fact, any two of these basidia might be interchanged without altering in any way the appearance of the table. Basidium 6 has spores belonging to only two of the sexual groups, but both of these groups are already represented by two of the spores from each of the other four basidia. By obtaining the four spores from a single basidium of the type shown in Table XIII it is, therefore, possible to obtain one representative of each of the four sexual groups found for *Coprinus lagopus*.

On theoretical grounds it might be expected that of the basidia bearing two kinds of spores in pairs some would be of the type (*A B*) (*A B*) (*a b*) (*a b*), while others would be of the type (*A b*) (*A b*) (*a B*) (*a B*). A reference to the diagrams for cases III and IV (p. 447) will make this

point clear. Little difficulty should be experienced in determining whether or not the two types of basidia actually occur in nature. If pairings were made between monosporous mycelia from a number of basidia bearing spores of two kinds in pairs, the presence of two types of basidia would readily become apparent, since monosporous mycelia from a basidium of

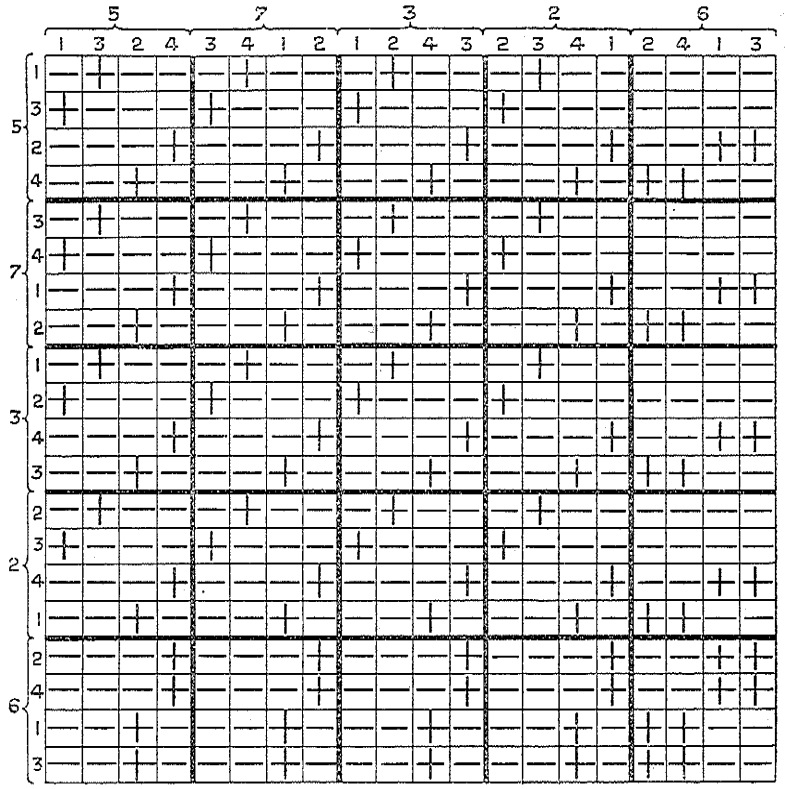


TABLE XIV. *Coprinus lagopus.* All possible pairings of twenty monosporous mycelia from five basidia of fruit-body 39 × 57.

type (*A B*) (*A B*) (*a b*) (*a b*) would fail to react with those from a basidium of type (*A b*) (*A b*) (*a B*) (*a B*).

Kniep (6), in his study of the basidia of *Aleurodiscus polygonius*, observed that frequently two of the spores from a basidium germinated and developed mycelia more rapidly than the remaining pair. When pairings of the monosporous mycelia were later made, the two spores which had germinated first were found to belong to the same sexual group, e. g. (*A B*), while the two which had germinated tardily were found to belong to another sexual group, e.g. (*a b*). In the present study of *Coprinus lagopus*, no correlation whatever has been observed between the sexual reaction and

the manner of germination of the spores and the growth of the mycelia. When grown on plates of sterile dung-agar, individual monosporous mycelia of *Coprinus lagopus* exhibit marked differences in rate and habit of growth and in the production of oidia. Some cultures produce oidia very sparingly, while others produce an abundance of oidia distributed evenly over the surface of the agar; in many of the cultures the oidia appear in clearly marked concentric rings exhibiting an apparent diurnal periodicity. In two of the basidia produced by fruit-body 54 × 58, distinct differences in the rate of spore germination and mycelial growth were observed. Two of the spores from each basidium had germinated at the end of twenty-four hours, and the resulting mycelia grew rapidly and produced an abundance of oidia; the remaining two spores from each basidium required over forty-eight hours for germination, while the mycelia produced few oidia and grew so slowly that at the end of three weeks the growth upon each agar plate was only about 5 cm. in diameter. When the pairings were made, one of the basidia proved to be of the type shown in Table XIII, with spores belonging to the four sexual groups; the other basidium was of the type shown in Table XII, with only two of the groups represented, but the two spores of slow growth belonged, not to the same group as might have been expected, but to different groups. In *Coprinus lagopus*, therefore, no evidence has been found to show that the manner of spore germination and rate of mycelial growth are in any way correlated with the segregation of sex-factors.

(6) *The Fruiting of Monosporous Cultures of Coprinus lagopus.*

Mlle Bensaude (1), working with *Coprinus fimetarius*, concluded that in a heterothallic species monosporous mycelia are always sterile. Since she made observations on only two cultures, the evidence which she obtaincd is not wholly conclusive. As already stated, Kniep (5) obtained a fruit-body from a monosporous mycelium of *Schizophyllum commune*; but the spores produced by this fruit-body were all of one sex. Vandendries (11) states that one of his monosporous cultures of *Panaeolus campanulatus* produced a fruit-body, but that the spores from it failed to germinate. In her second paper, Miss Mounce (10) records that she observed marked differences in the fruiting power of monosporous and polysporous mycelia of *Coprinus lagopus*. In the present study, upwards of eighty monosporous mycelia of *Coprinus lagopus* were grown in tubes of sterile horse-dung three inches long and nine-tenths of an inch in diameter; these cultures provided very suitable material for a study of the fruiting power of monosporous mycelia. All of these mycelia produced fruit-bodies, but with one exception, that of mycelium 53, which will be referred to later, the fruit-bodies produced were distinctly abnormal in appearance. Fruit-bodies arising on the bisporous

or polysporous mycelia having clamp-connexions were invariably few in number, generally not more than two or three to each tube culture, but all of these fruit-bodies elongated their stripes and shed an abundance of black spores in a normal manner. The monosporous fruit-bodies, on the contrary, were relatively much more numerous, each tube culture producing from eight to ten; some of these fruit-bodies remained as small rudiments, others developed pilei without maturing any spores or elongating their stipes, while still others elongated their stipes and produced a few ripe spores, but normal spore discharge was never observed. In all cases the pilei of the monosporous fruit-bodies remained white or pale grey in colour, owing to the small number or entire absence of ripe spores on the gills, in contrast with the black colour so characteristic of the normal secondary fruit-bodies.

Spores were obtained from a monosporous fruit-body of mycelium 1 of Table IV by touching a sterile platinum loop to the gills. The spores were then placed to germinate in five hanging drops of dung-agar, each drop containing about 100 spores. At the same time, spores from a normal secondary fruit-body (fruit-body 2 of Table II) were placed in two similar hanging drops. At the end of six hours, some of the spores from both fruit-bodies had started to germinate; at the end of twenty-four hours, about 85 per cent. of the spores in each of the seven hanging drops had germinated. The mycelia in all of the drops seemed to grow at the same rate, and all produced an abundance of the characteristic oidia. When examined seven days later, the mycelia from spores of the secondary fruit-body showed numerous clamp-connexions and had ceased producing oidia, while those from spores of the monosporous fruit-body had no clamp-connexions, but continued to produce oidia. Thus, we must conclude with Kniep that the spores from a monosporous fruit-body are all of one sex.

About 500 spores from the monosporous fruit-body referred to in the last paragraph were sown in polysporous culture, and the resulting mycelium was paired with the parent mycelium 1, and also with ten other monosporous mycelia derived from the same fruit-body as mycelium 1. The results of these pairings, given in Table IV under the column *M* 1, show that the spores from the monosporous fruit-body have the same sex as that of the parent.

Later, a portion of the polysporous mycelium *M* 1 was transferred to a tube of sterile dung. Fourteen days from the date of the transfer, a number of rudimentary fruit-bodies appeared on the surface of the dung, but only one of these fruit-bodies developed further. Four days later, this fruit-body elongated its stipe and shed a very few spores. A culture from the interior of the stipe of this fruit-body was made on a plate of sterile dung-agar, but no clamp-connexions ever developed. Spores were placed to germinate in two hanging drops of dung-agar, as well as in a plate of sterile dung-agar, each culture containing about 500 spores. Germination took place readily, but no clamp-connexions ever developed.

One of these polysporous cultures was then used for pairing with the eleven monosporous mycelia of Table IV and the polysporous mycelium *M* 1. From the results of the pairings given in Table IV (p. 437) under the column *M* 2, it will be seen that the polysporous mycelium has reacted in all respects like the original parent, mycelium 1. A portion of the polysporous culture *M* 2, when later transferred to a tube of sterile dung, gave rise to three fruit-bodies. These fruit-bodies elongated their stipes, but the pilei were pale grey in appearance and did not possess the normal number of spores. Unfortunately it was not found possible to make a study of these fruit-bodies.[1] Imperfect fruit-bodies of *Coprinus lagopus* have, therefore, been obtained from three successive generations of primary mycelia which originated from a single spore, the first two generations producing spores all of one sex, the sex being identical with that of the original parent mycelium.

It sometimes happens that secondary or diploid mycelia give rise to imperfect fruit-bodies similar in appearance to those produced by primary or haploid mycelia. A description of these imperfect fruit-bodies, with illustrations, will be found in Buller's 'Researches on Fungi', vols. ii and iii. As fruit-bodies of this kind might conceivably arise from hyphae of the compound mycelium which had not yet developed clamp-connexions, a portion of the interior of the stipe from an imperfect fruit-body was removed with a sterile scalpel and placed on a plate of sterile dung-agar in order to determine the condition of the mycelium growing out from it. When examined six days later, the mycelium was seen to be producing an abundance of clamp-connexions, thus proving that the imperfect fruit-body had arisen not from primary but from secondary mycelium.

From the above observations we may conclude that the primary or haploid condition of the mycelium of *Coprinus lagopus* is associated with the production of imperfect fruit-bodies ranging in size from tiny rudiments to almost perfect fruit-bodies, the spores of which are of only one sex, identical with that of the parent, while the secondary or diploid condition of the mycelium, although occasionally giving rise to imperfect fruit-bodies, is usually associated with the production of perfect fruit-bodies having spores of the four sexual groups.

(7) *The Abnormal Behaviour of certain Monosporous Mycelia of Coprinus lagopus.*

Kniep (5) states that when certain monosporous mycelia of *Schizophyllum commune* were kept in pure culture for a long time they developed clamp-connexions, thus passing definitely from the haploid to the diploid

[1] Four months later, spores from these fruit-bodies were sown in polysporous culture. The resulting mycelium remained primary, but produced two imperfect fruit-bodies (fourth generation), the spores of which, when sown in polysporous culture, produced only primary mycelium.

condition. Results analogous to these have been obtained with three monosporous mycelia of *Coprinus lagopus*, Nos. 26, 87, and 53.

After the work so far recorded in this paper had been completed, the various cultures of monosporous mycelia about to be discarded were given a final examination. It was then seen that mycelia 26 and 87 had small patches of hyphae bearing clamp-connexions. About twelve days prior to the time of this examination, a sub-culture of mycelium 26 had been made in a shallow glass bottle plugged with cotton-wool. A transfer was, therefore, made from this bottle to a plate of sterile dung-agar. When examined six days later, the mycelium in the plate culture was found to be in the primary condition and producing rings of oidia, showing no clamp-connexions whatever. A sub-culture of mycelium 87 had not been made, but on the date of the last transfer, about a week before the final examination, this mycelium was still in the primary condition.

After mycelium 53 had been used for pairing with other mycelia, it was sub-cultured in a tube of sterile horse-dung. About two weeks later, this mycelium gave rise to eight small fruit-bodies which at first were similar in appearance to the imperfect fruit-bodies produced by monosporous mycelia. Later, however, the eight fruit-bodies elongated their stipes, expanded their pilei, and shed an abundance of spores in a quite normal manner. When a portion of the mycelium from the dung tube was examined under the microscope, it was seen to possess clamp-connexions. Two sub-cultures of mycelium 53, previously made on plates of sterile dung-agar, were then examined and found to be still in the primary condition; moreover, sub-cultures from one of these plates remained in the primary condition for over a month longer and were only discarded at the conclusion of the work. In view of the fact that eight perfect fruit-bodies had been produced from a secondary mycelium on so small an amount of dung, while two sub-cultures of the same mycelium on dung-agar had remained in the primary condition, it seemed probable that mycelium 53 had changed spontaneously from the primary to the secondary condition. Spore-deposits were collected, therefore, from three of the fruit-bodies for the purpose of studying any abnormal changes which might have taken place. Unfortunately, only a short time could be devoted to this work, and the study is, therefore, very incomplete. Monosporous mycelia, however, were isolated from two of the fruit-bodies, five from fruit-body *F* and five from fruit-body *G*, and all possible pairings were made between them. The results of the pairings are given in Table XV. An examination of this table will show that spores of only three sexual groups have been obtained from each fruit-body, but spore *G* 4 is similar to spores *F* 4 and *F* 5, and spore *F* 2 is similar to spores *G* 1 and *G* 5, so that in the ten spores there are four sexual groups represented. It is probable that with a larger number of monosporous mycelia the four groups would have been found in each fruit-body.

The most striking part of the table lies in the reactions which have taken place between the different groups. Groups 1 and 2 have reacted together normally, as have also groups 3 and 4, but group 3 has reacted also with groups 1 and 2; in fact mycelia *F* 1 and *F* 3 exhibit the phenomenon of complete fertility with the other eight mycelia and conduct themselves in all respects like mycelia from a new sexual strain. One other point of interest in this table is the reaction which has taken place between mycelia *G* 2 and *G* 3, which belong to the same group.

From the preceding evidence it would be unwise to conclude that

		1			2			3		4	
		F4	F5	G4	F2	G1	G5	F1	F3	G2	G3
1	F4	—	—	—	+	+	+	+	+	—	—
	F5	—	—	—	+	+	+	+	+	—	—
	G4	—	—	—	+	+	+	+	+	—	—
2	F2	+	+	+	—	—	—	+	+	—	—
	G1	+	+	+	—	—	—	+	+	—	—
	G5	+	+	+	—	—	—	+	+	—	—
3	F1	+	+	+	+	+	+	—	—	+	+
	F3	+	+	+	+	+	+	—	—	+	+
4	G2	—	—	—	—	—	—	+	+	—	+
	G3	—	—	—	—	—	—	+	+	+	—

TABLE XV. *Coprinus lagopus.* All possible pairings of ten monosporous mycelia from fruit-bodies *F* and *G* of mycelium No. 53.

mycelia 26 and 87 of *Coprinus lagopus* suddenly became secondary in a spontaneous manner, since there is always the possibility that where so many cultures were being made contamination may have taken place from outside sources, not so much by means of spores as from oidia which may have been set free in the air. Such a possibility, however, cannot account for the abnormal behaviour of mycelium 53; the large number of fruit-bodies produced by this culture, together with the striking character of the reactions between monosporous mycelia from two of these fruit-bodies, point to the conclusion that this mycelium suddenly mutated from the primary to the secondary condition, and in so doing gave rise to a fruit-body having some spores of a new sexual strain. Mutations of this character may indeed account for the many different sexual strains of *Coprinus lagopus* which are to be found among wild fruit-bodies.

Summary.

1. A study has been made of the sex of *Coprinus lagopus*. The fruit-bodies employed in this work were collected from four widely separated points in Canada, and from three places at Birmingham, England.

2. All strains of *Coprinus lagopus* studied proved to be heterothallic. Miss Mounce's suggestion that there may exist both homothallic and heterothallic strains of this fungus has not been supported.

3. English and Canadian strains of *Coprinus lagopus* are similar morphologically and have been shown by the clamp-connexion criterion to be identical.

4. Sex in *Coprinus lagopus* is determined by certain factors which segregate out according to Mendelian principles.

5. Monosporous mycelia from any individual fruit-body of *Coprinus lagopus* belong to four sexual groups similar to those found by Kniep for *Schizophyllum commune*.

6. Complete fertility results when monosporous mycelia from wild fruit-bodies of different sexual strains are paired together.

7. Two fruit-bodies from a secondary bisporous mycelium produce spores belonging to but four sexual groups, the same four groups being present in both fruit-bodies.

8. Monosporous mycelia from a first-generation hybrid fruit-body react with the parent mycelia strictly in accordance with the theory of dihybridism.

9. The reduction of sex-factors in *Coprinus lagopus* takes place with the second division of the fusion nucleus, resulting in the production of (*a*) basidia each with spores of only two kinds, and (*b*) basidia each with spores of four kinds.

10. No correlation has been found between the rapidity of germination of spores from individual basidia and the segregation of sex factors, as observed by Kniep for *Aleurodiscus polygonius*.

11. The primary or haploid condition of the mycelium was found to be associated with the production of imperfect fruit-bodies bearing spores of only one sex, the sex being identical with that of the parent mycelium; the secondary or diploid condition of the mycelium was found to be associated with the production of perfect fruit-bodies bearing spores of the four sexual groups.

12. Imperfect fruit-bodies were produced by three successive generations of primary mycelia originating from a single spore; fruit-bodies of the first two generations had spores of only one sex, the sex being identical with that of the parent mycelium.

13. The imperfect fruit-bodies which are sometimes produced by secondary mycelia arise not from primary, but from secondary strands of mycelium.

14. One monosporous mycelium appears to have mutated from the primary to the secondary condition, and in so doing it gave rise to a fruit-body having some spores of a new sexual strain.

15. A method is described for removing the four spores from a living basidium, separating them from one another, and placing each one of them in a separate hanging drop of dung-agar.

The foregoing investigations were carried out in the Botanical Laboratory of the University of Manitoba during the tenure of a scholarship awarded by the Canadian Society of Technical Agriculturists; a grant in aid of this work was also made by the Canadian Honorary Advisory Council for Scientific and Industrial Research. The problem was suggested by Prof. A. H. R. Buller, whose valuable advice and stimulating criticism is gratefully acknowledged. The writer is also indebted to Miss Dorothy E. Newton, M.Sc., for examining some of the cultures for Table VIII.

Literature Cited.

1. Bensaude, M.: Recherches sur le cycle évolutif et la sexualité chez les Basidiomycètes, pp. 1–156, nine plates, Nemours, 1918.
2. Brefeld, O.: Untersuchungen, Heft 3, Taf. vi, Fig. 1, *a–g*, Leipzig, 1877.
3. Hanna, W. F.: The Dry-Needle Method of making Monosporous Cultures of Hymenomycetes and other Fungi. Ann. Bot., xxxviii, pp. 791–5, 1924.
4. Kniep, H.: Beiträge zur Kenntnis der Hymenomyceten, III, IV, and V. Zeitschr. f. Botanik, vii, pp. 369–98, 1915; viii, pp. 353–60, 1916; and ix, pp. 101–19, 1917.
5. ————: Über morphologische und physiologische Geschlechtsdifferenzierung. Verhandl. der Physikal.-Med. Gesellschaft zu Würzburg, 1919.
6. ————: Über Geschlechtsbestimmung und Reduktionsteilung. Ibid., Bd. 47, 1922.
7. ————: Über erbliche Änderungen von Geschlechtsfaktoren bei Pilzen. Zeitschr. f. induktive Abstammungs- und Vererbungslehre, Bd. xxxi, 1923.
8. Lange, J. E.: Studies in the Agarics of Denmark. Dansk Botanisk Arkiv, Copenhagen, Bd. ii, p. 41, 1915.
9. Mounce, I.: Homothallism and the Production of Fruit-bodies by Monosporous Mycelia in the Genus *Coprinus*. Trans. Brit. Mycolog. Soc., vii, pp. 198–217, 1921.
10. ————: Homothallism and Heterothallism in the Genus *Coprinus*. Ibid., viii, pp. 256–69, 1922.
11. Vandendries, R.: Recherches sur le déterminisme sexuel des Basidiomycètes. Bruxelles, 1923.
12. ————: Nouvelles recherches sur la sexualité des Basidiomycètes. Bull. de la Soc. Royale de Bot. de Belgique, tome xvi, 1923.
13. Buller, A. H. R.: Experiments on Sex in Mushrooms and Toadstools. Nature, cxiv, p. 826, 1924.

Sexual Stability in Monosporous Mycelia of Coprinus lagopus.

BY

W. F. HANNA, M.Sc.,

University of Alberta.

With one Figure in the Text.

1. Introduction.

RECENT researches have shown that the Hymenomycetes, from the point of view of sex, may be divided into two groups, the *homothallic* and the *heterothallic*.

Individual spores of a homothallic species, when germinated separately on suitable sterilized media, give rise to *haploid* (*primary*) mycelia. The hyphae of such haploid mycelia possess numerous simple transverse walls, and each cell encloses one or more independent nuclei. In the course of a few days each haploid mycelium passes spontaneously into the *diploid* (*secondary*) phase. This change is marked externally by the appearance of a clamp-connexion at each new transverse cell wall and internally by the nuclei becoming paired and dividing conjugately. Later on, each diploid mycelium gives rise to one or more perfect diploid fruit-bodies. Therefore, sexually, the spores of a homothallic species are of one kind only, and the normal development of the fungus throughout its complete life-cycle may be initiated by a single monosporous mycelium.

When individual spores of a heterothallic species are germinated separately, they also give rise to haploid mycelia, and these have the same essential characters as those of a homothallic species; but, whereas individual monosporous mycelia of a homothallic species become diploid spontaneously, those of a heterothallic species, so long as they are kept separate from one another, remain in the haploid condition indefinitely. However, if a number of monosporous mycelia derived from a fruit-body of a heterothallic species are paired together in all possible combinations so that the hyphae of the mycelia in each pair may anastomose, certain pairs continue to develop in a haploid manner, while others soon produce a

[Annals of Botany, Vol. XLII. No. CLXVI. April, 1928.]

diploid mycelium which bears clamp-connexions, has pairs of nuclei which divide conjugately, and later gives rise to one or more perfect fruit-bodies. It is therefore evident that a single perfect fruit-body of a heterothallic species must produce spores which are sexually of more than one kind, and that two monosporous mycelia of opposite sex must be brought together to enable the fungus to pass through its complete life-cycle with the production of perfect diploid fruit-bodies. Certain heterothallic species, e.g. *Coprinus radians* (8) and *C. Rostrupianus* (7), are bisexual, the spores from a single perfect fruit-body falling into two groups which sexually are opposite; while other species, e.g. *C. lagopus* (4), are quadrisexual, the spores from a single perfect fruit-body falling into four sexual groups.

It is not always possible to draw a clear distinction between homothallic and heterothallic species. Up to the present, all of the homothallic species investigated have proved to be strictly homothallic, the monosporous mycelia all changing from the haploid to the diploid condition within a few days after the germination of the spores and eventually producing perfect diploid fruit-bodies. In several heterothallic species, however, it has been found that a certain number of monosporous mycelia, without fusion with any other mycelia, may change spontaneously into the diploid condition. The first example of this kind was reported by Kniep (6) in his investigation of *Schizophyllum commune*, a heterothallic species of the quadrisexual type. He observed that two monosporous mycelia which, when young, had reacted as normal haplonts were, when examined a year later, in the diploid condition, as shown by their clamp-connexions. The nature of the change from the haploid to the diploid state was not investigated by Kniep, but it is reasonable to suppose that a sexual mutation occurred in each of the two mycelia concerned. Thus the transition from the haploid to the diploid condition, which in a monosporous mycelium of a homothallic species takes place normally a few days after the germination of the spore, took place in certain monosporous mycelia of the heterothallic species *S. commune* only after a prolonged period of culture on artificial media.

Mutations of a similar nature to those just described have been recorded for other heterothallic Hymenomycetes: by Vandendries for *Coprinus radians* (8, 9) and *C. micaceus* (11), by Newton for *C. Rostrupianus* (7), and by myself for *C. lagopus* (4). One of the monosporous mycelia in my cultures of *C. lagopus*, after being transferred to sterilized horse-dung, produced several fruit-bodies the pilei of which, instead of being pale and bearing but few spores like haploid fruit-bodies in general, were black and bore the full number of spores just like normal diploid fruit-bodies. Ten monosporous mycelia derived from as many spores taken from two of the fruit-bodies were investigated experimentally. It was found that the mycelia were not all of one and the same sex, as was to be expected if

the fruit-bodies had been haploid, but that they fell into four groups, thus proving that the fruit-body was diploid. Moreover, one of the groups of mycelia was completely fertile with the other three groups, thus indicating that a mutation had taken place with the production of a new sexual strain. Altogether, in my previous investigation, three monosporous mycelia of *C. lagopus* were observed which had mutated from the haploid to the diploid condition.

To explain the sexual mutations which occur in heterothallic Hymenomycetes, Vandendries has advanced the theory of *hetero-homothallism*, which may be stated as follows. With certain rare exceptions [1] basidiospores, on germination, give rise to haploid mycelia. In view of this fact it is probable that, in the early stages of development, all species of Basidiomycetes are heterothallic. Later, every haploid mycelium becomes diploid either by a pairing of the nuclei within itself or after conjugation with another mycelium of opposite sex. In the so-called homothallic species it has been determined that this change takes place spontaneously a few days after the germination of the spore, although future experiment may show that the diploid condition may also be brought about by conjugation between very young haplonts. In the so-called heterothallic species, the change from the haploid to the diploid condition may be brought about either by conjugation between two monosporous mycelia of opposite sex or by a sexual mutation which may take place many weeks or months after the germination of the basidiospore. Thus, according to the theory of Vandendries, homothallic species, e.g. *Coprinus sterquilinus*, and heterothallic species, e.g. *C. lagopus*, should be united into a single group which may be described as *hetero-homothallic*.

The hetero-homothallic theory suggested by Vandendries is of interest in that it offers an explanation for the mutations of monosporous mycelia from the haploid to the diploid condition so frequently observed in heterothallic species. Its validity, however, is based upon the following assumptions, which, as yet, have not been put to the test of experiment: (1) young monosporous mycelia of homothallic species, when paired, should in a certain number of pairings give rise to a diploid mycelium; and (2) *all* monosporous mycelia of heterothallic species should eventually change spontaneously from the haploid to the diploid condition. If these assumptions could be justified by experiment, the only fundamental distinctions between homothallic species and heterothallic species would disappear, and then the term *hetero-homothallic* could be applied to all the species which are now segregated into the homothallic and heterothallic groups.

The experiments about to be recorded were made upon one of the heterothallic Hymenomycetes—*Coprinus lagopus*—for the purpose of find-

[1] Kniep (5) showed that when a spore of *Hypochnus terrestris* germinates it gives rise at once to a binucleate mycelium.

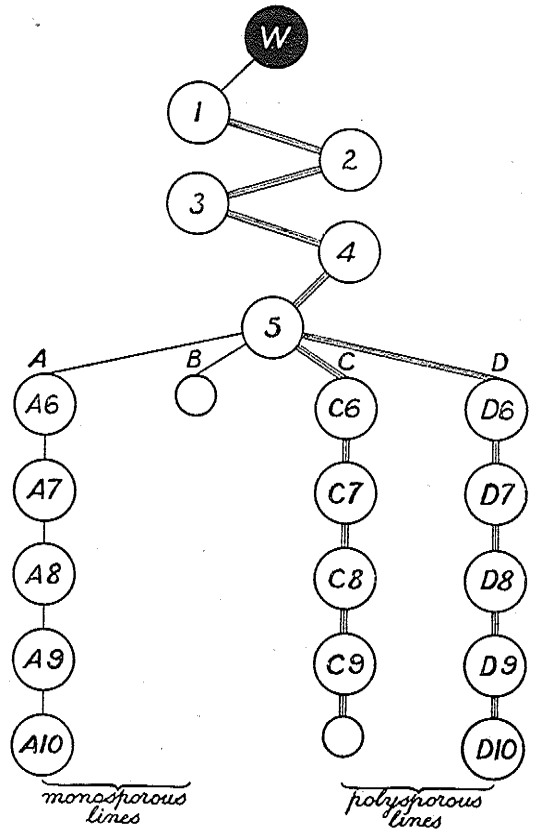
ing out: (1) to what extent in such a heterothallic fungus it is possible to rear a series of successive haploid generations; and (2) to determine whether or not haploid mycelia are sexually stable. *Coprinus lagopus* was chosen for the investigation because its normal life-cycle from spore to spore occupies only about a fortnight, because it can be readily grown on sterilized horse-dung, and because it has already been investigated by the author from the sexual point of view (4).

A single monosporous mycelium of *Coprinus lagopus* was isolated and, in the course of three years, several successive generations of haploid fruit-bodies have been produced from it. It is hoped that the results which have been obtained will throw some light on the question of hetero-homothallism in the Basidiomycetes.

2. Experiments on *Coprinus lagopus*.

As recorded in a previous paper (4), a certain number of haploid generations of *Coprinus lagopus* were raised as follows. A wild fruit-body (W in the figure opposite) appeared on horse-dung at Winnipeg. One of its spores was isolated and sown separately, and it gave rise to a mycelium. This *monosporous* mycelium was transferred to sterilized horse-dung. Soon it gave rise to a haploid fruit-body (No. 1 in the figure) which was proved by the mating method to be haploid; the spores borne by the fruit-body were all of one sex only, and this sex was identical with that of the monosporous mycelium which had given rise to the fruit-body. The production of this fruit-body with its spores completed the first haploid generation. A number of spores were taken from the fruit-body and were sown together. They germinated and produced what may be called a *polysporous* mycelium. This polysporous mycelium, which never bore any clamp-connexions—thus showing itself to be haploid—gave rise to another haploid fruit-body (No. 2 in the figure) which shed spores. This marked the completion of the second haploid generation. As before, the spores from the second-generation haploid fruit-body were found experimentally to be all of the same sex, and this sex was found to be identical with that of the original monosporous mycelium. By polysporous sowings third-generation and fourth-generation haploid fruit-bodies (Nos. 3 and 4 in the figure) were obtained in succession. About seven months had now elapsed since the original monosporous mycelium had been obtained by sowing a single spore derived from a wild fruit-body, but the polysporous mycelium of the fourth generation was still in the haploid condition, for it was producing the characteristic haploid oidia in great numbers, while its cross-walls were simple and not provided with clamp-connexions.

In four successive haploid generations no mutations to the diploid condition had taken place. This regular production of haploid fruit-bodies seemed of considerable interest in view of the fact that, as already mentioned in the introduction, three monosporous mycelia of *Coprinus lagopus* had been observed which had mutated spontaneously from the haploid to the diploid condition. With a view to investigating the sexual stability of



Scheme of ten haploid generations of *Coprinus lagopus* reared in pure cultures. Each generation consists of a haploid mycelium, shown by one or more lines, and of a haploid fruit-body, shown by a circle. A monosporous mycelium is represented by a single line, and a polysporous mycelium by three parallel lines. The black circle, W, represents the original wild diploid fruit-body. The circles containing numbers represent fruit-bodies which produced spores, and the small circles without numbers sterile fruit-body rudiments. The generations of the lines A and B were each initiated by the sowing of a single spore, while those of the lines C and D were each initiated by the sowing of many spores.

the haploid mycelia of *C. lagopus* still farther, it was therefore decided to continue the raising of haploid generations.

The method of germinating the spores and of obtaining fruit-bodies was described in a previous paper (4). In the further investigations, of which the details are now to be recorded, the mycelia were grown in Petri dishes containing sterile dung-agar. When it was desired to obtain fruit-bodies from any mycelium, a piece of the mycelium was transferred from its Petri dish to a test-tube containing sterilized horse-dung at its base. The

depth of the dung in the tube was about an inch and a quarter. The mycelium, after being placed on the dung, usually gave rise to fruit-bodies in the course of a few weeks.

The first four haploid generations, produced as described above, are indicated in the scheme shown on p. 383. A fifth-generation haploid fruit-body (No. 5 in the figure) was obtained from a polysporous mycelium by sowing many spores of fruit-body No. 4.

From the spores of fruit-body No. 5, as indicated in Fig. 1, four mycelial cultures were made. Two of these, A and B, were of monosporous origin, while the other two, C and D, were of polysporous origin. It was then sought to carry on the lines A and B by monosporous sowings and C and D by polysporous sowings.

The monosporous line A was continued successfully, five successive generations culminating in the spore-bearing fruit-bodies A 6 to A 10 being produced; but the monosporous line B failed because the mycelium gave rise only to small rudimentary fruit-bodies which never developed any spores.

The polysporous line D was continued successfully, five successive generations culminating in the spore-bearing fruit-bodies D 6 to D 10 being produced. The polysporous line C was carried on successfully for four generations, C 6 to C 9, but the fifth-generation fruit-bodies were quite rudimentary and failed to produce any spores.

By reference to p. 383, it will be seen that, starting with a spore of the original wild fruit-body W and passing to fruit-bodies A 10 and D 10, ten successive haploid generations of the heterothallic species *Coprinus lagopus* were successfully carried through. This, in itself, is an interesting result, for it once more shows that in a heterothallic species the union of two mycelia of opposite sex and the association of the nuclei in conjugate pairs in the mycelium are not required for the production and development of fruit-bodies. As we know from the work of Kniep and others, in each young basidium of a normal diploid fruit-body two nuclei are present, and these soon unite to form a fusion nucleus, whereas in each young basidium of a haploid fruit-body only one nucleus is present, so that nuclear fusion is impossible.

The haploid fruit-bodies of *Coprinus lagopus* differ from normal diploid ones not only in their nuclear condition (which the author has not investigated), but in their appearance and in the number of spores which they produce. A diploid fruit-body is easily distinguished by the dark colour of its pileus, which is due to the immense number of black spores upon the gills. The elongation of the stipe of a diploid fruit-body, followed by the expansion of the pileus, the discharge of the spores, and the autodigestion of the gills, takes place normally in a single night; and a heavy black spore-deposit is produced below the pileus. A haploid mycelium usually

gives rise to a number of fruit-body rudiments a few millimetres in length. Most of them do not develop farther, or much farther, but here and there one of them grows so as to form a fruit-body of normal size. Such a fruit-body may elongate its stipe and expand its pileus in the normal manner, but it can easily be distinguished from a diploid fruit-body by the greyish-white colour of its pileus. This light tint is due to the fact that the gills produce very few spores. These spores may not be discharged, but sometimes they are; and, in the course of the present investigation, several light spore-deposits from haploid fruit-bodies have been obtained. Some spores collected from the haploid fruit-bodies No. 5 and No. D 10 (see p. 383) were mounted in water and the lengths of 100 spores from each spore-deposit were measured. The mean length of the spores from fruit-body No. 5 and from fruit-body No. D 10 was found to be 13·5 μ and 12·2 μ respectively. These measurements are of the same order of magnitude as those for spores of normal diploid fruit-bodies of *C. lagopus*. The most fully developed haploid fruit-bodies, therefore, differ from normal diploid fruit-bodies in the smaller number of spores which they bear, but not in the size of the spores.

After transference to sterilized horse-dung, diploid mycelia fruit (produce fruit-bodies which expand) with great regularity within about fourteen days, whereas, while certain haploid mycelia fruit just as quickly, many others fruit much more slowly, so that three weeks or a month not infrequently elapses before a single fruit-body opens its pileus. The rudimentary fruit-bodies on some haploid mycelia never expand at all. On the whole, therefore, it appears that diploid mycelia fruit not only more certainly but also more quickly than haploid.

As a general rule, diploid fruit-bodies are more vigorous than haploid. Many haploid fruit-bodies do not elongate their stipes, or do not open their pilei, or do not produce any spores; and, as we have seen, the most highly developed haploid fruit-bodies produce but relatively very few spores, so that their pilei are pale in colour. The greater vigour of diploid fruit-bodies as compared with haploid is correlated with the fact that the cells of diploid fruit-bodies contain conjugate nuclei of opposite sex, while the cells of haploid fruit-bodies contain non-conjugate nuclei only. If we suppose that each nucleus possesses a factor for vigour which shows itself in fruit-body development, then diploid fruit-bodies, in general, should be more vigorous than haploid.

The lines B and C came to an end because the first-generation fruit-body of B and the fifth-generation fruit-body of C failed to develop sufficiently to produce any spores. In an endeavour to explain this failure two factors may be taken into account: (1) the age of the mycelium at the time of its transference from the Petri dish to a tube of sterilized horse-dung; and (2) heritable differences in vigour between mycelia derived from different spores of the haploid fruit-body No. 5.

The fruiting capacity of a haploid mycelium as affected by the age of the mycelium was investigated by transferring a number of mycelia of different ages from Petri dishes to tubes of sterilized horse-dung and observing to what extent the mycelia produced fruit-bodies. These mycelia, shortly after being isolated, had been tested on sterilized horse-dung, and at that time all of them had given rise to haploid fruit-bodies. The results of the experiments are embodied in Table I. In the last column the term *fruiting* means the production of one or more fruit-bodies which expand and bear spores. A mycelium is considered as non-fruiting if it bears fruit-body rudiments only. In the second column the fruit-body numbers are those of the figure as p. 383.

TABLE I.

Effect of Age on Fruiting Capacity in Haploid Mycelia of Coprinus lagopus.

Nature of Mycelium.	*Origin of Mycelium.*	*Age of Mycelium.*	*No. of Cultures made.*	*No. of Cultures fruiting.*
Monosporous	Fruit-body 5	514 days	10	0
,,	,, A 7	245 ,,	10	10
,,	,, A 9	160 ,,	10	8
Polysporous	,, 5	358 ,,	10	0
,,	,, D 7	260 ,,	10	2
,,	,, D 9	63 ,,	10	1

A consideration of the contents of Table I, and also of the fact that all the mycelia given in the table fruited readily when they were only a few weeks old, makes it obvious that prolonged growth in pure culture leads to a reduction in the capacity of haploid mycelia to produce haploid fruit-bodies, and that, with the passage of time, this loss of fruiting power may continue until finally only sterile fruit-body rudiments can be formed. In some cultures, moreover, the loss of fruiting power seems to take place much more rapidly than in others.

We may now return to the problem of the failure of lines B and C to produce fertile fruit-bodies. Culture B was about five months old when it was transferred to sterilized horse-dung. It is therefore possible that this culture, owing to age, had lost its power of producing fruit-bodies. However, the same explanation cannot account for the failure of the mycelium derived from fruit-body No. C 9 to produce fertile fruit-bodies, for only two weeks elapsed from the time of germination of the spores of fruit-body No. C 9 and the transference of the resulting mycelium to tubes of sterilized horse-dung. It would seem more reasonable to attribute the loss of fruiting capacity in culture C to some physiological peculiarity inherited by the mycelium.

A gradual diminution in the ability of sexual strains to react together has been noted by Blakeslee in *Mucor* (1), by Derx in *Penicillium* (3), and by Couch in *Dictyuchus* (2). A similar loss of sexual vigour has not been found to occur in haploid mycelia of *Coprinus lagopus*. About three years from the time the original monosporous mycelium obtained from a spore of the wild fruit-body shown in the figure was isolated, eighteen of the various monosporous mycelia which originated from it were separately paired with another haploid mycelium of a different sexual strain. All of the matings produced vigorous diploid mycelia with abundant clamp-connexions, thus showing that the haploid mycelia of the series under investigation had retained in a very high degree the power to conjugate with another haploid mycelium of opposite sex.

It seemed just possible that the haploid mycelia under investigation might have undergone some change in their sex factors which would make possible a positive reaction between two of the mycelia. The sexual reactions of the mycelia were therefore tested by pairing seven of the mycelia with one another in all possible combinations. The results of the pairings are given in Table II. The mycelia have the same symbols as the haploid fruit-bodies which produced the spores from which they originated. The fruit-bodies were those shown in the figure. A (—) sign indicates that no clamp-connexions appeared on the paired mycelium.

TABLE II.

The Results of Pairing in all Possible Ways seven Haploid Mycelia of Coprinus lagopus, derived from Spores of Different Generations of Haploid Fruit-bodies.

	A5	B5	C5	D5	A10	C9	D10
A5	—	—	—	—	—	—	—
B5	—	—	—	—	—	—	—
C5	—	—	—	—	—	—	—
D5	—	—	—	—	—	—	—
A10	—	—	—	—	—	—	—
C9	—	—	—	—	—	—	—
D10	—	—	—	—	—	—	—

From Table II it will be seen that no clamp-connexions appeared in any of the pairings. On the other hand, the pairs of mycelia continued to produce oidia. The presence of these oidia and the absence of clamp-connexions in all the pairings go to prove that, throughout the ten haploid

generations which were observed, the mycelia had undergone no change in sexual reaction, all of the mycelia and all of the fruit-bodies having been of one and the same sex.

In an earlier paper (4), as previously mentioned, it was recorded that three monosporous mycelia of *C. lagopus* had mutated from the haploid to the diploid condition. These three were the only mycelia that showed any evidence of mutation out of 167 monosporous mycelia which were kept under observation for periods varying from three weeks to five months. The new experimental evidence which has just been recorded indicates quite clearly that *certain monosporous mycelia of C. lagopus are perfectly stable and are capable of remaining permanently in the haploid condition.* In view of these facts, *C. lagopus* may be considered as a strictly heterothallic species in which sexual mutations take place only occasionally. The existence of such a species does not accord with the hetero-homothallic theory of Vandendries.

3. Summary.

1. Monosporous mycelia of *Coprinus lagopus*, one of the heterothallic Hymenomycetes, have been kept under observation for upwards of three years to determine whether or not prolonged culture on artificial media would lead to spontaneous mutations from the haploid to the diploid condition.

2. The parent mycelium was obtained from a single spore of a normal diploid wild fruit-body. From this mycelium have been obtained ten successive generations of haploid fruit-bodies. Spores from these fruit-bodies always produced haploid mycelia, and none of the mycelia was found to mutate to the diploid condition.

3. When cultivated on artificial media for a long time, haploid mycelia were found to lose their fruiting power, but not their power of uniting and sexually reacting with another mycelium of opposite sex.

4. The most perfect haploid fruit-bodies of *C. lagopus* differ from ordinary diploid fruit-bodies in the much smaller number of spores which they bear, but not in the size of their spores.

5. *C. lagopus* may be considered as a heterothallic species in which sexual mutations from the haploid to the diploid condition occur but rarely.

This investigation was begun in the Botanical Department of the University of Manitoba under the direction of Professor A. H. Reginald Buller, and was completed at the University of Alberta. I desire to thank Professor Buller for his interest in the work and for helpful suggestions.

Literature Cited.

1. Blakeslee, A. F.: Sexual Reproduction in the Mucorineae. Proc. Amer. Acad. Arts and Sciences, vol. xl, pp. 205–319, 1904.
2. Couch, J. N.: Heterothallism in *Dictyuchus*, a Genus of the Water Moulds. Ann. Bot., vol. xl, pp. 849–81, 1926.
3. Derx, H. G.: Heterothallism in the Genus *Penicillium*. Trans. Brit. Mycol. Soc., vol. xi, pp. 108–12, 1926.
4. Hanna, W. F.: The Problem of Sex in *Coprinus lagopus*. Ann. Bot., vol. xxxix, pp. 431–57, 1925.
5. Kniep, H.: Beiträge zur Kenntnis der Hymenomyceten, I, II. Zeitschr. f. Bot., Bd. v, pp. 593–637, 1913.
6. ———: Über morphologische und physiologische Geschlechtsdifferenzierung. Verhandl. der Physikal.-med. Gesellsch. zu Würzburg, Bd. xlvi, Heft 1, 1920.
7. Newton, D. E.: The Bisexuality of Individual Strains of *Coprinus Rostrupianus*. Ann. Bot., vol. xl, pp. 105–28, 1926.
8. Vandendries, R.: Recherches expérimentales sur la bipolarité sexuelle des Basidiomycètes. Bull. de la Soc. royale de Bot. de Belgique, tom. lvii, fasc. 1, 1924.
9. ———: Contribution nouvelle à l'étude de la sexualité des Basidiomycètes. La Cellule, tom. xxxv, pp. 129–55, 1924.
10. ———: L'hétéro-homothallisme dans le genre *Coprinus*. Bull. de la Soc. royale de Bot. de Belgique, tom. lvii, fasc. 2, 1925.
11. ———: La tétrapolarité sexuelle des *Coprins*. Ibid., tom. lviii, fasc. 2, 1926.
12. ———: Les mutations sexuelles, l'hétéro-homothallisme, et la stérilité entre races géographiques de *Coprinus micaceus*. Mémoires de l'Académie royale de Belgique, tom. ix, pp. 1–50, 1927.

NOTE.

THE DRY-NEEDLE METHOD OF MAKING MONOSPOROUS CULTURES OF HYMENOMYCETES AND OTHER FUNGI.—Students of mycology and phytopathology frequently find it necessary to cultivate certain species of fungi from single spores. The procedure employed for isolating and germinating the individual spores should permit of the work being performed simply, rapidly, and with perfect accuracy.

The poured-plate method, used so generally by bacteriologists, has often been employed in mycological investigations. While satisfactory in some respects, it falls far short of perfection; for it is slow and cumbersome, and if the medium used for germinating the spores is not perfectly clear there is always the possibility, when the transfer is being made from the Petri dish, that the mycelium taken up may have been derived from more than one spore. Furthermore, if one requires to sow a spore of particular size or shape, the poured-plate method is of little or no value.

Edgerton[1] has described a method of isolating a particular spore from a liquid medium by means of a small capillary tube suitably attached to the substage of the microscope. The upper end of the tube is sealed, while the lower end is drawn out to a fine point. This point is lowered carefully until it comes in contact with the spore, whereupon a small drop of ether is placed on the upper closed end of the tube, thus causing the spore to be sucked up into the tube's interior. By gently heating the tube with a small flame, the spore, together with the liquid in which it is enveloped, is then driven from the tube and deposited upon the medium on which it is to germinate. Recently, Roberts[2] has suggested certain improvements which might be made to this apparatus, particularly in respect to the adjustment of the capillary tube to the substage.

While working with certain species of the genus *Coprinus*, the following method of making monosporous cultures from particular spores was devised. It is simple and accurate, and yet permits of the cultures being made very rapidly. No apparatus is required, other than that which is to be found in every mycological laboratory.

If a sterilized glass slide is placed under a fruit-body, such as that of *Coprinus sterquilinus*, which is rapidly shedding spores, a suitable spore-deposit may be obtained in from one to two minutes. An examination of the slide under the microscope will show that the spores are well separated from one another, thus making the selection of individual spores a comparatively simple matter. The slide should be kept in a sterile Petri dish until required. If spore-deposits of this kind have not been procured, and fruit-bodies are not available, a relatively thicker spore-deposit which has been taken on a glass slide for ordinary cultural purposes may serve equally well, as certain spots in it will generally be found where the spores are sufficiently separated from one another.

[1] Edgerton, C. W. (1914): A Method of picking up Single Spores. Phytopathology, vol. iv, No. 2, pp. 115–17.

[2] Roberts, J. W. (1923): A Method of isolating Single Spores. Ibid., vol. xiii, No. 12, pp. 558–60.

A number of Petri dishes should be fitted up with ring-cells and cover-slips (Fig. 1), as described by Duggar.[1] A large Petri dish, 14 cm. in diameter and 2 cm. high, has been found to be very suitable for this work. In the bottom of the dish is placed a sheet of filter-paper, in which ten circular holes have been cut. In each hole a ring-cell, 17 mm. in diameter and 10 mm. high, is inserted, and cover-

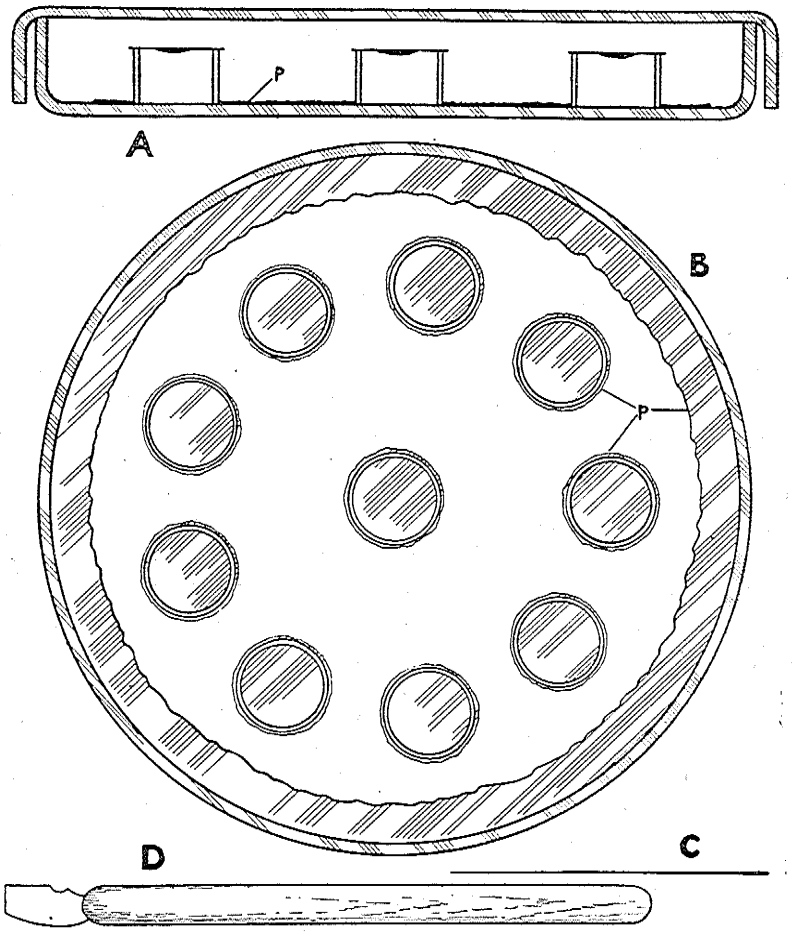


FIG. 1. A and B, a Petri dish shown in section and in surface view respectively. The ring-cells are set in holes in a sheet of wet filter-paper, *p*. The cover-slips in A bear hanging drops of agar. C, a needle used for making monosporous cultures. D, an agar spade, used for transferring hanging drops containing mycelia from ring-cells to poured plates. A, B, and D, reduced to 2/3; C, natural size.

slips (without vaseline) are placed on the tops of the ring-cells. The whole is then sterilized in hot air. When cool, a little water is poured over the filter-paper, and a drop of the germinating medium is touched to the bottom of each cover-slip. A Petri dish fitted up in the manner described is shown in section, in Fig. 1, A, and in surface view in Fig. 1, B.

[1] Duggar, B. M. (1909): Fungus Diseases of Plants, New York, p. 95.

When single spores are to be isolated, a slide bearing a spore-deposit is placed under the low power of the microscope, and moved about until a spore of the required shape or size is brought into the centre of the field. Then, holding a fine sewing-needle, such as is shown in Fig. 1, C, between the thumb and forefinger of the right hand, the point is lowered slowly downwards until it comes into contact with the desired spore. When touched, the spore leaves the glass slide and adheres readily to the needle-point. Fig. 2 represents a small portion of a glass slide bearing spores of *Coprinus sterquilinus* sufficiently separated from one another to permit of any one of them being easily picked up without danger of touching any of its fellows. The needle-point shown in this figure is drawn on the same scale as the spores, and has

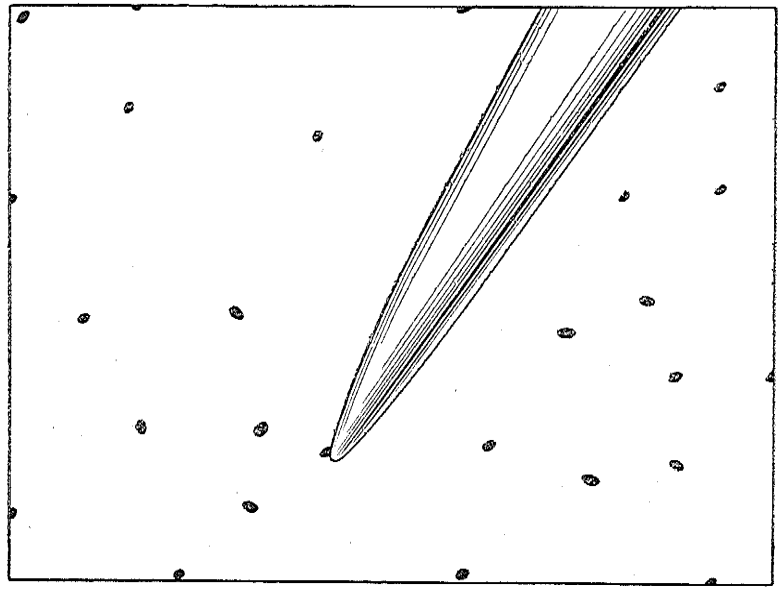


FIG. 2. Piece of a glass slide with a dry spore-deposit of *Coprinus sterquilinus*. In the middle, a dry needle-point used to pick up individual spores. A spore is attached to its tip. Magnification, 107.

a single spore adhering to its point. When the spore is seen to be separated from the slide and to be attached securely to the needle-point, it is transferred to the germinating medium simply by touching the end of the needle to the drop on the lower side of one of the cover-slips. By examining the drop under the microscope, the spore is quickly located; and thus a final and absolute proof is obtained that the particular spore selected on the slide, and no other, has been transferred to the drop. With a little practice, spores may be picked up very rapidly. With *Coprinus sterquilinus*, for example, ten monosporous cultures were made without difficulty in ten minutes.

Later on, when germination has taken place, the mycelium is removed from the cover-slip to a plate of sterile agar or gelatine. A platinum loop may be employed in making this transfer, but the best results have been obtained by using a piece of safety-razor blade about 1 cm. wide, placed in the end of a wooden handle, as shown

in Fig. 1, D. With this little instrument, which may be called an *agar spade*, the young mycelium can be removed from the cover-slip without injury. I have found by experience that the use of the agar spade in the manner just described greatly increases the chance of making transfers successfully.

The adhesion of the spore to the needle-point does not seem to be an ordinary electrical phenomenon, as spores deposited on a thin brass plate were picked up without difficulty, although both plate and needle were grounded by means of fine copper wires. The amount of moisture in the atmosphere also seems to be of no consequence, as spores could be picked up equally well in a dry or saturated atmosphere. Spores seem to behave exactly like small particles of glass, wool, or gelatin; for these, when touched, also attach themselves readily to the needle-point. This property of adhering to other objects, with which very small objects are endowed, in all probability is due to the fact that very small objects possess a very large surface relatively to their mass, in consequence of which the force of adhesion becomes of great importance relatively to the force of gravitation. In some instances the spores could be moved about on the slide without becoming readily attached to the needle-point; but, even under these conditions, it was always found possible to pick up any required spore after a few trials.

Cultures of a number of species have been successfully made with the dry-needle method. Of these species the following may be mentioned:

Coprinus sterquilinus	*Coprinus cordisporous*
,, *stercorarius*	,, *atramentarius*
,, *lagopus*	,, *Rostrupianus*
,, *curtus*	*Panaeolus campanulatus*
,, *ephemerus*	*Stropharia semiglobata*
,, *niveus*	*Bolbitius*, sp.

While all of these species belong to the Hymenomycetes, there is every reason to believe that the dry-needle method might be employed with equal success in dealing with other groups of the Basidiomycetes, as well as with many of the Ascomycetes.

SUMMARY.

A dry-needle method of making monosporous cultures of Hymenomycetes and other fungi has been described. The method is at once very simple, very rapid in its application, and extremely precise. Relatively to the poured-plate method, when it is necessary to make numerous cultures, it effects a considerable saving of time and energy and gives reliable results.

The dry-needle method was devised during the course of certain mycological researches carried out in the Department of Botany at the University of Manitoba. The work was made possible by a research scholarship granted by the Canadian Society of Technical Agriculturists. The writer wishes to express his indebtedness to Professor A. H. R. Buller for his valuable suggestions and stimulating criticism.

W. F. HANNA.

[REPRINTED FROM THE
TRANSACTIONS OF THE BRITISH MYCOLOGICAL SOCIETY,
VOL. XI, PARTS III AND IV, DECEMBER 1926.]

THE INHERITANCE OF SPORE SIZE IN COPRINUS STERQUILINUS.

(With 2 Text-figs.)

By W. F. Hanna, M.Sc. (Alberta).

I. Introduction.

The possibilities of increasing the food value and productivity of the higher plants by selection of superior individuals must have been recognised in a general way by all primitive agricultural peoples. However, the fundamental principles which are involved in the process of selection have only recently been studied. Johannsen's* classical researches on pure lines form one of the foundation stones upon which the present-day conception of selection has been built. He chose for his experiments a certain variety of the common garden bean, *Phaseolus vulgaris nana*, known as the Princess bean. In 1901, he planted a number of bean seeds of different sizes and known weights. At the end of the season each plant was harvested separately and an exact record was kept of the weight of each seed. When the weights

* Johannsen, W. Elemente der exakten Erblichkeitslehre. Jena, 1909.

of the mother beans were compared with the weights of the progeny, it was evident that the original population from which the mother beans had been selected was a mixed one and that selection had resulted in the sorting out of a number of already existing types or "pure lines." Johannsen then proceeded to determine the effect of the selection of plus and minus variants within his pure lines. In these experiments he employed nineteen pure lines, and the work was carried on for six years, from 1902 to 1907 inclusive. Each year, every pure line was represented by two lots of plants, one a plus strain grown from large beans and the other a minus strain grown from small beans. Continued selection, however, failed to produce permanent departure in either direction and the offspring of the plus and minus variants exhibited complete regression to the mean of the particular line. A series of similar experiments using the character length and breadth of seed gave the same result. Johannsen, therefore, concluded that individual variations within a pure line are not inherited and that selection within such a line is without effect.

Johannsen's experiments and those of other investigators have shown that in the higher plants the species must be regarded as consisting of various groups of individuals which possess a general similarity but which, nevertheless, differ from one another in respect to minor heritable characters. Environmental conditions may bring about further variation in the individuals making up each group or pure line; but, on the basis of Johannsen's work, modifications of this kind will not be transmitted to succeeding generations and will no longer appear when the influences which brought them into existence have ceased to operate. Heritable modifications within pure lines, therefore, can arise only by mutation.

Biologic forms are known to be of relatively common ocurrence in many species of parasitic fungi. Furthermore, Arthur*, Gäumann†, Levine‡, and others have shown that biologic specialisation is accompanied in many instances by a certain degree of morphological differentiation. By using single-spore cultures Brierley§ demonstrated the existence of a number of races, morphologically distinct, within species of *Botrytis*, *Penicillium*, and *Stysanus*. On the basis of such evidence, we may conclude that species of fungi are not homogeneous in composition but, like those of the higher plants, are made up of a number of separate races or pure lines.

* Arthur, J. C. Cultures of Uredineae in 1916 and 1917. Mycologia, IX (1917), 295–312.

† Gäumann, E. Ueber die Formen der *Peronospora parasitica* (Pers.) Fries. Beih. z. Bot. Centr. (Abth. 1), XXXV (1917–18), 395–533.

‡ Levine, M. N. A statistical study of the comparative morphology of biologic forms of *Puccinia graminis*. Jour. Agr. Res. XXIV (1923), 539–567.

§ Brierley, W. B. Experimental studies in the specific value of morphological characters in the fungi. Proc. Lin. Soc. London, Oct. 1918.

Very little experimental evidence is available as to the value of continued selection within pure lines of fungi. On *a priori* grounds it might be expected that selection in fungi would obey the same general laws as in the higher plants, *i.e.* that continued selection within a pure line would fail to bring about any permanent departure in either morphological or physiological characters. It is well known that fungi possess a high degree of variability and that, under cultural conditions, many species are extremely unstable. The possibilities of selection in fungi, therefore, would seem to be worthy of serious consideration. Burkholder* has recently shown that long-continued culturing of *Fusarium Martii Phaseoli* on artificial media may bring about changes in both morphological and physiological characters, which persist for some time after the fungus has been again transferred to its host plant. The strain with which he worked was isolated from a diseased bean root, and the first culture was made from a single spore. Six years of culture on artificial media was effective in bringing about distinct changes in the colour of the mycelium and in the size and number of the conidia produced and, in addition, the fungus had lost much of its former virulence. When this attenuated form was allowed to infect the bean plant and was then re-isolated, its original characters were partly restored and, after two such passages, the organism was found to possess all its former virulence. Rosenbaum†, on the contrary, found that selection of large conidia of *Phytophthora infestans* for five generations was ineffective in producing any change either in the size of the conidia or in the relative numbers of large and small conidia.

La Rue has carried out extensive experiments on the effect of selection in *Pestalozzia Guepini*‡ and *Helminthosporium teres*§. All the cultures used by him originated from single spores. Selections according to progeny in *Pestalozzia Guepini* were made for length of spore for ten generations and for length of spore appendages for twenty-five generations; but, at the end of the selection period, no permanent modification had been

* Burkholder, W. H. Variation in a member of the genus *Fusarium* grown in culture for a period of five years. Amer. Jour. Bot. XII (1925), 245–253.

† Rosenbaum, J. Studies of the genus *Phytophthora*. Jour. Agr. Res. VIII (1917), 233–276.

‡ La Rue, C. D. The results of selection within pure lines of *Pestalozzia Guepini* Desm. Genetics, VII (1922), 142–201.

§ La Rue, C. D. The results of selection within pure lines of the genus *Helminthosporium*. Paper read before Amer. Assoc. for the Advancement of Science, Dec. 1925.

brought about in respect to either of these characters. Similarly, when individual large and small spores were selected, in one experiment for six generations and in another experiment for ten generations, no increase or decrease in the average size of the spores was effected. The experiments with *Helminthosporium teres* were carried out for thirty generations, and the selections were made on the basis of spore length. A long line was propagated from the longest individual spore of each generation, and a short line was propagated from the shortest individual spore of each generation, while a third unselected control line was propagated from spores taken at random. The mean spore length of each generation was obtained by measuring 150 spores from each culture. Throughout the experiment the average length of the spores in the three lines paralleled one another in a remarkable manner, and the selecting of long and short spores seemed to be without effect in altering the average spore length of the species.

From the experimental evidence that is available it would appear that the spore characters of fungi are comparatively stable and cannot be readily altered by selection. Final conclusions, however, will be warranted only after a careful study of many groups of fungi.

The size, shape, and colour of the reproductive bodies of the higher fungi are generally regarded as reliable characters for systematic purposes. In the classification of the Agaricaceae much importance is placed upon macroscopic characters, such as the shape and colour of the pileus and the attachment of the gills; but spore size is also known to be of great value in the identification of species, and in most modern works on mycology spore measurements are included in the description of each species. It is well known that there may be considerable variation in the size of the spores collected from a single fruit body. Differences have also been noted in the average size of spores from different fruit bodies of the same species. Buller* determined the average diameters of the spores of three wild fruit bodies of *Amanitopsis vaginata* by measuring 50 spores from each fruit body and found them to be 10·19 μ, 10·87 μ, and 11·65 μ respectively. When systematic works are consulted for the spore dimensions of a particular species, it is frequently found that there is considerable disagreement between the measurements recorded by different authors. Differences of this kind might reasonably be expected if the spore sizes given were based on the measurement of a few spores from a single fruit body. There is also the possibility that within species of the Agaricaceae there may exist numerous strains or pure lines

* Buller, A. H. R. Researches on Fungi, I (1909), 161.

each having a characteristic size of spores. As far as the writer is aware, however, no experiments on the inheritance of spore size in this group of fungi have been made. The present paper is a contribution to this subject.

The fungus selected for experiment was *Coprinus sterquilinus*, a large coprophilous species which occurs commonly on horse dung in both Europe and North America. It has been described and fully illustrated by Buller*. Miss Mounce† has shown that this species is homothallic, a fact since confirmed by Brunswik‡.

In the experiments to be recorded later, *Coprinus sterquilinus* was successfully cultivated in monosporous culture for several successive generations. In one series of experiments each generation was started from as small a spore as possible, and in another series of experiments each generation was started from as large a spore as possible. Both series of experiments were carried out with a view to determine whether or not the ordinary fluctuating variations in spore size are inherited.

II. Methods.

In studying the effect of selection on a particular organism, careful consideration must be given to the genetic purity of the material from which selections are to be made. If a normally self-fertilised species such as the bean is employed, the progeny of a single seed may be regarded as a pure line. If, however, a cross-fertilised species is employed, selection of self-fertilised individuals for several generations will be necessary before an approximately homozygous condition is reached.

In view of recent progress in our knowledge of sex in the higher fungi, *Coprinus sterquilinus* may be regarded as a particularly suitable species for use in an experimental study of the inheritance of spore size. The work of Miss Mounce§, supplemented by the nuclear studies of Brunswik‖ has shown that a single spore of *Coprinus sterquilinus*, when germinated on dung agar, gives rise to a haploid mycelium characterised by simple septa and isolated nuclei and that, after a few days, this haploid mycelium spontaneously becomes diploid, the diplophase being indicated by the presence of clamp connections

* Buller, A. H. R. Researches on Fungi, III (1924), 177–257.

† Mounce, Irene. Homothallism and the production of fruit bodies by monosporous mycelia in the genus *Coprinus*. Trans. Brit. Mycolog. Soc. VII (1921), 198–217.

‡ Brunswik, H. Untersuchungen über die Geschlechts- und Kernverhältnisse bei der Hymenomycetengattung *Coprinus*. K. Goebel's Botanische Abhandlungen, V (1924), 1–152, Jena.

§ Mounce, Irene. Loc. cit. pp. 203–205.

‖ Brunswik, H. Loc. cit. pp. 15–19.

and the occurrence of the nuclei in pairs. The nuclei in this diploid mycelium divide conjugately. After a few weeks the diploid mycelium gives rise to perfect fruit bodies. In each young basidium there are two nuclei of opposite sex which fuse together. The fusion nucleus divides twice, as shown by Buller*, and the four nuclei pass upwards through the sterigmata into the four spores. Thus the life history of the fungus passes through all its possible stages beginning with a single spore; in other words, *C. sterquilinus* is homothallic. The progeny of a single spore of *C. sterquilinus*, therefore, may be regarded as a pure line.

Cultures of *C. sterquilinus* were made from spore deposits collected from a number of wild fruit bodies which appeared on dishes of horse dung kept in the laboratory. A spore deposit was obtained as follows. When a fruit body had begun to shed its spores, the pileus was removed by cutting through the stipe at the level of the pileus periphery. The pileus was then pinned through its centre to a small cork which had previously been attached with sealing wax to a circular glass plate. A sterilised glass slide was placed in the bottom of a crystallising dish and the glass plate, with the pileus suspended on its lower side, was placed as a cover over the dish. In this way the pileus came to hang at a height of from two to three inches immediately above the glass slide. As soon as the slide had become coated by a thin spore deposit—of such a nature that, when viewed under the microscope, the spores were seen to be fairly close together although not touching one another—it was removed, labelled, and placed in a cardboard case.

Single spores were removed from the dry spore deposit on a glass slide by the dry-needle method† and were sown singly in hanging drops of nutrient gelatine having the following composition:

Dextrose	10 gm.	Sodium chloride	5 gm
Peptone	10	Gelatine	100
Beef extract	5	Water	1000 cc.

Out of a total of 433 spores selected from 50 different fruit bodies and placed in hanging drops of gelatine for germination, 101 or 23·3 per cent. germinated. There was considerable variation in the viability of spores from different fruit bodies. In some fruit bodies the number of spores germinating was higher than 23·3 per cent., while in other fruit bodies the percentage was much lower than this and the spores could be induced to

* Buller, A. H. R. Researches on Fungi, III (1924), 208.

† Hanna, W. F. The dry-needle method of making monosporous cultures of Hymenomycetes and other fungi. Ann. Bot. XXXVIII (1924), 791–794.

germinate only with the greatest difficulty. In general, the small spores were less viable than the large ones. Of the 433 spores selected and sown 229 were between 10·7 μ and 16·7 μ in length, and of these only 16·2 per cent. germinated; the remaining 204 spores were between 17·2 μ and 23·3 μ in length, and of these 31·4 per cent. germinated. The larger spores, therefore, germinated about twice as well as the smaller ones.

Miss Mounce* has already referred to a paper by Miss Baden† on the germination of spores of *C. sterquilinus*. Miss Baden found that the spores of *C. sterquilinus* germinated only when certain bacteria about 1·2 μ in length and 0·8 μ in breadth were present in the culture medium. Other longer bacteria inhibited the growth of the mycelium. She states that "hanging-drop cultures were made with and without the bacteria. In those with the bacteria germination took place within twenty-four hours, but it never occurred at all without them. These experiments seem to show that the bacteria are in some way necessary for the germination of the spores." She further found that the spores of *C. sterquilinus* did not mature until about three weeks after they had been shed but that if dried for two days at 40° C., they would germinate at once. This she regarded as an adaptation on the part of the spores to retard germination until the substratum had become fairly dry and such fungi as *Mucor* had disappeared.

The results which Miss Baden obtained have never received any confirmation. In 1911, A. H. R. Buller and S. G. Churchward carried out a series of experiments‡ which proved conclusively that the spores of *C. sterquilinus* will germinate satisfactorily in a number of sterile synthetic media. Later, Miss Mounce§ germinated the spores and obtained fruit bodies without difficulty under sterile conditions of culture. In the present study, many strains of *C. sterquilinus* have been grown in pure culture throughout several generations. The spores were found to germinate at room temperature in hanging drops of *sterile* nutrient gelatine or dung agar. Moreover, no difference was observed in the germination of spores which had just been shed and those which had been kept for several weeks. On one occasion, ten spores which had just been shed were placed in hanging drops of sterile nutrient gelatine and within twenty-four hours all had germinated. My own observations, therefore, confirm those of Buller and Churchward and of Miss Mounce; they seem

* Mounce, Irene. Loc. cit. p. 213.

† Baden, M. L. Observations on the germination of spores of *Coprinus sterquilinus* Fr. Ann. Bot. XXIX (1915), 135–142.

‡ Communicated to me in MS. by Professor Buller.

§ Mounce, Irene. Loc. cit. pp. 203–205.

to provide convincing evidence that Miss Baden was entirely mistaken in concluding (1) that the spores of *C. sterquilinus* germinate only in the presence of certain bacteria, and (2) that they are not ready to germinate when they are shed.

Fruit bodies were obtained by transferring the monosporous mycelia to dishes of sterile horse dung. Small crystallising dishes, five inches in diameter and three inches in depth, were found to be suitable for the purpose; they were half-filled with horse dung, covered with Petri-dish covers, and autoclaved for one hour at fifteen pounds pressure. A small piece of sterile dung agar was placed on the dung in each crystallising dish and a hanging drop of gelatine with its monosporous mycelium was transferred to the dung agar. By this method fruit bodies were generally obtained twenty-five days from the time of spore germination, although cultures were frequently observed to fruit in twenty-two days. No culture fruited in less than twenty-two days.

All spore measurements were made with the Poynting Plate Micrometer. This instrument has been described and illustrated by Buller* who was the first to employ it in biological research. The instrument used for the experiments recorded in the present paper was attached to a Watson microscope equipped with a mechanical stage which could be rotated when necessary. The mechanism for rotation was not present in the original instrument used by Buller. The Poynting Plate Micrometer combines speed of manipulation with a high degree of accuracy and has given most satisfactory results throughout the present work.

The method finally adopted for measuring spores was somewhat different from that which is generally employed and, therefore, will be described in detail. In preliminary experiments the spores were mounted in a drop of water on a glass slide, covered with a coverglass, and measured wet; this is the method usually adopted. It was noticed, however, that the true lengths of some spores were difficult to obtain owing to the fact that, in water, the long axes of the spores were not parallel to the face of the glass slide. If the cover glass was pressed down slightly with a view to remedying this defect, the spores often became appreciably compressed, thus bringing about a change in the ratio of length to breadth. It was then found that *the spores could be measured much more accurately when dry than when wet.* As is recorded elsewhere, the author working in conjunction with Buller† discovered that spores of *C. sterquilinus* which have fallen from a pileus and have settled upon a dry glass slide always have their long axes parallel to the surface

* Buller, A. H. R. Researches on Fungi, I (1909), 158–163.

† Ibid. III (1924), 224–230.

of the slide, and that each spore is held in this position by a thin colourless adhesive layer on its more rounded side which is always directed towards the surface of the glass. These dry spores never alter in shape or position. When viewed under the microscope, they appear perfectly regular in outline and are perfectly still, so that they can be measured with great accuracy. All spore dimensions which are recorded in the following experiments are based on the measurement of dry spores collected on glass slides.

The dimensions which are given for the dry spores may be compared with similar measurements presented by other workers for spores mounted in water by referring to the following table:

Table I.

Condition of spores	Mean length of spores in microns	Mean breadth of spores in microns
Dry	17·4	12·6
Wet	18·6	11·8

The sizes given in Table I were calculated from the measurements of 36 spores. Each spore was measured separately on the dry slide and was then transferred with the needle to a drop of water and measured again. On the basis of these measurements, a calculation shows that dry spores, on being wetted, increase in length 6·9 per cent., and decrease in breadth 6·4 per cent.; but that the product of their length and breadth remains practically constant.

In comparing the sizes of the spores from different fruit bodies, lengths only have been considered. The breadth of the spores, however, may be calculated from the data in Table I by taking the ratio of length to breadth of dry spores as 1 : 0·72 and that of wet spores as 1 : 0·63.

The number of measurements which must be made to determine accurately the average length of the spores from a given fruit body depends upon the variability in size of the spores which are to be measured. If the range of variability is small, a few measurements may suffice; while, if the variability is great, a correspondingly larger number of measurements will have to be made. For two fruit bodies, one with an average spore length of 18·7 μ, and the other of 18·4 μ, the probable error in the measurement of 100 spores was found to be $\pm$ 0·08 μ. When groups of 20 spores were measured from the same two fruit bodies, the experimental error in determining the average spore length was found to be as high as $\pm$ 0·5 μ. In the following experiments the average lengths recorded for spores of different fruit bodies are based on the measurement of 100 spores

from each fruit body. When this number of spores is employed, the error in determining the average length should be small even for fruit bodies with a wide range of variability in spore size.

III. Variation in the Size of Spores of Different Wild Fruit Bodies.

An examination of spore deposits of *Coprinus sterquilinus* collected from wild fruit bodies, which appeared upon horse dung cultures in the laboratory, showed that a wide variation in size may be found among the spores from a single fruit body. Considerable differences were also observed in the mean size of the spores produced by individual fruit bodies. The extent of these variations is brought out in Table II which gives the mean spore size and the limits in variation in spore size for five wild fruit bodies.

Table II.

Fruit body	Mean spore length in microns	Range of variation in spore length in microns
1	19·9	17·7–21·9
2	18·7	16·3–21·4
3	18·7	13·5–21·4
4	16·1	13·0–19·5
5	15·9	11·6–18·1

Table III.

Authority	Length in microns	Breadth in microns
Baden†	15–18*	8–12
Carleton Rea‡ ...	14–23	9–14
Kauffman§	18–25	—
Murrill‖	18	12
Ricken¶	18–22	12–14

* The length actually given is 0·15 mm.–0·18 mm., but this is obviously intended for 0·015 mm.–0·018 mm.

† Baden, M. L. Loc. cit. p. 140.

‡ Rea, Carleton. British Basidiomycetae, p. 501. Cambridge, 1922.

§ Kauffman, C. H. The Agaricaceae of Michigan, Lansing, I (1918), 211.

‖ Murrill, W. A. Mycologia, III (1911), 167.

¶ Ricken, A. Die Blätterpilze, I, 57. Leipzig, 1915.

As may be seen from an inspection of Table II, the limits of variation in the lengths of individual spores from different fruit bodies are from 11·6 μ to 21·9 μ, while the mean lengths of the spores from different fruit bodies range from 15·9 to 19·9 μ. Converted into lengths of wet spores with the help of the data for dry and wet spores given in connection with Table I, the lengths just referred to become 12·4 μ to 23·4 μ, and 17·0 μ to 21·3 μ respectively. It is, therefore, quite evident that *the measurement of any number of spores from a single fruit body would be entirely insufficient to give a true picture of either the range in variation or the mean size of the spores for the species as a whole.*

Reference to a number of works showed that mycologists are by no means agreed as to the size of the spores of *C. sterquilinus*. The size of the spores, as given by several authorities, is set down in Table III.

The range of spore length given is from 14 μ (Carleton Rea) to 25 μ (Kauffman). Whether the limits of variation given by these authorities apply to the spores of a single fruit body or to those of several fruit bodies is uncertain and, in the absence of such information, these data are of limited value.

IV. Experiments on the Inheritance of Spore Size.

To determine whether or not individual variations in spore size are inherited in succeeding generations, single spore selections were made from the wild fruit bodies Nos. 1 to 5 of Table II and from another fruit body not included in this table. The results of these experiments are shown in Table IV. From fruit body No. 1, large spores were selected for five generations; from fruit body No. 2, large spores were selected in three lines of experiment and small spores in two lines for five generations; from fruit body No. 3, small spores were selected for three generations; from fruit bodies Nos. 4 and 5, small spores were selected for two generations; from fruit body No. 6, small spores were selected for five generations. The original spore deposit of fruit body No. 6 was unfortunately lost before measurements from it could be made.

As may be inferred from a study of Table IV, no particular relationship appears to exist between the size of the spore selected and the mean size of the spores from the resulting fruit body. Furthermore, although the original fruit bodies from which selections were made varied considerably in respect to the size of their spores, these differences do not appear to have been inherited as they have not been consistently retained in the succeeding generations.

The results presented in Table IV may be studied more easily by comparing the mean size of the spores from all the fruit bodies produced by monosporous mycelia arising from large spores with similar data for all the fruit bodies produced by monosporous mycelia arising from small spores. This information is summarised in Tables V and VI.

The mean length of the spores from the 21 fruit bodies resulting from large-spore selections was 18·1 μ, while an equal number of fruit bodies resulting from small-spore selections had a mean spore length of only 17·7 μ. At first sight these data seem to

Table IV. *The effect of selecting large and small spores of* Coprinus sterquilinus *throughout several successive generations.*

( ) indicates length of mother spore in microns. O indicates mean length of 100 spores from the progeny fruit body.

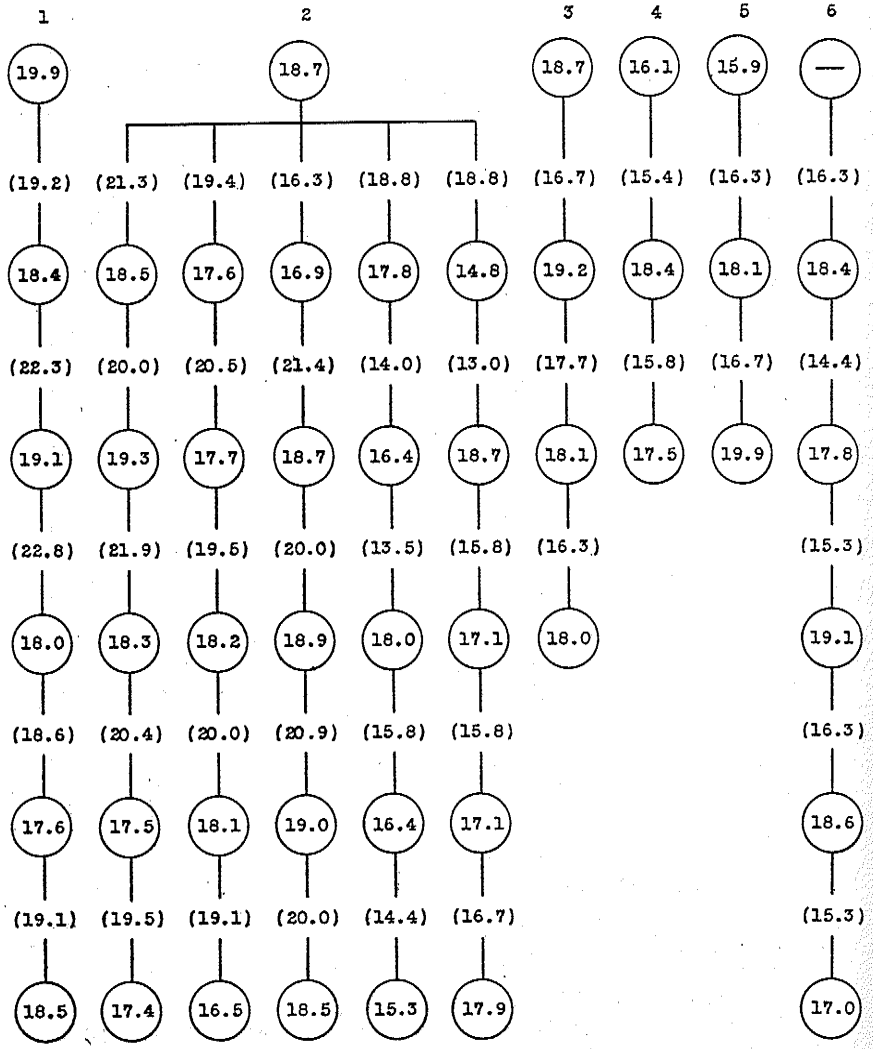


Table V. *Fruit bodies produced by monosporous mycelia arising from large spores.*

Generation	Number of fruit bodies	Mean spore length of progeny fruit bodies in microns
1	5	17·4
2	4	18·7
3	4	18·4
4	4	18·1
5	4	17·7

Table VI. *Fruit bodies produced by monosporous mycelia arising from small spores.*

Generation	Number of fruit bodies	Mean spore length of progeny fruit bodies in microns
1	5	18·2
2	6	18·1
3	4	18·1
4	3	17·4
5	3	16·7

suggest that the selection of small spores has had a slight effect in reducing the size of the spores in the progeny. However, when considered in relation to the differences in the mean size of the spores from individual fruit bodies, the effect of selection in modifying spore size appears to be without significance.

The coefficient of correlation between the size of the mother spores and the mean size of the spores produced by the progeny fruit bodies was calculated by Karl Pearson's formula. The value obtained was 0·257 ± 0·097. When considered in relation to the probable error, the evidence of correlation is very slight. The following conclusions may therefore be drawn: (1) the original wild fruit bodies from which selections were made did not represent a number of strains possessing spores of a particular size, and the differences in spore size which they exhibited were merely the result of fortuitous variations which were not inherited in succeeding generations; and (2) the continued selection of large and small spores for five generations did not result in any material increase or decrease in the size of the spores produced by the progeny fruit bodies.

In view of the experiments which have just been described, spore size may be regarded as a stable character for systematic purposes provided sufficient consideration is given to variability in the size of spores from individual fruit bodies.

The large number of spore measurements which were made in studying the inheritance of spore size in *C. sterquilinus* provide very suitable material for an analysis of the variations in spore size in this species, and an account of these results will now be given.

V. Variation in Spore Size during the Spore-discharge Period.

Considerable variation may be found in the mean size of the spores collected from a fruit body at different times during the spore-discharge period. Measurements of the mean length of spores collected from three fruit bodies at the beginning, middle, and end of the spore-discharge period are given in Table VII.

Table VII.

Period of spore discharge	Fruit body *A* Mean length of spores in microns	Fruit body *B* Mean length of spores in microns	Fruit body *C* Mean length of spores in microns
Beginning ...	17·4	17·3	18·7
Middle	18·4	17·7	18·7
End	20·0	16·9	19·1

From the data embodied in Table VII it is clear that there is considerable variation in the mean length of the spores produced by a fruit body at different times during the spore-discharge period. While in fruit bodies *A* and *C* there is an increase in the size of the spores produced at the end of the spore-discharge period, in fruit body *B* there is a decrease. To what these differences are due is by no means evident. That spores which develop under conditions of desiccation may be subnormal in size has been shown by Cotton* who found that there was a gradual diminution in the size of the spores shed by a pileus of *Stropharia semiglobata* which had been severed from its stipe and placed in the warm dry air of a room: the spores collected during the first hour of spore discharge measured 18 μ in length, while those collected in the eighty-third hour measured only 12 μ in length and were pale in colour. In the present investigation the spore deposits of *Coprinus sterquilinus* were collected from pilei suspended in closed crystallising dishes, so that very little drying of the gills, if any, could have taken place. Moreover, in *Coprinus sterquilinus*, as shown by Buller†, the spores on the gills all attain their maximum size before the pileus expands and begins to shed its spores from below upwards. It is clear, therefore, that transpiration during the spore-discharge period cannot have affected the size of the spores liberated. The spores in the Coprini ripen on each gill in succession from below upwards and are shed from below upwards; so that the spores collected from a fruit body at the beginning, middle, and end of the spore-discharge period are derived respectively from the

* Cotton, A. D. On the production of imperfectly developed spores in the Agaricaceae. Trans. Brit. Mycolog. Soc. IV (1914), 298–300.
† Buller, A. H. R. Researches on Fungi, III (1924), fig. 73, p. 184.

lower parts, middle parts, and upper parts of the gills. Possibly, therefore, the variations in spore size given in Table VII were due to an irregular flow of food materials to the hymenium during its development.

VI. A Correlation between the Size of the Spores and the Width of the Pileus.

The size of the spores produced by a fruit body is correlated to some extent with the size of the pileus of the fruit body. Buller* found that the spores of dwarf fruit bodies of *Coprinus lagopus*, while of about the same breadth as those of larger fruit bodies, are distinctly shorter. He has also shown† that the hairy scale cells on the pilei of fruit bodies of *Coprinus lagopus* are larger in large fruit bodies than in small fruit bodies. Table VIII shows the relation between the diameter of the pileus and the size of the spores produced for twenty-three fruit bodies of *C. sterquilinus*.

Table VIII.

Number of fruit bodies considered	Diameter of pileus in cm.	Mean length of spores in microns
2	1	15·5
2	5	16·8
5	6	18·3
4	7	18·4
9	8	18·2
1	9	18·3

From a consideration of the data set forth in Table VIII we may conclude: (1) that fruit bodies with small pilei produce small spores; (2) that the size of the spores increases with the diameter of the pileus until the latter reaches 7–8 cm. which is normal for the species; and (3) that a further increase in the diameter of the pileus is not correlated with any further increase in the size of the spores.

VII. Viability of Large and Small Spores.

It is evident from the data which have already been presented that spores of *C. sterquilinus* vary greatly in size. In the many spore deposits examined, the largest spore found measured 23·3 μ in length, while the smallest spore found measured 10·7 μ in length. The largest and smallest spores observed to germinate were respectively 22·8 μ and 12·6 μ in length. Extremely large or extremely small spores must be regarded, therefore, not as abnormal or imperfectly formed

* Buller, A. H. R. Researches on Fungi, II (1922), 86.
† Ibid. III (1924), fig. 141, p. 320.

structures, but as viable reproductive bodies capable of performing their normal functions.

As already pointed out in Section II, larger spores were found to germinate about twice as well as smaller ones. Hence we must conclude that small spores are less viable than large ones.

VIII. The General Range of Variation in Spore Size for the Species.

The extent of the variation in size of the spores of *C. sterquilinus* is shown by the frequency curve in Fig. 1. This curve was constructed from measurements of the lengths of a hundred

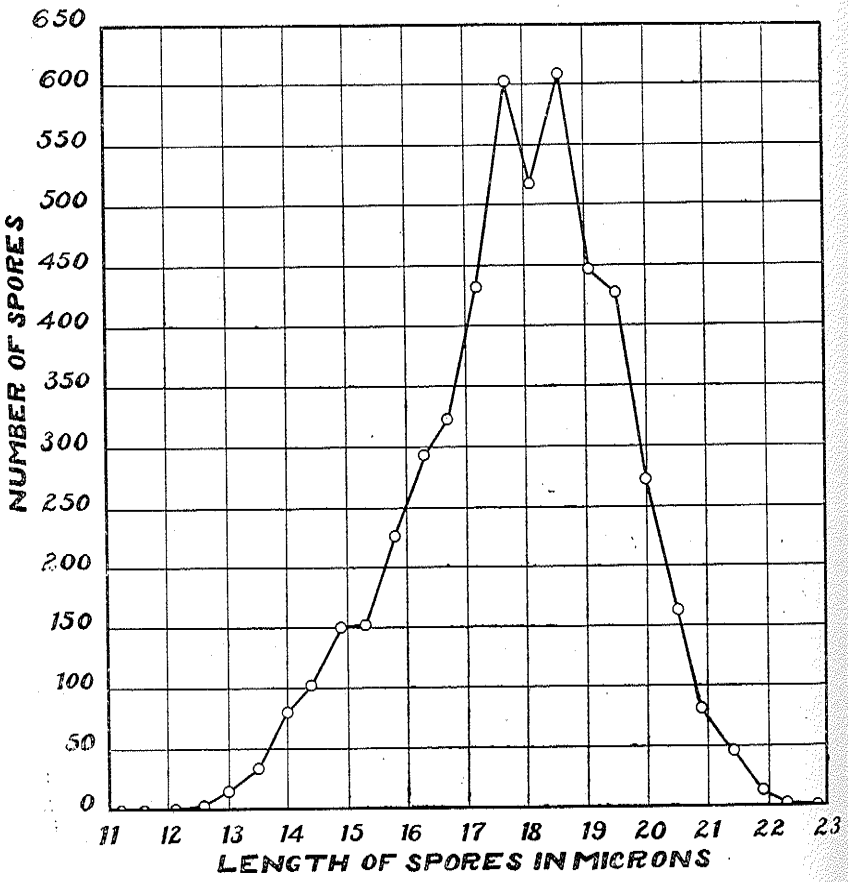


Fig. 1. Frequency distribution of spores from fifty fruit bodies of *Coprinus sterquilinus*, according to spore length. One hundred spores from each fruit body were measured. The class interval is 0·465 μ.

spores from each of fifty fruit bodies. As is shown graphically, the 5000 spores measured vary in length from 11·2 μ to 22·8 μ with a mean at 17·8 μ. Notwithstanding the large number of spores measured, the distribution is very irregular. This unevenness, undoubtedly, is due to the fact that the curve is multimodal in nature, with the spores from each fruit body, or from each group of similar fruit bodies, occupying a particular position on the curve.

Fig. 1 presents a general picture of the complete range of variation in spore size for the species, but gives little information as to the frequency distribution of the spores from the different types of fruit bodies which go to make up the compound curve. In view of the difficulty of showing frequency curves for a range of fruit bodies having modes for spore length from 14·4 μ to 20·5 μ, only three types of fruit bodies will be considered: (1) those having modes for spore length of 14·4 μ–15·3 μ; (2) those having modes for spore length of 15·8 μ–16·7 μ; and (3) those having modes for spore length of 20·0 μ–20·5 μ. The first group is made up of four fruit bodies, the second of three fruit bodies, and the third of three fruit bodies; and 100 spores from each fruit body were measured. The three types of frequency distribution are shown in Fig. 2. Two of the curves are asymetrical in form; the curve for spores from large-spored fruit bodies has a negative skew, while that for spores from small-spored fruit bodies has a positive skew. The distribution of the spores from fruit bodies having spores with modes for length from 15·8 μ–16·7 μ approaches in form the normal frequency curve. A measure of the skewness may be obtained from the formula $\frac{\text{mean mode}}{\sigma}$, where σ refers to the standard deviation. The coefficients of skewness, as calculated from this formula, are as follows:

Group 1 (Modes 14·4 μ–15·3 μ) = + 0·294
,, 2 (Modes 15·8 μ–16·7 μ) = 0
,, 3 (Modes 20·0 μ–20·5 μ) = − 0·557

In fruit bodies having modes for spore length of 14·4 μ–15·3 μ, the lower limit of spore size seems to have been reached. If smaller spores than these were to form on the gills of a fruit body of *C. sterquilinus*, they would probably not mature and, therefore, would not be shed by the fruit body. For this reason, a frequency curve for spores from such fruit bodies falls off rapidly on the lower side and gradually on the upper side, as is shown by the coefficient of skewness. In fruit bodies having modes for spore length of 15·8 μ–16·7 μ, a wide range of variation is possible both below and above the mode, so that spores from

such fruit bodies show a normal frequency distribution. In fruit bodies having modes for spore length of 20·0 μ–20·5 μ, the upper limit of spore size is approached and variation is possible only in the direction of smaller spores; a frequency distribution

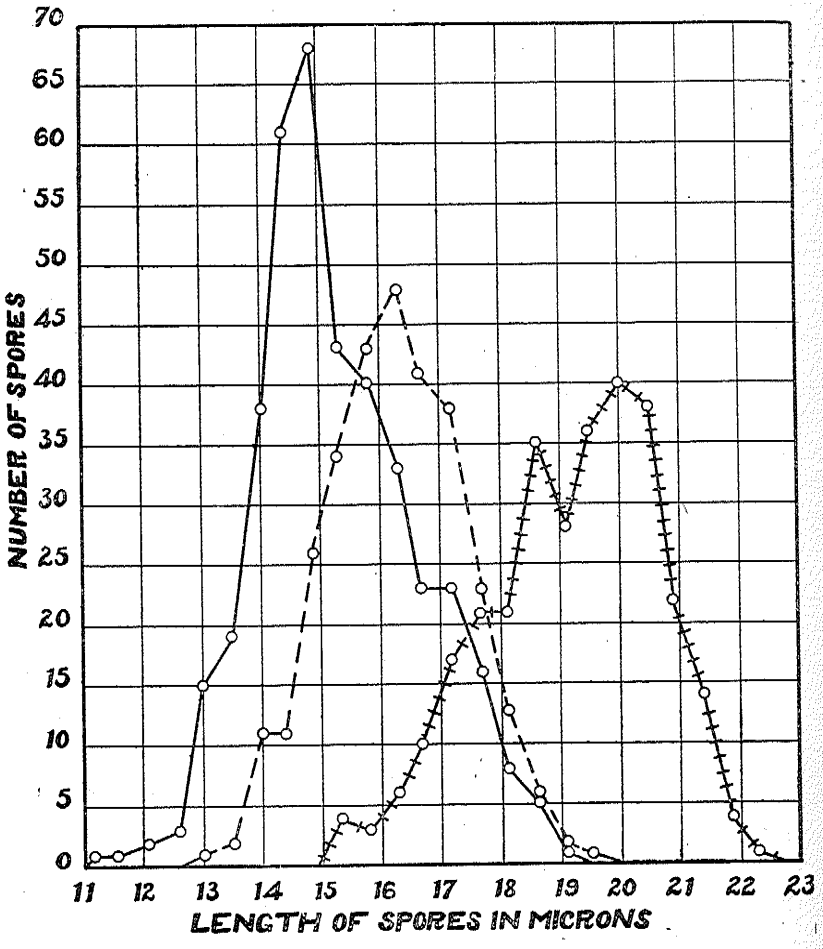


Fig. 2. Frequency distribution of spores from three groups of fruit bodies of *Coprinus sterquilinus*, according to spore length. Group 1, four fruit bodies with modes for spore length 14·4 μ–15·3 μ; group 2, three fruit bodies with modes for spore length 15·8 μ–16·7 μ; group 3, three fruit bodies with modes for spore length 20·0 μ–20·5 μ. One hundred spores from each fruit body were measured. The class interval is 0·465 μ.

for spores from such fruit bodies, therefore, shows negative skewness. If the fruit bodies which have been studied had

represented distinct races of *C. sterquilinus* differing from one another in respect to the size of their spores and breeding true for such a character, these distinct types of frequency distribution would not have been expected to occur. The nature of the variations in spore size in the fruit bodies studied, therefore, would seem to provide additional evidence that fruit bodies of *C. sterquilinus* which produce large spores do not differ genetically from those which produce small spores. Variations in spore size must be attributed rather to the particular physiological conditions under which the fruit bodies develop.

IX. Summary.

1. An experimental study has been made of the inheritance of spore size in *Coprinus sterquilinus*, one of the homothallic Hymenomycetes.

The character on which selection was based was length of spore. The original spore selections were made from six wild fruit bodies. In one series of experiments, each generation was propagated from the smallest spore which could be found; in another series of experiments, each generation was propagated from the largest spore which could be found. Selections from three fruit bodies were carried on for five generations, those from one fruit body for three generations, and those from two fruit bodies for two generations. Records were kept of the length of each mother spore, and of the mean length of the spores from the progeny fruit body of each monosporous mycelium. All spores were measured when *dry* with the Poynting Plate Micrometer.

2. The attempt to produce large-spored strains and small-spored strains in pure lines of *Coprinus sterquilinus* by the continuous selection of large and small spores respectively failed. No satisfactory evidence of the inheritance of individual variations in spore size was found. In this respect the spores of *Coprinus sterquilinus* resemble the seeds of the garden bean *Phaseolus vulgaris nana* investigated by Johannsen, the conidia of *Phytophthora infestans* investigated by Rosenbaum, and the spores of *Pestalozzia Guepini* and *Helminthosporium teres* investigated by La Rue.

3. There is considerable variation in the mean length of the spores produced by a fruit body at different times during the spore-discharge period.

4. Fruit bodies having small pilei produce smaller spores than those having pilei of the normal size or larger.

5. Since the mean size of the spores of different fruit bodies of one and the same species of Hymenomycete vary considerably, the spore size given by systematists for determining hymenomycetous species ought to be based on measurements of the spores of a number of fruit bodies obtained in different places.

6. The largest and smallest spores found were respectively 23·3 μ and 10·7 μ in length; the largest and smallest spores observed to germinate were respectively 22·8 μ and 12·6 μ in length.

7. The percentage germination of spores between 17·2 μ and 23·3 μ in length was 31·4; that of spores between 10·7 μ and 16·7 μ in length was only 16·2. Thus larger spores were found to germinate twice as well as smaller ones.

8. Frequency curves of spore length are presented for fruit bodies having modes for spore length of (1) 14·4 μ–15·3 μ, (2) 15·8 μ–16·7 μ, and (3) 20·0 μ–20·5 μ. The relation between the forms of these curves and the nature of the variation in spore size in fruit bodies of *Coprinus sterquilinus* is discussed.

9. Miss Baden's conclusions (1) that spores of *Coprinus sterquilinus* germinate only in the presence of certain bacteria and (2) that they are not ready to germinate when they are shed have not been confirmed.

The foregoing investigation was carried out in the Botanical Laboratory of the University of Manitoba during the tenure of a scholarship awarded by the Canadian Society of Technical Agriculturists; a grant in aid of this work was also made by the Canadian Honorary Advisory Council for Scientific and Industrial Research. The problem was suggested by Professor A. H. R. Buller, whose valuable advice and stimulating criticism is gratefully acknowledged.