HOMOTHALLISM AND HETEROTHALLISM IN THE GENUS COPRINUS.

by

Irene Mounce.

M. Sc. 1929

Homothallism and Heterothallism in the Genus Coprinus.

By Irene Mounce, M.A. (British Columbia);
Hudson's Bay Company Research Fellow,
University of Manitoba.

I. Introduction.

In 1918 Mlle Bensaude[1] brought forward experimental and cytological evidence which appeared to prove that Coprinus fimetarius is heterothallic; and in 1919 Hans Kniep[2], after making a similar investigation, came to the conclusion that heterothallism is characteristic of Schizophyllum commune and of a number of other Hymenomycetes.

In 1921, in a paper published in these Transactions, I recorded that clamp-connections had regularly appeared in mycelia of monosporous origin in Coprinus sterquilinus, C. stercorarius, C. lagopus, and C. niveus, and that I had therefore come to the conclusion that all these four species are homothallic.[3] This paper was sent to England for publication in May, 1921. The following autumn, on resuming my investigations, I made a large number of monosporous cultures of Coprinus lagopus but found to my surprise that in not a single one of them did any clamp-connections make their appearance. The spore-deposits used for making

1) Mathilde Bensaude, Recherches sur le cycle évolutif et la sexualité chez les Basidiomycètes, Nemours, 1918, pp. 1-156, nine plates.

2) Hans Kniep, Über morphologische und physiologische Geschlechtsdifferenzierung, Verhandl. der Physikal.-med. Gesellschaft zu Wurzburg, 1919, pp. 1-18.

3) Irene Mounce, Homothallism and the Production of Fruit-bodies by Monosporous Mycelia in the Genus Coprinus, Trans. Brit. Mycolog. Soc., Vol.vii, Dec., 1921, pp. 198-217.

the cultures described in my previous paper had been destroyed, so that it was unfortunately impossible to investigate them again. The spores in my new cultures, therefore, had a different origin to those previously employed. Suspecting that the strains of the fungus used in my new investigation were heterothallic, I tried the effect of pairing the mycelia. The results of this operation were similar to those obtained by Mlle Bensaude and Hans Kniep; certain pairs of mycelia produced clamp-connections in large numbers and others never produced any clamp-connections at all. Moreover, the pairs of mycelia which produced clamp-connections gave rise to perfect fruit-bodies, whilst those pairs which did not product clamp-connections gave rise only to imperfect fruit-bodies. I therefore came to the conclusion that the strains of Coprinus lagopus used for my new investigation were heterothallic. It then seemed highly desirable that I should re-investigate the mycelia of Coprinus sterquilinus, C. stercorarius, and C. niveus. I therefore made a new series of cultures for each of these species. With Coprinus sterquilinus and C. stercorarius, I obtained results exactly similar to those already recorded: clamp-connections appeared regularly on all the mycelia of monosporous origin. My conclusion that these two species are homothallic was therefore confirmed and strengthened. With Coprinus niveus, just as with C. lagopus, I found that in a series of monosporous cultures not a single mycelium gave rise to clamp-connections, but that, on pairing the mycelia, some pairs produced clamp-connections abundantly and some pairs no clamp-connections whatever. I have therefore convinced myself that the strain of Coprinus niveus used for my new investigation is heterothallic.

The object of this paper is to bring forward further evidence in support of my previous conclusion that Coprinus sterquilinus and C. stercorarius are homothallic and to show that Coprinus lagopus and C. niveus are not always homothallic, as I at first believed, but are often, and perhaps as a rule, heterothallic.[1)]

1) These results were communicated to the Mycological Section of the American Botan-

II. Criteria of Sex.

This paper is to be regarded as a continuation of the one already published. Here, therefore, it will not be necessary to discuss the criteria for distinguishing a homothallic species of Coprinus from a heterothallic one in detail; and it will be sufficient for our present purpose to remind the reader of the following facts. Mlle Bensaude and Hans Kniep, working independently, have shown that the presence of clamp-connections on a mycelium is associated with dicaryons and the conjugate division of the nuclei. Clamp-connections, therefore, are an outward and visible sign that the mycelium on which they arise is in the diploid and not the haploid condition. A species of Coprinus producing clamp-connections is homothallic if clamp-connections are regularly formed on mycelia of monosporous origin, or if clamp-connections are formed on compound mycelia arising from spores all of which have been derived from a single fruit-body produced from a mycelium of monosporous origin. A species of Coprinus producing clamp-connections is heterothallic if clamp-connections are not formed on mycelia of monosporous origin but only upon compound mycelia produced by the union of two monosporous mycelia presumably of opposite sex.

Mlle Bensaude, working with Coprinus fimetarius, came to the conclusion that, in a heterothallic species, monosporous mycelia are always sterile. However, Kniep has discovered that, in Schizophyllum commune and certain other species of Hymenomycetes which are heterothallic, fruit-bodies sometimes appear on mycelia of monosporous origin and of haploid nature, one nucleus finally entering each basidium instead of two. Fruit-body production in a heterothallic species, therefore,

1) Cont.
ical Society at the Toronto meeting of the American Association for the Advancement of Science, December, 1921, in a paper entitled: Homothallism and Heterothallism in the Genus Coprinus; but no abstract of this paper has been published.

is not necessarily bound up with the diploid condition of the mycelium; so that if, on experimenting with a new species, one finds that a fruit-body is produced on a monosporous mycelium, one is not justified by that fact alone in regarding the species as homothallic. Nevertheless, from my own experience with heterothallic strains of Coprinus lagopus and C. niveus, Mlle Bensaude's experience with Coprinus fimetarius, and Knæp's experience with Schizophyllum commune, I am of the opinion that, in a heterothallic species, fruiting takes place either not at all or much less readily and less perfectly on a monosporous mycelium than on a compound mycelium formed by the union of two mycelia presumably of opposite sex.

III. Methods.

The isolation of mycelia of monosporous origin by plating out spores in dung agar was accomplished in the manner already described in my first paper. However, owing to the provision of a new and improved autoclave, the culture medium was sterilised at 15 pounds pressure instead of 7 pounds.

The pairing of mycelia of monosporous origin in the tests for heterothallism was effected as follows. The monosporous mycelia were first grown separately upon dung-agar plates or in wide test-tubes containing horse dung, and then portions of the mycelia were placed in pairs on dung-agar plates. In making an inoculation from two plate cultures, a small piece of mycelium-covered agar about 7 - 10 mm. square was removed by means of a sterile platinum loop from each of two plates, and these two pieces of agar were placed about 2 cm. apart in the middle of a freshly-poured dung-agar plate. In making an inoculation from two dung-tubes, the procedure was the same, except for the fact that the two pieces of mycelium-covered dung were removed from the tubes by means of sterilised forceps instead of by a platinum loop.

After portions of two monosporous mycelia had been deposited near one another in an agar plate, the hyphae soon began to grow radially outwards through the agar; and, after a few days, the hyphae of the two mycelia came into contact in a line passing through the centre of the plate, so that fusions could take place between them. At the end of 7 - 14 days from pairing, the compound mycelium was examined under the high power of the microscope for the presence or absence of clamp-connections.

In the heterothallic strains of Coprinus lagopus and of C. niveus, a primary (haploid) mycelium differs from a secondary (diploid) mycelium in several ways. (1) A primary mycelium produces oidia in great abundance but a secondary does not. (2) A primary mycelium does not bear clamp-connections, whereas a secondary one does on all the stouter hyphae. (3) The branching of a primary mycelium is relatively irregular, whereas in a secondary mycelium it takes place on all the leading hyphae at a definite angle. (4) It was also noticed that in a primary mycelium the aerial hyphae are more abundant and, therefore, collectively, are more woolly in appearance than in a secondary mycelium. Owing to these differences between a primary and a secondary mycelium, one could usually tell microscopically, 7 -14 days after pairing, whether the sexes of any two paired mycelia were the same or different. Microscopic observation as to the presence or absence of clamp-connections, therefore, usually confirmed what had been surmised by examination with the naked eye.

IV. Coprinus sterquilinus.

The series of monosporous cultures of Coprinus sterquilinus of which I gave an account in my first paper has been extended, and now fruit-bodies have been obtained from monosporous mycelia for seven successive generations. When grown under similar conditions, the mycelia of the seventh monosporous generation were found to fruit just as readily and as perfectly as compound mycelia derived from many spores of a wild fruit-body.

Clamp-connections were found in the new cultures: (1) on each of two mycelia which originated from single spores produced by a fruit-body of the sixth successive monosporous generation, and (2) on each of four mycelia which originated from single spores produced by a fruit-body of the seventh successive monosporous generation. These results are in complete accord with those given in my first paper.

The additional facts just described, which again show that monosporous mycelia produce clamp-connections and fruit readily and perfectly, afford strong confirmatory evidence of the correctness of the conclusion to which I came in my first paper, namely, that Coprinus sterquilinus is homothallic.

V. Coprinus stercorarius.

In a new series of monosporous cultures of Coprinus stercorarius, clamp-connections were formed: (1) on each of three mycelia which originated from single spores of a wild fruit-body, and (2) on each of six mycelia which originated from single spores produced by a fruit-body of monosporous origin. All these nine mycelia produced clamp-connections two days after isolation and transference to a new plate. They, therefore, behaved exactly like the monosporous mycelia described in my first paper.

Three of the mycelia of the second series (2) were allowed to continue their development on dung-agar plates, and there they produced small perfect fruit-bodies which shed spores. It thus was proved that monosporous mycelia of Coprinus stercorarius, even of the second monosporous generation, are able to fruit in a perfectly normal manner.

The additional facts just described, which again show that monosporous mycelia produce clamp-connections and fruit readily and perfectly, afford strong confirmatory evidence of the correctness of the conclusion to which I came in my first paper, namely, that Coprinus stercorarius is homothallic.

Brefeld[1] shows clamp-connections as occurring on a monosporous mycelium of C. stercorarius. His observations and my own are, therefore, in accord. On the other hand, Kniep,[2] without giving details of his evidence, states that this fungus is heterothallic. It is, therefore, possible that there may be in existence both homothallic and heterothallic strains of C. stercorarius. Further experiment with diverse strains obtained from different localities can alone teach us the truth about this matter.

1) O. Brefeld, Untersuchungen, Leipzig, Heft III, 1877, p. 206, under Fig. 3, b; also Taf. I, Fig 3, b.

2) Hans Kniep, loc. cit., p. 13.

VI. Coprinus lagopus.

In the series of experiments upon Coprinus lagopus recorded in my first paper I found that clamp-connections developed: (1) on each of several mycelia of monosporous origin derived from the spores of a wild fruit-body; (2) on each of three mycelia of monosporous origin derived from spores produced by a fruit-body of monosporous origin; and (3) on a compound mycelium of polysporous origin derived from many spores produced by a fruit-body of monosporous origin. I therefore came to the conclusion that C. lagopus is homothallic. There was nothing to suggest that this species might be heterothallic.

After sending my first paper to the press in May, 1921, I isolated another series of monosporous mycelia of C. lagopus and, as I was leaving Winnipeg for Vancouver, took the cultures with me. To my great surprise, I found that these mycelia did not produce any clamp-connections. During the summer of 1921, circumstances prevented me from making any further experiments; but, on returning to Winnipeg in September, I at once began to investigate Coprinus lagopus again with the object of solving the problem of the sex of the mycelia in this species.

Altogether, since writing my first paper, I have made fifty-nine new monosporous cultures of C. lagopus. Owing to the fact that some of the spore-deposits of C. lagopus had been destroyed after the completion of my first paper, I was compelled to use new spore material. The new spores were provided by ten different fruit-bodies. To the best of my knowledge the mycelia were isolated in exactly the same manner as formerly. They were all transferred to agar plates and, later, twenty-nine of them were transferred to sterilised dung in wide test-tubes; and they were all kept in pure culture for fifty days and most of them for from two to three months. Examination with the microscope yielded a result just the opposite of that found in the experiments recorded in my first paper, for not a single one of all the fifty-nine monosporous mycelia produced any clamp-connections whatever.

On the other hand, polysporous mycelia derived from several spores produced clamp-connections within four or five days after the spores were sown.

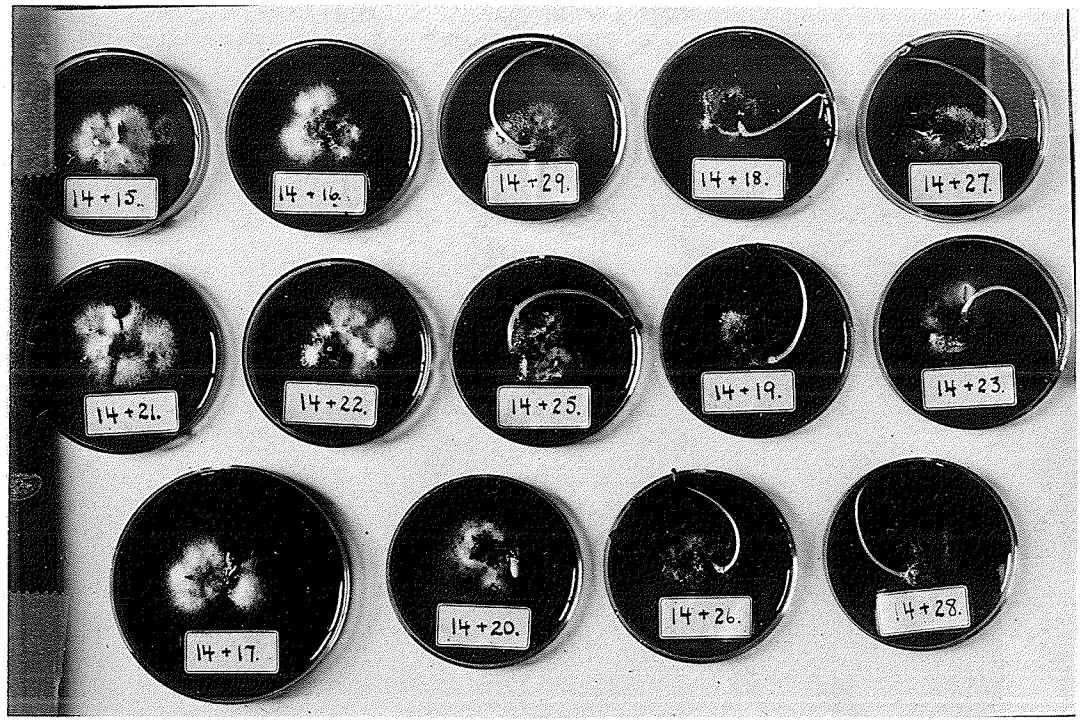
These new observations naturally suggested that the strains of Coprinus lagopus with which I was working were heterothallic. Therefore, assuming that the monosporous mycelia were all unisexual and would behave in the same manner as Blakeslee's (+) and (-) strains of Mucor and as Mlle Bensaude's (+) and (-) strains of her Coprinus fimetarius, I paired twenty-three of the mycelia with no. 14 and seven with no. 46, nos. 14 and 46 being taken as standards. The results of making these thirty pairs are shown in Table I. In the fourth column of this Table the blanks indicate that no observations with regard to fruiting were ~~made.~~ recorded.

Table I.

Coprinus lagopus: The Effect of Pairing Monosporous Mycelia.

Mycelial pairs	Sexual signs	Clamp-connections 4 - 7 days after pairing	Fruit-body development in the plates.
3 x 14	+ +	absent	imperfect
4 x 14	+ +	absent	imperfect
6 x 14	+ +	absent	imperfect
7 x 14	+ +	absent	imperfect
9 x 14	+ +	absent	imperfect
10 x 14	- +	present	perfect
11 x 14	- +	present	———
12 x 14	- +	present	perfect
13 x 14	+ +	absent	———
15 x 14	+ +	absent	imperfect
16 x 14	+ +	absent	imperfect
17 x 14	+ +	absent	imperfect
18 x 14	- +	present	perfect
19 x 14	- +	present	perfect
20 x 14	- +	present	perfect
21 x 14	+ +	absent	imperfect
22 x 14	+ +	absent	imperfect
23 x 14	- +	present	perfect
25 x 14	- +	present	perfect
26 x 14	- +	present	perfect
27 x 14	- +	present	perfect
28 x 14	- +	present	perfect
29 x 14	- +	present	perfect
47 x 46	- -	absent	———
48 x 46	- -	absent	———
51 x 46	+ -	present	———
52 x 46	+ -	present	———
[illegible]	+ -	present	———
54 x 46	+ -	present	———
55 x 46	+ -	present	———

Figure I.



Coprinus lagopus. Results of pairing monosporous mycelia Nos. 15-29 with No. 14. Nos. 18, 19, 20, 23, 25, 26, 27, 28, and 29 formed clamp-connections with No.14, and the remaining five did not. The nine pairs which formed clamp-connections also produced normal fruit-bodies as the photograph shows, but the five did not fruit normally. When the photograph was taken all the fruit-bodies, except that in the culture Nos. 14 x 20, had shed their spores, but the spore deposits and stipes are still visible. About one-third natural size.

Of the thirty pairs of mycelia, seventeen developed clamp-connections in a regular manner, while thirteen did not develop any clamp-connections whatever. It was, therefore, supposed that a(+) and a (-) sexual strain must have been present in each of the seventeen pairs in which clamp-connections had appeared. No. 14 was given a (+) sign, but this was done quite arbitrarily, for morphologically no. 14 did not differ from the other mycelia. The mycelia with which no. 14 had produced clamp-connections were then given a (-) sign, and the mycelia with which no. 14 had not produced clamp-connections were given a (+) sign. Subsequently it was found that clamp-connections were formed when no. 46 was mated with no. 14. Since no. 14 had a (+) sign, it was therefore necessary to give no. 46 a (-) sign. Nos. 51-55 all produced clamp-connections with no. 46. Hence they were given a (+) sign; and, since nos. 47 and 48 did not yield clamp-connections with no. 46, they were given a (-) sign. All these signs are shown in Table I.

The pairs of mycelia which developed clamp-connections also developed normal fruit-bodies. See figure I. About a week after the matings had been effected, these fruit-bodies expanded and shed an abundance of their black spores. On the contrary, the pairs of mycelia which remained in the primary condition and did not develop clamp-connections, gave rise only to imperfect fruit-bodies. These varied from about the size of a pin's head to rudiments 3 - 4 mm. high. In the larger rudiments, the stipe and pileus were clearly differentiated, but no expansion took place and no spores were ever liberated. It was therefore clear that rapid and perfect production of fruit-bodies was associated in my cultures with the formation of clamp-connections, i.e. with the secondary or diploid condition of the mycelium, and that the production of imperfect fruit-bodies was associated with the non-formation of clamp-connections, i.e. with the primary or haploid condition of the mycelium.

After the results embodied in Table I had been obtained, it was to be expected that mycelia of like signs, if paired, would not produce any clamp-connections. Each of six plates was therefore inoculated with two mycelia of (+) sign, and each

of eighteen plates with two mycelia of (-) sign. The results of this pairing, which are shown in Table II, did not conform with expectation, for only sixteen of the twenty-four pairs remained without clamp-connections, while clamp-connections soon made their appearance in one of the six (++) pairs and in seven of the eighteen (- -) pairs. It therefore became apparent that the phenomenon of heterothallism in Coprinus lagopus is much more complicated than I had at first imagined.

Table II.

Coprinus lagopus: The Effect of Pairing Monosporous Mycelia of like Sign.

Mycelial pairs	Sexual Signs	Clamp-connections after pairing	Fruit-body development in the plates.
15 x 16	+ +	absent	———
16 x 17	+ +	absent	———
17 x 21	+ +	absent	———
21 x 22	+ +	absent	———
27 x 28	- -	absent	———
23 x 26	- -	present	———
28 x 19	- -	absent	———
25 x 46	- -	absent	———
48 x 47	- -	absent	———
18 x 47	- -	present	———
51 x 15	+ +	absent	———
55 x 16	+ +	present	———
23 x 18	- -	present	perfect
23 x 19	- -	present	perfect
23 x 25	- -	absent	imperfect
23 x 27	- -	absent	imperfect
23 x 28	- -	absent	imperfect
23 x 29	- -	absent	imperfect
26 x 18	- -	present	perfect
26 x 19	- -	present	imperfect
26 x 25	- -	absent	imperfect.
26 x 27	- -	present	perfect
26 x 28	- -	absent	imperfect
26 x 29	- -	absent	imperfect

On the strict (+) and (-) theory of sex, one would suppose that if a mycelium a were to yield clamp-connections when paired with either b or c, the sex of the mycelia b and c would be identical; so that, if b and c were to be paired, no clamp-connections would be formed. But this is not always the case, for sometimes we may have clamp-connections developed in all the pairs a x b, a x c, and b x c. Thus, as shown in Table I, mycelium no. 14 forms clamp-connections with both no. 23 and no. 26; but the mycelia nos. 23 and 26 are not identical in sex, for, as shown in Table II, when paired, they yield clamp-connections. With four mycelia instead of three, even greater complications may arise. It thus appears that the factors or genes for sex in Coprinus lagopus are not of a simple but of a compound nature.

In the fourth column of Table II are given the results of observation of fruit-body production for twelve (- -) pairs of mycelia. Here again, as in the experiments recorded in Table I, rapid and perfect production of fruit-bodies was associated always with the production of clamp-connections, i.e. with the secondary or diploid condition of the mycelium, while the production of imperfect fruit-bodies was associated always with the absence of clamp-connections and the retention by the mycelium of the primary or haploid condition.

As a result of the experiments which have just been described it became necessary to discard the strict (+) and (-) theory of sexual strains for C. lagopus.

Table III shows the results that were actually obtained when seven monosporous mycelia were paired in all the possible ways. In this Table, for the sake of conformity with similar Tables made by Kniep for Schizophyllum commune , the (+) and (-) signs are introduced, but here they have not the same significance as in Tables I and II, for they do not indicate sex but merely the presence (+) or absence (-) of clamp-connections in the pairs.

Table III.

Coprinus lagopus: All Possible Pairings of Seven Monosporous Mycelia.

	21	22	23	25	26	27	29
21	-	-	+	+	+	+	+
22	-	-	+	-	+	+	+
23	+	+	-	-	+	-	-
25	+	-	-	-	-	-	+
26	+	+	+	-	-	+	-
27	+	+	-	-	+	-	-
29	+	+	-	+	-	-	-

When the experiments recorded in Table III were being made, a check upon the purity of the seven monosporous mycelia was obtained by setting out portions of the mycelia in plates by themselves. In none of these sub-cultures were clamp-connections ever produced.

Since, as indicated in Table I, nos. 23, 25, 26, 27, and 29 all produced clamp-connections with no. 14, it was to be expected on the strict (+) and (-) theory of sex that they would all behave uniformly with respect to other mycelia; but, by looking along the horizontal rows in Table III, it will be at once seen that this expectation was not justified by experience. Thus although with no. 21 they all gave clamp-connections, ~~except no. 25~~ yet, with nos. 22, 23, and 25 they did not all behave uniformly; for with no. 22 they all produced clamp-connections except no. 25, with no. 23 they none of them produced clamp-connections except no. 26, and with no. 25 they none of them produced clamp-connections except no. 29.

The results given in Table III are identical in their nature with those obtained by Hans Kniep with Schizophyllum commune.[1)]

VII. Coprinus niveus.

In the series of experiments upon Coprinus niveus recorded in my first paper, I found that clamp-connections developed: (1) on each of several mycelia of monosporous origin derived from the spores of a wild fruit-body; (2) on each of two monosporous mycelia derived from spores produced by a fruit-body of monosporous origin, and (3) on a compound mycelium of polysporous origin derived from many spores produced by a fruit-body of monosporous origin. I therefore came to the conclusion that Coprinus niveus is homothallic.

In the winter of 1921-22, I made a new series of experiments which yielded results quite different to those previously obtained. The source of the spores was a wild fruit-body which came up spontaneously on horse dung in the laboratory.

---

1) H. Kniep, loc. cit., p.12.

Nine monosporous mycelia were isolated and transferred to dung-agar plates and afterwards sub-cultured in other plates; but, even after the lapse of thirty-two days, they all failed to produce clamp-connections. On the other hand, a compound mycelium of polysporous origin derived from many spores produced clamp-connections in abundance ten days after inoculation.

The presence of clamp-connections on the mycelium of polysporous origin and their absence from the nine mycelia of monosporous origin at once suggested that my new strain of Coprinus niveus was heterothallic. A complete series of crossings with the nine mycelia was therefore undertaken. The results are shown in Table IV. As before, the numbers above and to the left indicate the numbers given to individuals mycelia, while the (+) and (-) signs indicate that clamp-connections were present or absent respectively after the crossings had been effected.

Table IV.

Coprinus niveus: All Possible Pairings of Nine Monosporous Mycelia.

	1	2	3	4	5	6	7	8	9
1	-	-	-	+	-	-	-	-	-
2	-	-	-	-	-	-	+	+	+
3	-	-	-	+	-	-	-	-	-
4	+	-	+	-	+	+	-	-	-
5	-	-	-	+	-	-	-	-	-
6	-	-	-	+	-	-	-	-	-
7	-	+	-	-	-	-	-	-	-
8	-	+	-	-	-	-	-	-	-
9	-	+	-	-	-	-	-	-	-

As is shown in Table IV, nos. 2 and 3 both failed to form clamp-connections with nos. 1, 2, 3, 5 and 6. Yet they are not identical sexually, for no. 2 formed clamp-connections with nos. 7, 8, and 9, whereas no. 3 did not. Similarly, nos. 4 and 5 did not form clamp-connections with nos. 2, 7, 8, and 9 and yet are not identical sexually, for no. 4 formed clamp-connections with nos. 1, 3, 5, and 6, whereas no. 5 did not. Moreover, when nos. 4 and 5 were paired, they yielded clamp-connections. That the monosporous mycelia were pure was ascertained by making sub-cultures of these mycelia in plates at the same time that the pairings were effected. In none of these nine sub-cultures did any clamp-connections make their appearance. This is indicated in Table IV by the (-) sign for 1 x 1, 2 x 2, 3 x 3 ..... 9 x 9.

The results embodied in Table IV seem to show conclusively that the strain of Coprinus niveus with which my new work has been carried out is heterothallic but that the individual mycelia cannot be regarded as belonging strictly to two opposite strains, (+) and (-). Thus in my new work C. niveus has behaved sexually in the same manner as C. lagopus.

VIII. Discussion.

In both of my investigations upon the sex of Coprinus sterquilinus and C. stercorarius, mycelia of monosporous origin yielded clamp-connections regularly and readily gave rise to normal fruit-bodies. All the evidence so far obtained, therefore, strongly supports the view already expressed in my first paper, namely, that Coprinus sterquilinus and C. stercorarius are homothallic.

So far as concerns Coprinus lagopus and C. niveus, the results of my two investigations are discordant; for in the first, monosporous mycelia gave rise regularly to clamp-connections, whilst in the second (described in this paper), clamp-connections never appeared in monosporous mycelia (59 isolations for C. lagopus and

9 for C. niveus) but only after the pairing of two monosporous mycelia presumably of opposite sex. My first investigation, therefore, led me to believe that Coprinus lagopus and C. niveus are homothallic, whereas my second one has provided conclusive evidence that there are strains of both these fungi which are heterothallic.

It may be asked; admitting that all the new observations go to show that Coprinus lagopus and C. niveus are heterothallic, how is it that different results pointing to homothallism were obtained in the first investigation? There appear to be two alternative explanations: (1) in my first investigation some error crept into the work, (2) there are homothallic strains of both these fungi as well as heterothallic.

I have not been able to think of a single source of error in my first work that could account for the results obtained. So far as I know, I used the same method for isolating and cultivating the mycelia for my second investigation as for my first. I do not think that the plates became infected by spores from the air, or that I confused species or spore-deposits; and I certainly saw the clamp-connections described, for I sketched some of them with the camera-lucida. If I made some mistake in method, I must have repeated it quite consistently in every one of my first experiments, for all the results were consistent, all the monosporous mycelia having produced clamp-connections, and I must have avoided this error consistently in my second experiments, for no clamp-connections ever made their appearance in the 68 new monosporous cultures. On the other hand, it is to be remembered that my first investigation was chiefly devoted to the fruiting of monosporous mycelia, that the question of the presence or absence of clamp-connections was taken up only toward its conclusion, and that the number of observations was relatively few. It is also noteworthy that in my second series of experiments with Coprinus lagopus, although I used ten different fruit-bodies, I was unable

to obtain any more homothallic strains, and therefore at no time have I been able to grow simultaneously homothallic and heterothallic strains of this fungus.

Blakeslee[1] found that in the heterothallic Mucors, each monosporous mycelium is either (+) or (-), and that in these fungi there is an absence of sexual intergrades. Kniep[2], on the other hand, working with Schizophyllum commune, found that, while the monosporous mycelia of this fungus usually are unisexual and do not produce clamp-connections, yet occasionally, when kept in pure culture for a long time, a monosporous mycelium may begin to produce clamp-connections and thus pass definitely from the haploid to the diploid condition. Thus we can speak of Schizophyllum commune as being heterothallic but as sometimes producing homothallic strains.

Owing to the fact that I am unable to explain my first results on the basis of error in experiment or observation, and taking into account Kniep's work upon Schizophyllum commune, it seems difficult to escape the conclusion that in my first investigation I was studying strains of Coprinus lagopus and C. niveus which were homothallic. On the other hand, my later results, described in this paper, certainly point to the possibility of some error having occurred in my earlier work. I think, therefore, that it is advisable to lay most weight on my second investigation and to conclude that, while Coprinus lagopus and C. niveus are undoubtedly heterothallic, it is possible that homothallic strains of both these species exist. The question of the existence or non-existence of these homothallic strains will doubtless be decided by future workers.

In finding that the strict (+) and (-) theory of sex is inapplicable to Coprinus lagopus and C. niveus, I have but confirmed what Hans Kniep discovered in

---

1) A.F. Blakeslee, Sexual Dimorphism in Cunninghamella, Botanical Gazette, Vol.LXXII, 1921, p. 186.

2) H. Kniep, loc. cit., p.16.

Schizophyllum commune. His Table for the latter fungus and my Tables III and IV for the two Coprini are identical in general form.

There can be no doubt that in such fungi as Schizophyllum commune, Coprinus lagopus, and C. niveus, the factors or genes for sex are not simple but complex in their nature. To what extent this applies to the Hymenomycetes in general can only be determined by new and extensive investigations.

Recently, B. O. Dodge[1] working with Ascobolus magnificus has found that, so far as sex-organs and asocarps are concerned, monosporous mycelia are self-sterile, but that sexual organs and ascocarps are produced in cultures containing two strains properly chosen. It thus appears that there are heterothallic species not only in the Basidiomycetes but also in the Ascomycetes.

In my first paper, I pointed out that the Coprinus fimetarius used by Mlle Bensaude and my own C. lagopus might be identical species. I now think them to be identical and shall so regard them in future. Mlle Bensaude worked with only two monosporous strains of her fungus. When grown separately, they did not produce clamp-connections and remained sterile; when paired, they produced clamp-connections and fruited well. She therefore regarded them as (+) and (-) strains; but, had she used more sexual strains, she no doubt would have found out how complex sex is in this fungus and also that the strict (+) and (-) theory of sex is here inapplicable. In my experiments, the development of rudimentary fruit-bodies on monosporous mycelia has been very variable. However, it is somewhat remarkable that Mlle Bensaude's two sexual strains, when grown separately, remained entirely sterile.

1) B.O. Dodge, The Life History of Ascobolus magnificus; Origin of the Ascocarp from two Strains, Mycologia, Vol. XII, 1920, pp. 115-134.

IX. Conclusions.

1. Coprinus sterquilinus and C. stercorarius, as shown by the production of clamp-connections and fruit-bodies in monosporous cultures, are homothallic.

2. Coprinus sterquilinus has been successfully cultivated with the production of perfect fruit-bodies for seven successive monosporous generations. The mycelium of the seventh generation was just as vigorous and fruited just as rapidly as the mycelium of the first generation.

3. There are heterothallic strains of Coprinus lagopus and C. niveus. Fifty-nine monosporous mycelia of C. lagopus, grown separately, never produced clamp-connections. When brought together in suitable pairs, the monosporous mycelia soon produced clamp-connections and later perfect fruit-bodies. Similar results were obtained with nine monosporous mycelia of C. niveus.

4. Coprinus lagopus and C. niveus are heterothallic species; but, as indicated by previous experiments, it is possible that they both sometimes give rise to homothallic strains.

5. The question of sex in heterothallic Coprini is complicated by the fact that the sexual strains cannot be strictly divided into (+) and (-) groups.

6. The sexual reactions between the monosporous mycelia in Coprinus lagopus, or in C. niveus, are similar to those described by Kniep for Schizophyllum commune.

The investigations recorded above were carried out in the Botanical Department of the University of Manitoba during my tenure of the Hudson's Bay Company Research Fellowship. In conclusion, I desire to express my sincere thanks to Professor A.H. Reginald Buller for his continued encouragement and valuable advice.

****