

CONTRIBUTIONS TO OUR KNOWLEDGE OF SEX
AND THE DIFFERENTIATION OF SPECIES
IN THE HIGHER FUNGI

1. THE BISEXUALITY OF INDIVIDUAL STRAINS OF
COPRINUS LAGOPUS.
2. THE DISTRIBUTION OF SPORES OF DIVERSE SEX
ON THE HYMENIUM OF COPRINUS LAGOPUS.
3. THE MATING METHOD OF IDENTIFICATION OF
A COPRINUS GROWING ON GERMINATING
SEEDS OF MANGEL AND SUGAR-BEET.

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by

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1
NEWTON, DOROTHY E.
THE BISEXUALITY OF INDIVIDUAL STRAINS OF
COPRINUS ROSTRUPIANUS.

The Bisexuality of Individual Strains of *Coprinus Rostrupianus*.

BY

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With Plate VI and seventeen Figures in the Text.

I. INTRODUCTION.

IN 1918 Mlle Bensaude, employing experimental and cytological methods, proved conclusively that the phenomenon of heterothallism occurs in the Hymenomycetes (1). She isolated two monosporous mycelia of *Coprinus fimetarius* and found that they remained in the haploid (primary) condition during eight months of continuous cultivation, without any sign of fruiting, but that when they were paired they united and produced a diploid (secondary) clamp-bearing mycelium which fruited readily. In 1919 Kniep showed by experiment that *Schizophyllum commune* is heterothallic, and stated that many other Hymenomycetes also exhibit this sexual condition (11). Further discoveries of heterothallism in Hymenomycetes have been made: by Kniep, working with *Aleurodiscus polygonius* (12); by Miss Mounce, working with *Coprinus lagopus* and *C. niveus* (15, 16); by Vandendries, working with *Collybia velutipes*, *Hypholoma fasciculare*, *Panaeolus campanulatus*, *P. separatus* (= *Anellaria separata*), *P. fimicola*, and *Coprinus radians* (18, 19, 20); and by Brunswik working with sixteen species of the genus *Coprinus* (3).

In papers published in 1921 and 1922, Miss Mounce first definitely established that the phenomenon of homothallism occurs in the Hymenomycetes (15, 16). She made monosporous cultures of *Coprinus sterquilinus* and of *C. stercorarius* and found that the mycelia soon developed clamp-connexions and subsequently produced perfect fruit-bodies. She succeeded in growing *C. sterquilinus* in pure monosporous cultures for seven successive generations. In unpublished work, reported by Buller (5), she also found that *C. narcoticus* is homothallic. Confirmation of homothallism, as found

by Miss Mounce, has been obtained in the Winnipeg laboratory: for *C. sterquilinus* by Hanna and the writer, working independently; and for *C. stercorarius* by the writer.¹ The species of *Coprinus* determined by Brunswik as being homothallic are: *C. sterquilinus*, *C. stercorarius*, *C. narcoticus*, and *C. ephemeroideus* (3).

Individual sexual strains of *Schizophyllum commune* and *Aleurodiscus polygonius* have each been shown by Kniep to possess four sexually different kinds of spores (12, 13). In order to explain his results, Kniep assumed that in these strains sex is determined by two allelomorphic pairs of factors which are present in the fusion nucleus of the basidium and which, during the two subsequent nuclear divisions, become segregated in accordance with Mendelian principles. If these pairs of factors are represented by the symbols (Aa) and (Bb), then the fusion nucleus of the basidium must have the genetic constitution (AaBb). During reduction and division of the fusion nucleus, the sex factors become segregated, with the result that, finally, there are formed four sexually different kinds of spores: (AB), (ab), (Ab), and (aB). Only those spores without a common factor unite sexually in the mycelial stage. Thus, monosporous mycelia bearing the factors (AB) and (ab), when paired, unite sexually to form a diploid clamp-bearing mycelium; whereas monosporous mycelia bearing the factors (AB) and (aB) are not able to do this because (B) is a common factor and two like sex factors repel one another. Similarly, while mycelia bearing the factors (Ab) and (aB) can unite sexually, mycelia bearing the factors (AB) and (Ab) cannot.

Kniep observed that, under certain abnormal culture conditions, each basidium of *Aleurodiscus polygonius* shoots away its four spores in a single mass (12). He isolated thirty-five of these spore-masses, and shook the four spores of each mass apart in a flask containing nutrient agar. Subsequently, the four mycelia produced from the four spores were removed from the flask, grown separately, and then mated in all possible ways. As a result, Kniep found that each basidium of *Aleurodiscus polygonius* bears two pairs of spores, one pair of one sex, and the other pair of another and opposite sex. He also found that the basidia of a single sexual strain were of two kinds, one kind bearing spores (AB), (AB), (ab), and (ab), and the other bearing spores (Ab), (Ab), (aB), and (aB). Kniep concluded from these results that the reduction of the chromosomes takes place in *A. polygonius* in the *first* division of the fusion nucleus, and not in the second.²

Hanna (8), working with *Coprinus lagopus*, found that the spores from any individual fruit-body belong to four sexually different groups, and thus

¹ This confirmation has not previously been announced.

² Since this paragraph was written Professor Kniep has informed Professor Buller *in litt.* that, since the publication of his paper, he has found a few spore-masses which included spores of all four sexes: (AB), (ab), (Ab), (aB).

resemble those found by Kniep in *Schizophyllum commune* and *Aleurodiscus polygonius*. However, Hanna's basidial analyses show that, while some basidia bear spores of two sexes only, a pair of one sex and a pair of another and opposite sex, other basidia bear spores of all four sexes: (AB), (ab), (Ab), and (aB). The occurrence of four sexually different kinds of spores on a single basidium in *Coprinus lagopus* is regarded by Hanna as proving that the reduction process in the basidium in this species takes place in the second division of the nucleus, and not in the first.

Funke (6) isolated the four spores of individual basidia of *Hypholoma fasciculare*, *H. capnoides*, and *Collybia velutipes* and determined their sex, with results similar to those obtained by Hanna. Like Hanna, from the fact that some of the basidia had produced spores of four different kinds, he concluded that reduction takes place during the second division of the basidial nucleus and not during the first.

In view of the fact that homothallic and heterothallic quadrisexual species had already been found in *Coprinus*, it seemed not unlikely that this genus might also include heterothallic bisexual species. Such a species the writer has had the good fortune to find in *Coprinus Rostrupianus*. While the investigations here recorded were in progress, Vandendries (20) and Brunswik (3) announced that they also had discovered bisexual species of *Coprinus*.

Experiments made by Kniep, Vandendries, Brunswik, Hanna, and the writer indicate that every species of Hymenomycetes is made up of many different sexual strains which are perfectly fertile *inter se* (*vide infra*). Thus Hanna (8) found six interfertile geographical strains of *Coprinus lagopus*. While each of these strains was quadrisexual, the six strains collectively represented twenty-four different sexes. In this paper, when a species is spoken of as *bisexual* or *quadrisexual*, it must be understood that these terms have reference to the sexual condition of each of the individual strains of which the species is composed and not to all the strains taken collectively. When all the sexual strains are considered together, each species of *Coprinus*, &c., must be regarded as *multisexual*.

In his most recent paper Vandendries (21) records that a large percentage of the monosporous mycelia of his bisexual species, *Coprinus radians*, in the course of six months, changed spontaneously from the haploid to the diploid condition, in consequence of which he now regards *C. radians* not as heterothallic but as *hetero-homothallic*. As will be seen from a series of observations given at the end of this paper, *C. Rostrupianus* behaves in exactly the same manner as *C. radians*; for, in the course of some months, a large percentage of the haploid mycelia change spontaneously into diploid mycelia. However, since actual tests made at intervals showed that each of twenty-six monosporous mycelia of *C. Rostrupianus* retained its unisexual character for the first nine weeks of its

existence, the individual strains of the fungus have been regarded as strictly bisexual during this period.

Coprinus Rostrupianus is a large-spored sclerotium-producing species. On November 14, 1923, in a meadow at the Manitoba Agricultural College, Winnipeg, a number of large irregular blackish sclerotia (cf. Pl. VI, Figs. 1 and 2) were found by Professor A. H. R. Buller embedded in an old cow-dung plat. They were brought to the laboratory, where, in a moist chamber, in the course of about two weeks, they began to produce fruit-bodies (Pl. VI, Figs. 3 and 4). Each fruit-body took about ten days to develop to maturity. The scales on the pileus were white and made up of slender septate hyphae, the individual cells of which were often branched. The cystidia were cylindrical and very long—up to 0.3 mm. in length—and they could be seen with the naked eye in expanding pilei bridging adjacent gills as described by Buller for *C. atramentarius* (5). The spores were densely black, spade-like in general appearance, having three dimensions (Text-fig. 1, *a, b, c*), the length in dry spore-deposits (Pl. VI, Fig. 5) being 14–16 μ and the breadth 11.5–13 μ , and provided with an unusually prominent black apiculus. In October, 1924, Professor Buller found the fungus again, once more in a meadow at the Manitoba Agricultural College and a second time at Kenora, Ontario, about 100 miles east of Winnipeg.

Coprinus Rostrupianus was first described in 1897 by E. Chr. Hansen, who found it in Denmark coming up on cow-dung (9). The fruit-bodies always originated from a sclerotium, and the sclerotia were larger and more irregular than those of *C. stercorearius*. Hansen illustrated his paper with sketches of the spores, the pilear covering, &c.; but, unfortunately, he did not give a drawing of the whole fruit-body. However, from his description, there was no difficulty in identifying the fungus found at Winnipeg as *Coprinus Rostrupianus*. Lange (14), in his monograph of the genus *Coprinus* (1915), includes *C. Rostrupianus*; but, as he could not find any sclerotia attached to the fruit-bodies, his identification seems doubtful.¹ *C. Rostrupianus* is not mentioned by Ricken (17) in 'Die Blätterpilze' (1915), and hitherto it has not been recorded for North America. Hence we may conclude that *C. Rostrupianus* is a somewhat rare fungus or, at least, one that is easily overlooked by mycologists.

A preliminary announcement of some of the chief results of this paper has been made by Buller (4).

II. METHODS.

Spore-deposits from each fruit-body were collected separately on dry sterilized glass slides (Pl. VI, Fig. 5). The slides bearing spore-deposits

¹ In laboratory cultures, without exception, it was observed that fruit-bodies of *C. Rostrupianus* never developed directly on ordinary mycelium, but always from sclerotia.

taken from a single fruit-body were wrapped together in white writing-paper and labelled with the number of the fruit-body and the date on which the spores were shed. Thus the wrapped slides were stored free from danger of contamination by spores from other fruit-bodies.

The spores were isolated and sown by the dry-needle method (7), and dung-agar was used as the medium for germination. In preparation for removing a new spore from a dry spore-deposit, the sewing needle was never heated in a flame, but was always run through clean linen. The surface of the slide or cover-glass which receives the spore-deposit should be flamed to remove any organic film which, if present, may make it difficult or even impossible to pick up the spores. These details were not mentioned by Hanna (7).

The germination of fresh spores taken from deposits made in the spring of 1923 varied from 60 to 80 per cent., while that of spores taken from deposits made in November, 1924, and tested in lots of fifteen to twenty spores, showed 100 per cent. germination. After spores had been stored for a month or longer, their power of germination was found to have considerably decreased. Some germinating spores are shown in Text-figs. 2-6.

The four spores of single basidia were obtained for isolation by what may be called the *coverglass-contact method* (4, 8), which will now be described. A fruit-body about to undergo autodigestion is removed from the culture-dish in which it is growing, and one of its gills is cut away and laid flat on a glass slide. A cover-glass, held with forceps, is then lowered until it touches the hymenium lightly, whereupon it is raised, inverted, and examined under the microscope. In certain places on the cover-glass the spores can be seen adhering by their apices in groups of four (Pl. VI, Fig. 6). The spatial arrangement of each tetrad is exactly like that of the four spores on a basidium when the hymenium is examined microscopically from above, so that there can be no doubt that each tetrad has actually been derived from the four spores of a single basidium. By means of the dry-needle method (7) the spores surrounding a well-defined spore-tetrad are first removed, and then the four spores are picked up one by one and sown separately in the culture medium.

The dung-agar used as the culture medium was prepared as follows: About 300 grm. of fresh horse-dung were stirred up with 1,000 c.c. water. The mixture was boiled for fifteen minutes, and then filtered twice through cotton-wool. Water equivalent to that lost through boiling and filtering was then added, agar at the rate of 1.2 per cent. was stirred in, and the whole boiled again until all the agar had melted. The medium was then filtered through cotton-wool, tubed, and sterilized for one hour at fifteen pounds pressure.



TEXT-FIGS. 1-16. *Coprinus Rostrupianus*. × 470.

III. CRITERIA OF SEX.

Hans Kniep (10) and Mlle Bensaude (1), working independently, discovered that in a *haploid* mycelium in which the nuclei occur singly and separated from one another, often one in each cell, the septa are always simple and never accompanied by clamp-connexions; but that in a *diploid* mycelium in which the nuclei occur in pairs and divide conjugately clamp-connexions are present. Thus the presence of clamp-connexions is an outward and visible sign that the mycelium of which they form a part is in the diploid and not in the haploid condition. This criterion of sex, i. e. the presence or absence of clamp-connexions, was the chief one employed in determining the nature of the sexual reaction when two mycelia of *Coprinus Rostrupianus* were mated. A haploid mycelium of *C. Rostrupianus* with simple septa is shown in Text-fig. 7, and a diploid mycelium with the septa accompanied by clamp-connexions in Text-figs. 13-16.

A haploid mycelium of *Coprinus Rostrupianus* bears numerous oidia at the surface of the culture medium (Text-figs. 8-12), whereas a diploid mycelium never bears any. Hence, macroscopically, a haploid mycelium has a somewhat floury appearance, whereas a diploid has not. Moreover, a haploid mycelium sends out its lateral branches at a greater angle than

TEXT-FIGS. 1-16. *Coprinus Rostrupianus*. × 470.

TEXT-FIG. 1. Ripe spores viewed: *a*, from in front or from behind; *b*, from the side; and *c*, from the top.

TEXT-FIG. 2. A spore germinating in dung-extract: an irregular vesicle has grown out from the germ-pore.

TEXT-FIGS. 3, 4, and 5. Spores germinating in dung-extract, showing the production of germ-tubes from the vesicles at the germ-pores.

TEXT-FIG. 6. A spore, germinating in dung-extract, which has produced a haploid or primary mycelium which is as yet unicellular.

TEXT-FIG. 7. A branch of a haploid mycelium developed in dung-agar, showing simple septa and lateral hyphae growing outwards from the leading hypha at various angles.

TEXT-FIGS. 8-12. These show the production of aerial chains of oidia on haploid hyphae submerged in dung-agar.

TEXT-FIG. 8. A haploid hypha with an oidiophore bearing three hyphae which are developing into chains of oidia.

TEXT-FIG. 9. A haploid hypha with two oidiophores, one bearing a single chain of oidia, the other bearing two chains.

TEXT-FIG. 10. An irregularly thickened terminal haploid hypha bearing numerous simple or branched oidiophores, each continued into a chain of oidia.

TEXT-FIG. 11. Several isolated oidia and one chain of oidia which became separated from the parent mycelium on the addition of water.

TEXT-FIG. 12. A diagram of a vertical section through dung-agar showing a submerged haploid hypha with three oidiophores which have grown towards the surface of the medium. The basal oidiophore bears a single chain of aerial oidia and the central one two chains of oidia (one branched), while the apical one is about to produce oidia.

TEXT-FIGS. 13-16. Hyphae of a diploid or secondary mycelium developed on dung-agar, showing septa with clamp-connexions. The two clamp-connexions in Fig. 13 are seen from above, those in Figs. 14, 15, and 16 from the side. In Fig. 15 there is a plain septum as well as one with a clamp-connexion. The lateral hyphae shown in Figs. 13 and 15 make an acute angle with the parent hyphae. In Fig. 13 the two terminal hyphae, as indicated by the broken lines, have been slightly shortened.

a diploid mycelium (cf. Text-figs. 7 and 13). Thus the presence or absence of oidia and the nature of the branching form two further criteria for distinguishing between haploid and diploid mycelia; but, in the present investigation, the criterion finally relied upon for determining sex was always the presence or absence of clamp-connexions.

Both haploid mycelia of monosporous origin and diploid mycelia of polysporous origin were grown on horse-dung in pure cultures, and it was found that both haploid and diploid mycelia gave rise to sclerotia (for diploid sclerotia *vide* Pl. VI, Figs. 1 and 2). However, when the sclerotia were set out in damp sand, only those of diploid origin gave rise to perfect fruit-bodies (Pl. VI, Figs. 3 and 4), whereas those of haploid origin either grew out into the sand in the form of a thick mycelium or produced rudimentary fruit-bodies only. It therefore appears that in *Coprinus Rostrupianus* the production of spore-bearing fruit-bodies is limited to those sclerotia which arise from a diploid mycelium. Mlle Bensaude (1) used the criterion of fruiting in the determination of the sexual reactions of *C. fimetarius*, but this criterion could not be conveniently applied in the present investigation, owing to the fact that the mycelium of *C. Rostrupianus* first forms sclerotia, and the sclerotia usually require a period of rest of some weeks or months before they germinate.

IV. EXPERIMENTAL RESULTS.

By employing the methods described, the four spores from each of a number of basidia of fruit-body No. 1 were transferred to dung-agar for germination. All four spores of one basidium germinated perfectly, three of another basidium, and two of each of two other basidia. All possible crossings of these eleven mycelia were made on dung-agar plates in the manner described by Hanna for *Coprinus lagopus* (8). The plates were examined six days after the pairings had been made. When two mycelia of like sexual reactions happened to be paired on a plate, the compound mycelium resulting remained in the haploid condition; whereas, when two mycelia of opposite sex happened to be paired, the compound mycelium resulting became diploid and could easily be distinguished from a haploid mycelium by the presence of clamp-connexions. Before examining the hyphae for clamp-connexions microscopically, the plates were always viewed macroscopically, and then, as a rule, the haploid mycelia could be distinguished by the presence or absence of the floury oidia at the surface of the agar.

In quadrisexual Hymenomycetes, e. g. in *Schizophyllum commune* (13), *Aleurodiscus polygonius* (12), *Coprinus lagopus* (8), *C. niveus* (3, 16), *C. micaceus* and *C. picaceus* (3), investigated by others, as well as in *Collybia velutipes* and *Coprinus curtus* studied by the writer before the present investigation was undertaken, when all possible pairings are made between

ten or more monosporous mycelia of an individual sexual strain, the mycelia fall into *four* sexual groups, the mycelia of any one group giving a positive reaction with only one of the three other groups. When the plates containing all possible crossings of the eleven monosporous mycelia of *Coprinus Rostrupianus* came to be examined, it was found that the mycelia fell into *two* groups only and not into four. It therefore seemed possible that the strains of *C. Rostrupianus* are bisexual and not quadrisexual. Of the four spores of one basidium, two were of one sex and the other two were of another and opposite sex; of the three spores of another basidium, two were of one sex and one of another and opposite sex. The remaining spores were all of one and the same sex. Of the eleven spores which had developed mycelia eight were of one sex and three of another and opposite sex.

It seemed possible that the occurrence of only two sexually different kinds of monosporous mycelia in the experiments just described might have been due to the fact that, while the species was in reality quadrisexual, the eleven mycelia chosen might have belonged by chance to only two of the four possible sexual groups. A new set of experiments was therefore undertaken. Twelve spores derived from six different basidia germinated, and the resulting mycelia were then crossed in all possible ways. The results of the crossings are embodied in Table I. A (-) sign denotes that the two mycelia placed together in the Petri dish remained in the haploid condition and therefore did not give a positive sexual reaction, while a (+) sign denotes that the two mycelia developed a diploid, clamp-bearing mycelium, and therefore reacted positively towards one another.

It will be seen from this table that the twelve mycelia, as in the previous experiment, fall into only two groups, mycelia Nos. 1, 2, 6, 8, 10, 11, and 12 being of one sex, and mycelia Nos. 3, 4, 5, 7, and 9 being of another and opposite sex. The chances that with twelve monosporous mycelia only two sexual groups should be represented out of a possible four seemed very slight, and the evidence therefore pointed to each individual fruit-body of *Coprinus Rostrupianus* as being bisexual. However, as no clear-cut case of bisexuality in the Hymenomycetes had previously been recorded,¹ it seemed advisable to extend the investigation with a view to substituting a certainty for a probability. Therefore, in the autumn of 1924, more extensive experimental work was undertaken.

By means of the coverglass-contact and dry-needle methods already described, all the spores from fourteen different basidia of fruit-bodies Nos. 3 and 4 were sown separately in the culture medium. All the fifty-six spores germinated.

¹ Vandendries (18), in 1923, had reported 'bipolarity' in *Panaeolus separatus* and *P. campanulatus*, but his tables contained so many exceptions, or 'hermaphrodites' as he called them, that his experiments did not prove conclusively that bisexuality occurs in the Hymenomycetes.

TABLE I.

All Possible Pairings of Twelve Monosporous Mycelia derived from Six Different Basidia.

		1		2		3		4		5 6			
		1	2	3	4	5	6	7	8	9	10	11	12
1	1	-	-	+	+	+	-	+	-	+	-	-	-
	2	-	-	+	+	+	-	+	-	+	-	-	-
	3	+	+	-	-	-	+	-	+	-	+	+	+
	4	+	+	-	-	-	+	-	+	-	+	+	+
2	5	+	+	-	-	-	+	-	+	-	+	+	+
	6	-	-	+	+	+	-	+	-	+	-	-	-
3	7	+	+	-	-	-	+	-	+	-	+	+	+
	8	-	-	+	+	+	-	+	-	+	-	-	-
4	9	+	+	-	-	-	+	-	+	-	+	+	+
	10	-	-	+	+	+	-	+	-	+	-	-	-
5	11	-	-	+	+	+	-	+	-	+	-	-	-
6	12	-	-	+	+	+	-	+	-	+	-	-	-

TABLE II.

All Possible Pairings of Four Monosporous Mycelia derived from the Four Spores of a Single Basidium.

		1	4	2	3
1		-	-	+	+
4		-	-	+	+
2		+	+	-	-
3		+	+	-	-

The sexual constitution of the four mycelia produced by the four spores of each of the fourteen basidia was first determined. The four mycelia were crossed with one another in all possible ways. It was found that each of the fourteen basidia had produced two pairs of spores, one pair of one sex,

and the other pair of another and opposite sex, as shown in Table II, which is representative of the behaviour of all fourteen sets of spores. The

TABLE III.

All Possible Pairings of Twenty-eight Monosporous Mycelia derived from Seven Different Basidia of Fruit-body No. 4.

		7				8				9				10				11				12				14			
		1	4	2	3	3	4	1	2	1	3	2	4	1	2	3	4	1	3	2	4	1	2	3	4	1	4	2	3
7	1	-	-	+	+	-	-	+	+	-	-	+	+	-	-	+	+	-	-	+	+	-	-	+	+	-	-	+	+
	4	-	-	+	+	-	-	+	+	-	-	+	+	-	-	+	+	-	-	+	+	-	-	+	+	-	-	+	+
	2	+	+	-	-	+	+	-	-	+	+	-	-	+	+	-	-	+	+	-	-	+	+	-	-	+	+	-	-
	3	+	+	-	-	+	+	-	-	+	+	-	-	+	+	-	-	+	+	-	-	+	+	-	-	+	+	-	-
8	3	-	-	+	+	-	-	+	+	-	-	+	+	-	-	+	+	-	-	+	+	-	-	+	+	-	-	+	+
	4	-	-	+	+	-	-	+	+	-	-	+	+	-	-	+	+	-	-	+	+	-	-	+	+	-	-	+	+
	1	+	+	-	-	+	+	-	-	+	+	-	-	+	+	-	-	+	+	-	-	+	+	-	-	+	+	-	-
	2	+	+	-	-	+	+	-	-	+	+	-	-	+	+	-	-	+	+	-	-	+	+	-	-	+	+	-	-
9	1	-	-	+	+	-	-	+	+	-	-	+	+	-	-	+	+	-	-	+	+	-	-	+	+	-	-	+	+
	3	-	-	+	+	-	-	+	+	-	-	+	+	-	-	+	+	-	-	+	+	-	-	+	+	-	-	+	+
	2	+	+	-	-	+	+	-	-	+	+	-	-	+	+	-	-	+	+	-	-	+	+	-	-	+	+	-	-
	4	+	+	-	-	+	+	-	-	+	+	-	-	+	+	-	-	+	+	-	-	+	+	-	-	+	+	-	-
10	1	-	-	+	+	-	-	+	+	-	-	+	+	-	-	+	+	-	-	+	+	-	-	+	+	-	-	+	+
	2	-	-	+	+	-	-	+	+	-	-	+	+	-	-	+	+	-	-	+	+	-	-	+	+	-	-	+	+
	3	+	+	-	-	+	+	-	-	+	+	-	-	+	+	-	-	+	+	-	-	+	+	-	-	+	+	-	-
	4	+	+	-	-	+	+	-	-	+	+	-	-	+	+	-	-	+	+	-	-	+	+	-	-	+	+	-	-
11	1	-	-	+	+	-	-	+	+	-	-	+	+	-	-	+	+	-	-	+	+	-	-	+	+	-	-	+	+
	3	-	-	+	+	-	-	+	+	-	-	+	+	-	-	+	+	-	-	+	+	-	-	+	+	-	-	+	+
	2	+	+	-	-	+	+	-	-	+	+	-	-	+	+	-	-	+	+	-	-	+	+	-	-	+	+	-	-
	4	+	+	-	-	+	+	-	-	+	+	-	-	+	+	-	-	+	+	-	-	+	+	-	-	+	+	-	-
12	1	-	-	+	+	-	-	+	+	-	-	+	+	-	-	+	+	-	-	+	+	-	-	+	+	-	-	+	+
	2	-	-	+	+	-	-	+	+	-	-	+	+	-	-	+	+	-	-	+	+	-	-	+	+	-	-	+	+
	3	+	+	-	-	+	+	-	-	+	+	-	-	+	+	-	-	+	+	-	-	+	+	-	-	+	+	-	-
	4	+	+	-	-	+	+	-	-	+	+	-	-	+	+	-	-	+	+	-	-	+	+	-	-	+	+	-	-
14	1	-	-	+	+	-	-	+	+	-	-	+	+	-	-	+	+	-	-	+	+	-	-	+	+	-	-	+	+
	4	-	-	+	+	-	-	+	+	-	-	+	+	-	-	+	+	-	-	+	+	-	-	+	+	-	-	+	+
	2	+	+	-	-	+	+	-	-	+	+	-	-	+	+	-	-	+	+	-	-	+	+	-	-	+	+	-	-
	3	+	+	-	-	+	+	-	-	+	+	-	-	+	+	-	-	+	+	-	-	+	+	-	-	+	+	-	-

numbers 1, 2, 3, and 4 are merely arbitrary numbers used to designate the four mycelia before the pairings were made. As shown in the table, spores Nos. 1 and 4 belong to one sexual group, and spores Nos. 2 and 3 to another and opposite sexual group.

In order to determine whether or not the two sexes represented by the

two pairs of spores of any one basidium are identical with the two sexes represented by the two pairs of spores of any other basidium, all possible crossings between twenty-eight spores derived from seven basidia of fruit-body No. 4 were made. The results are embodied in Table III.

As shown in the table, the four mycelia derived from any one of the seven basidia behaved sexually in exactly the same manner as the four mycelia derived from any of the other six basidia. The results embodied in Table III therefore justify us in concluding that in a fruit-body of *Coprinus Rostrupianus*: (1) there are only two sexes, 50 per cent. of the spores being of one sex and 50 per cent. of the other and opposite sex; (2) every basidium bears two spores of one sex and two spores of the other and opposite sex; and (3) the hymenium bears only one sexual type of basidium.

As the work recorded here was nearing completion, the occurrence of *Coprinus* species the strains of which are bisexual was announced by both Vandendries and Brunswik. Thus these two investigators and the writer, working independently, all discovered bisexual Coprini simultaneously.

Vandendries (20), in a paper in 'La Cellule', showed that each strain of *Coprinus radians*¹ sexually has only two kinds of spores. His conclusions were based on two sets of experiments in which he made all possible crossings: (1) between twenty-three monosporous mycelia derived from one fruit-body, and (2) between twenty-five monosporous mycelia derived from another fruit-body. In the first set of experiments the spores without exception proved to be of only two sexes. In the second set twenty-four out of twenty-five spores also proved to be of only two sexes, but a twenty-fifth spore reacted positively with all the other spores, thus being anomalous in its sexual behaviour.

Vandendries, in his work on *Coprinus radians*, did not analyse the sexual reactions of the four spores of individual basidia; but it seems very probable that, had he done so, he would have obtained results similar to those recorded here for *C. Rostrupianus*, i. e. he would have found that each basidium bears two spores of one sex and two of another and opposite sex.

Vandendries, as already mentioned, met with an exceptional spore in his second set of experiments. He states that the spore was derived from the same fruit-body as the other twenty-four spores, but he admits that it behaved sexually as though it had come from another fruit-body belonging to a different sexual strain. In the experiments recorded in Table III with twenty-eight spores derived from the basidia of a single fruit-body of *Coprinus Rostrupianus* there was *not one single exception*, so that the evidence for bisexuality is even more satisfactory for *C. Rostrupianus* than for *C. radians*.

The sexual reactions of *Coprinus comatus*, *C. curtus*, *C. deliquescens*, *C.*

¹ *Coprinus radians* is a synonym for *C. domesticus*. Vide A. H. R. Buller, *Researches on Fungi*, vol. iii, pp. 41-2, 1924.

ephemerus, *C. radians*, and *C. velaris*, as determined by Brunswik (3), are similar to those of *C. radians* as determined by Vandendries, and to those of *C. Rostrupianus* as determined by the writer. However, for none of these species has Brunswik so far given us individual basidial analyses such as those of Tables II and III. Brunswik's theoretical conceptions differ from those of Kniep in that he explains the reactions between paired mycelia as due to the presence or absence of sterility factors rather than of sex factors.

Segregation of the sex factors in the basidia of *Coprinus Rostrupianus* results, as we have seen, in the production of two sexually different kinds of

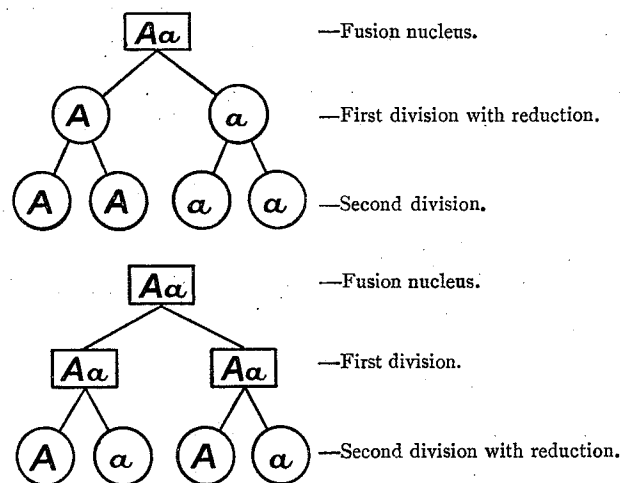
TABLE IV.

All Possible Pairings of Four Monosporous Mycelia derived from the Four Spores of a Single Basidium, with a Mendelian Interpretation of the Results.

		A		a	
		1	4	2	3
A	1	-	-	+	+
	4	-	-	+	+
a	2	+	+	-	-
	3	+	+	-	-

spores or haploid genotypes. Just as Kniep for *Schizophyllum commune* and Hanna for *Coprinus lagopus* have assumed that quadrisexuality may be explained on the assumption that two pairs of Mendelian factors are involved in the determination of sex, (Aa) and (Bb), both being present in the fusion nucleus of the basidium, so we may perhaps explain the bisexuality of *C. Rostrupianus* by supposing that sex in each strain of this species is determined by the presence, not of two, but of one pair of Mendelian factors, which we may call (Aa). On this assumption, when segregation of the sex factors takes place during the two divisions of the fusion nucleus, each spore will come to carry one or other of the factors (Aa), but not both. For twenty-one basidia altogether it has been found that each basidium bears two spores of one sex and two of another and opposite sex. This is exactly what we should expect on our assumption. When segregation of sex factors takes place in a basidium, two spores should come to carry the (A) factor and two the (a) factor. If now we rewrite Table II, assigning the symbols (A) and (a) to spores of opposite sex, it will have the appearance shown in Table IV.

It is impossible to determine without the use of cytological methods, whether the reduction process in the basidium takes place with the first division of the fusion nucleus or with the second; for in either case, as shown below, there would be produced two spores of one sex and two spores of the other and opposite sex.



TEXT-FIG. 17. Two possible modes of reduction in the basidium of *Coprinus Rostrupianus*.

Basidial dimorphism, or the presence of long and short basidia, as pointed out by Buller (5), occurs in many species of *Coprinus*; and it is also characteristic of *C. Rostrupianus*. It seemed possible that, when spore-tetrads were being removed from the hymenium of the fungus by means of the coverglass-contact method, the cover-glass might have touched the long basidia only. In order to find out whether or not the spores of the short basidia behave sexually like those of the long basidia, a general spore-deposit was obtained to which both long and short basidia had contributed: a slide was set under a mature fruit-body and a thin dry spore-deposit was collected. Nine of the spores were then sown separately in the culture medium, and the nine mycelia which resulted were then crossed with one another in all possible ways. The results of the crossings are embodied in Table V.

It will be observed from an inspection of the table that, from their reactions, mycelia Nos. 1, 2, 6, 8, and 9 are of one sex, and Nos. 3, 4, 5, and 7 are of another and opposite sex. If now we rearrange Table V so as to place like mycelia together and assign to each of the two groups of mycelia Mendelian symbols, we obtain Table VI.

An inspection of Table VI shows still more clearly than that of Table V that the nine mycelia fall into two opposite groups, those mycelia of like sex when paired remaining in the haploid condition, and those of

TABLE V.

All Possible Pairings of Nine Monosporous Mycelia derived from Nine Spores collected from a General Spore-deposit.

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

TABLE VI.

Table V rearranged, with a Mendelian Interpretation of the Results.

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

unlike sex when paired yielding a diploid clamp-bearing mycelium. Furthermore, the Mendelian interpretation, which here involves the assumption that mycelia which are sexually alike repel one another, while mycelia

of unlike sexes attract one another or react positively, fits the reactions contained in the table; for it is clear that (A) fails to mate with (A), and (a) with (a), while (A) and (a) always give positive results. Since, doubtless owing to the method of obtaining the spores, some of the mycelia were derived from the spores of long basidia and other mycelia from the spores of short basidia, we may conclude that the dimorphism of the basidia is in no way linked with sexual phenomena, i. e. that both long and short basidia bear spores of two sexes and of two sexes only.

Vandendries (19) collected spores from the wild fruit-bodies of *Panaeolus campanulatus* and *P. separatus* and analysed their sexual reactions. He found that while any individual strain of *P. campanulatus* and *P. separatus* was in general 'bipolar', i. e. bisexual, there were numerous exceptions to this rule, a number of spores being 'hermaphrodite', i. e. reacting positively with both sexual groups. However, when he analysed the sexual reactions of spores derived from first-generation fruit-bodies reared in the laboratory from two spores of opposite sex, he found that the spores showed distinct sexual 'bipolarity' with few exceptions. Vandendries collected spores from wild fruit-bodies of *Coprinus radians*; but in this species, as we have seen, there was only one exception to the rule of bisexuality. Basing his views on these results, Vandendries offered the suggestion that, in wild fruit-bodies, the two sexes are not strictly segregated from one another ('dans une sporée sauvage les sexes ne sont pas rigoureusement opposés'). He supposed that, in nature, fruit-bodies often arise from mycelia derived from many diploid mycelia, each mycelium owing its origin to the union of two haploid mycelia produced by two spores of opposite sex; and he believed that this accounts for the irregularity of his experimental results with the two species of *Panaeolus*. On the other hand, to account for the almost uniform 'bipolarity' with spores obtained from wild fruit-bodies of *Coprinus radians*, he supposed that these wild fruit-bodies happened each to have been derived from a single pair of spores (20).

Having observed that a considerable percentage of the monosporous mycelia of *Coprinus radians* in course of time pass spontaneously from the haploid to the diploid (clamp-bearing) condition, Vandendries has recently expressed the view that each of the wild fruit-bodies, to which reference has just been made, may have been derived not from two spores of opposite sex but from a single spore (21).

Against Vandendries's theory that wild fruit-bodies are derived from many diploid mycelia various arguments may be adduced. Firstly, Brefeld (2) has shown that a fruit-body of *Coprinus stercorarius* or *C. lagopus* arises from a single hypha bearing clamp-connexions, and that, having had this origin, the fruit-body develops to maturity. Brefeld's observations on *C. stercorarius* have been confirmed by the writer. There is every reason to suppose that all *Coprinus* fruit-bodies spring from a single diploid hypha,

and that what happens in Petri dishes in the laboratory also happens in nature. Secondly, Hanna analysed the sexual reactions of spores of *Coprinus lagopus* (a species with quadrisexual strains) collected from six wild fruit-bodies derived from six geographically different places, and found that the spores in each case fell into four groups with perfect regularity (8). Had any one of these fruit-bodies been derived from several diploid mycelia, the chances are that the mycelia contributing to the formation of the fruit-body might well have belonged to two or more sexual strains (which are perfectly fertile *inter se*), and this would have been revealed by the increase in the number of positive reactions when the monosporous mycelia obtained from the spores of the wild fruit-body were crossed. Since there was no such increase in positive results, the evidence points to each of Hanna's six wild fruit-bodies having had a bisporous origin. Thirdly, two of the fruit-bodies of *Coprinus Rostrupianus* used for the present investigation were derived from sclerotia grown from mycelia which were produced from a mixture of many spores of two perfectly interfertile sexual strains sown thickly together in the laboratory. An analysis of the sexual reactions of the spores derived from each of the two fruit-bodies showed that the spores of each fruit-body fell into sexually opposite groups without exception. If we represent the two sex factors of one strain as (A) and (a), and the two sex factors of the other strain as (A¹) and (a¹), then the possible unions would be as follows: (Aa), (AA¹), (Aa¹), (A¹a), (A¹a¹), (aa¹). A compound mycelium composed of two or more of these six diploid mycelia would contain three or four of the factors (A), (a), (A¹), (a¹); so that, if a compound mycelium of the type indicated were to enter into the composition of a single fruit-body, that fruit-body would bear spores of three or four different kinds; and, on making the crosses, the sexes of the spores would fall not into two groups but into three or four. Thus our three arguments all point to the conclusion that a wild or cultivated fruit-body of any species of *Coprinus*, *Panaeolus*, &c., is derived from a single diploid mycelium which owes its origin to the union of only two haploid mycelia which have been developed from two spores of opposite sex.

It has been shown by Kniep for *Schizophyllum commune* (12), Vandendries for *Panaeolus campanulatus* (19) and *Coprinus radians* (20), Hanna for *Coprinus lagopus* (8), and Brunswik for *Coprinus comatus*, *C. picaceus*, *C. niveus*, *C. lagopus*, *C. fimetarius* and *C. Friesii* (3) that in each of these species there are different sexual strains. Thus in a quadrisexual species, e. g. *Schizophyllum commune*, and *Coprinus lagopus*; in each strain there are four sexually different kinds of spores, yet the four kinds of spores of one strain differ in their sexual constitution from the four kinds of any other strain, with the result that any spore of one sexual strain is perfectly fertile with any spore of any other sexual strain. Brunswik reports the finding of twenty-seven such perfectly interfertile strains in *Coprinus fimetarius* (3).

Coprinus Rostrupianus was found to resemble the Hymenomycetes just mentioned in possessing sexual strains which *inter se* are perfectly fertile, any spore of one strain producing a diploid clamp-bearing mycelium with any spore of another strain. Eight monosporous mycelia derived from one wild fruit-body were crossed with eight other monosporous mycelia derived from a second wild fruit-body. As shown by the results embodied in Table VII

TABLE VII.

All Possible Pairings between Eight Monosporous Mycelia of a Fruit-body of One Sexual Strain and Eight Monosporous Mycelia of a Fruit-body of Another Sexual Strain.

		A'				a'			
		1	2	3	4	5	6	7	8
A	I	+	+	+	+	+	+	+	+
	II	+	+	+	+	+	+	+	+
	III	+	+	+	+	+	+	+	+
	IV	+	+	+	+	+	+	+	+
a	V	+	+	+	+	+	+	+	+
	VI	+	+	+	+	+	+	+	+
	VII	+	+	+	+	+	+	+	+
	VIII	+	+	+	+	+	+	+	+

complete interfertility resulted. If (A) and (a) be used as symbols for the spores of opposite sex of one of the two fruit-bodies, then we may use the symbols (A') and (a') for the spores of opposite sex of the other fruit-body, as shown in the table, where it has been arbitrarily assumed that in one fruit-body the spores 1-4 were of one sex and 5-8 of the other sex, and that in the other fruit-body the spores I-IV were of one sex and V-VIII of the other sex.

As an illustration of the danger of drawing false conclusions when these are based on an insufficient number of observations, an experiment may be mentioned in which ten monosporous mycelia of one fruit-body were crossed in all possible ways, with the result shown in Table VIII. It will be seen that in no case did clamp-connexions develop, showing that all ten spores were of one sex.

Now the chances that ten spores picked successively at random from

a deposit of equal numbers of spores of two sexes shall all be of the same sex is, according to the law of probability, 1 : 1,024; and it seemed possible that the fruit-body from which the spores had been derived might have originated from a sclerotium produced by the mycelium of a single spore, in which case, as would be expected from the previous experience of Kniep (11) and Hanna (8), all the spores of the fruit-body should be of one sex. But

TABLE VIII.

All Possible Pairings of Ten Monosporous Mycelia derived from Ten Spores of a Single Fruit-body.

	1	2	3	4	5	6	7	8	9	10
1	-	-	-	-	-	-	-	-	-	-
2	-	-	-	-	-	-	-	-	-	-
3	-	-	-	-	-	-	-	-	-	-
4	-	-	-	-	-	-	-	-	-	-
5	-	-	-	-	-	-	-	-	-	-
6	-	-	-	-	-	-	-	-	-	-
7	-	-	-	-	-	-	-	-	-	-
8	-	-	-	-	-	-	-	-	-	-
9	-	-	-	-	-	-	-	-	-	-
10	-	-	-	-	-	-	-	-	-	-

that the fruit-body was not of monosporous origin and did actually have spores of two different sexes was shown by sowing many of the spores together and obtaining therefrom a diploid clamp-bearing mycelium. Evidently, therefore, the selection of ten spores of one sex was a remarkable chance occurrence.

About October 15 twenty-eight spores derived from seven basidia were successfully germinated. In the third week of October the four mycelia resulting from the four spores of each basidium were crossed in all possible ways. Towards the end of October the plates were examined for clamp-connexions, and it was found that each basidium had borne two spores of one sex and two of another and opposite sex (Table III). It was clear that all the twenty-eight mycelia were unisexual. In order to find out whether or not this unisexuality would be retained by the mycelia indefinitely, on November 3 the mycelia were transferred from the agar plates to sterilized

quart jars half filled with horse-dung and plugged with cotton-wool. On December 17, i. e. eight weeks after the spores had been sown, pieces of the twenty-eight mycelia were transferred to as many agar plates. About December 24 all the plates were examined with the microscope. Two of the mycelia showed no growth. The other twenty-six mycelia all proved to be devoid of clamp-connexions. Therefore these twenty-six mycelia had retained their unisexual character for about nine weeks.

On December 17 transfers of the twenty-eight mycelia were made not only to agar plates, as recorded above, but also to sterilized pint jars half filled with dung and plugged with cotton-wool. As some of the mycelia failed to grow, a few second inoculations were made. Finally twenty-six of the mycelia grew well, while two failed to develop. On January 21 the twenty-six mycelia were transferred from the dung to as many agar plates. About February 7 all the plates were examined with the microscope. It was now found that while twenty-one of the mycelia were still unisexual, the other five mycelia exhibited clamp-connexions, thereby showing that they had passed from the unisexual (haploid) condition to the bisexual (diploid) condition. Thus between the ninth and the sixteenth week of their existence five mycelia had changed spontaneously from the haploid to the diploid condition.

On January 31 transfers of the twenty-six mycelia were made not only to agar plates, as recorded above, but also to sterilized quart jars half filled with dung and plugged with cotton-wool. Two of the mycelia did not grow: on February 7 one of these had been found to be haploid and the other diploid. On March 28 the twenty-four living mycelia were transferred from the dung to as many agar plates. About April 4 all the plates were examined with the microscope. It was found that the four mycelia which had become diploid by February 7 still continued to be diploid, and that of the other twenty mycelia which on February 7 had all been haploid nine had now become diploid, the total now being: eleven haploid and thirteen diploid. Thus between the sixteenth and the twenty-fourth week of their existence, nine mycelia had changed spontaneously from the haploid to the diploid condition.

Summarizing the series of observations just recorded, we have clear evidence that, in the course of six months, of twenty-five monosporous mycelia continuously cultivated eleven mycelia retained their haploid condition, while fourteen mycelia, or 56 per cent. of the whole, changed spontaneously from the haploid to the diploid condition.

As already indicated in the introduction, the sexual change in the monosporous mycelia of *Coprinus Rostrupianus* here recorded resembles that found by Vandendries (20) for the monosporous mycelia of *C. radians*. Of his mycelia twenty-seven changed from the haploid to the diploid condition in the course of six months.

Vandendries (21) has advanced the theory that all species of *Coprinus*, &c., are at first heterothallic: i. e. that the spores of the so-called homothallic species, as well as those of heterothallic species, are all unisexual, and that the mycelia which the spores produce are at first also unisexual. He suggests that in a homothallic species, e. g. *Coprinus sterquilinus*, the mycelia change from the haploid to the diploid condition at a very early stage in their development, whilst in a heterothallic species the change is delayed and may take place only after several weeks or months, as actually occurs with *C. radians*. Vandendries therefore regards *C. sterquilinus* not as homothallic and *C. radians* not as heterothallic, but both of them as *hetero-homothallic*.

Let us suppose that in *Coprinus radians* or *C. Rostrupianus* two haploid mycelia bearing the sex-factors (A) and (a) are mated and give rise to a diploid mycelium from which a fruit-body is produced. This fruit-body will yield 50 per cent. of spores with the (A) factor and 50 per cent. with the (a) factor. Let us suppose further that a mycelium which has come from a spore with the (A) factor spontaneously becomes transformed into a diploid mycelium. We do not as yet know whether the two sexes represented in each pair of nuclei in the diploid phase of this mycelium are identical with the parent sexes and can be represented by the symbols (A) and (a), or whether the two sexes differ from the parental sexes and must therefore be represented by two new symbols, say (A¹) and (a¹), in which latter case we should be obliged to explain the spontaneous change from the haploid to the diploid condition by the theory of mutation. To solve the problem of the nature of the two sexes in our diploid mycelium, it would be necessary to obtain a fruit-body from the diploid mycelium, collect the spores therefrom, germinate them, and back-cross their mycelia with portions of the original haploid parental mycelia or their sister mycelia. If the species is hetero-homothallic, the crossings should yield only two groups of mycelia of opposite sex; but if the spontaneous change to the diploid condition has been due to mutation, there should be three or four groups corresponding to the factors (A), (a), (A¹), and (a¹). The true explanation of the spontaneous change from the haploid to the diploid condition in *C. radians* and *C. Rostrupianus*, therefore, can be given only after further critical experiments have been made.

Finally, we can ask: Do the wild fruit-bodies of *Coprinus Rostrupianus* originate from a diploid mycelium which has been produced by the union of two haploid mycelia of opposite sex, or from a diploid mycelium which has come into existence from a single haploid mycelium by a spontaneous change? The writer is of the opinion that, owing to the large number of spores liberated by each fruit-body and their prevalence on the grass of the meadows where the fungus exists, it is likely that, as a rule, a dung-plot will contain not one spore of *C. Rostrupianus* but many spores, thus giving

ample opportunity for diploid mycelia to be rapidly produced by the union of haploid mycelia of opposite sex. Probably, therefore, the great majority of wild fruit-bodies of *C. Rostrupianus*, and perhaps all of them, arise individually not from a single haploid mycelium which, in the course of many weeks, has spontaneously become diploid, but from two haploid mycelia of opposite sex which have fused and formed a diploid mycelium.

V. SUMMARY.

1. *Coprinus Rostrupianus* is heterothallic, in that its monosporous mycelia during the first few weeks of their existence are all unisexual, half of them being of one sex and the other half of another and opposite sex.

2. Of twenty-five monosporous mycelia, which were cultivated continuously for six months, eleven remained haploid while fourteen, or 56 per cent. of the whole, spontaneously became diploid.

3. *C. Rostrupianus* resembles *C. radians*, as investigated by Vandendries, in that all the spores and young mycelia of a single sexual strain belong to one of two sexes and in that, in the course of some months, many of the monosporous mycelia change spontaneously from the haploid to the diploid condition. Whether or not both of these species should be regarded as hetero-homothallic can only be decided by further investigation.

4. In any individual strain of *C. Rostrupianus* only one type of basidium exists. Each basidium bears one pair of spores of one sex and another pair of spores of another and opposite sex. In any spore-deposit half the spores are of one sex and the other half of another and opposite sex.

5. It is impossible to decide by the experimental methods used whether the reduction process in the basidium takes place with the first or with the second division of the fusion nucleus.

6. Dimorphism of the basidia is not linked with differences in their sexual constitution. Both long and short basidia bear spores of both sexes.

7. Both haploid and diploid mycelia of *C. Rostrupianus* produce sclerotia; but, whereas sclerotia of diploid origin fruit perfectly, those of haploid origin give rise to infertile mycelia or to fruit-body rudiments which soon cease to develop.

8. It seems probable that every wild fruit-body of *C. Rostrupianus*, *C. lagopus*, and many other heterothallic Agaricineae develops from a single diploid mycelium formed by the union of two haploid mycelia of opposite sex, and not from several or many diploid mycelia.

9. *C. Rostrupianus* comprises sexually different strains which show complete interfertility, so that, while each sexual strain is bisexual, the species as a whole must be regarded as multisexual.

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BIBLIOGRAPHY.

1. BENSAUDE, MATHILDE : Recherches sur le cycle évolutif et la sexualité chez les Basidiomycètes. Nemours, pp. 1-156, nine plates, 1918.
2. BREFELD, O. : Untersuchungen. Leipzig, 1877, Heft 3, Taf. I, Figs. 5 and 6; Taf. II; Taf. VI, Fig. 5.
3. BRUNSWIK, H. : Untersuchungen über die Geschlechts- und Kernverhältnisse bei der Hymenomycetengattung *Coprinus*. In K. Goebel's Botanische Abhandlungen, Jena, Heft 5, pp. 1-152, 1924.
4. BULLER, A. H. R. : Experiments on Sex in Mushrooms and Toadstools. Nature, vol. cxiv, p. 826, 1924.
5. ——— : Researches on Fungi. London, vol. iii, 1924.
6. FUNKE, G. L. : Über die Isolierung von Basidiosporen mit dem Mikromanipulator nach Janse und Péterfi. Zeitschr. f. Bot., Jahrg. xvi, pp. 619-23, 1924.
7. HANNA, W. F. : The Dry-needle Method of making Monosporous Cultures of Hymenomycetes. Ann. Bot., vol. xxxviii, pp. 791-4, 1924.
8. ——— : The Problem of Sex in *Coprinus lagopus*. Ibid., vol. xxxix, 1925.
9. HANSEN, E. CHR. : Biologische Untersuchungen über mistbewohnende Pilze. {Bot. Zeit., Bd. xlv, 1897.
10. KNIEP, HANS : Beiträge zur Kenntnis der Hymenomyceten; III, IV, and V. Zeitschr. f. Bot., Bd. vii, 1915; Bd. viii, 1916; Bd. ix, 1917.
11. ——— : Über morphologische und physiologische Geschlechtsdifferenzierung. Verhandl. der Physikal.-Med. Gesellschaft zu Würzburg, Bd. xlvi, pp. 1-18, 1919.
12. ——— : Über Geschlechtsbestimmung und Reduktionsteilung. Ibid., Bd. xlvii, pp. 1-29, 1922.
13. ——— : Über erbliche Änderungen von Geschlechtstfaktoren bei Pilzen. Zeitschr. f. indukt. Abstammungs- und Vererbungslehre, Bd. xxxi, pp. 170-83, 1923.
14. LANGE, J. E. : Studies in the Agarics of Denmark. Dansk Botanisk Arkiv, Copenhagen, 1915.
15. MOUNCE, IRENE : Homothallism and the Production of Fruit-bodies by Monosporous Mycelia in the Genus *Coprinus*. Trans. Brit. Myc. Soc., vol. vii, pp. 198-217, 1921.
16. ——— : Homothallism and Heterothallism in the Genus *Coprinus*. Ibid., pp. 256-69, 1922.
17. RICKEN, A. : Die Blätterpilze. Leipzig, 1915.
18. VANDENDRIES, RENÉ : Recherches sur le déterminisme sexuel des Basidiomycètes. Bruxelles, pp. 1-98, 1923.
19. ——— : Nouvelles recherches sur la sexualité des Basidiomycètes. Bull. Soc. Royale de Bot. de Belgique, t. lvi, pp. 73-97, 1923.
20. ——— : Contribution nouvelle à l'étude de la sexualité des Basidiomycètes. La Cellule, t. xxxv, pp. 129-55, 1924.
21. ——— : L'hétéro-homothallisme dans le genre *Coprinus*. Bull. Soc. Royale de Bot. de Belgique, t. lvii, pp. 1-8, 1925.

EXPLANATION OF PLATE VI.

Illustrating Miss Dorothy Newton's paper on *Coprinus Rostrupianus*.All figures are those of *Coprinus Rostrupianus*.

Fig. 1. Two sclerotia found at Winnipeg in old cow-dung, photographed when dry and shrunken. Natural size.

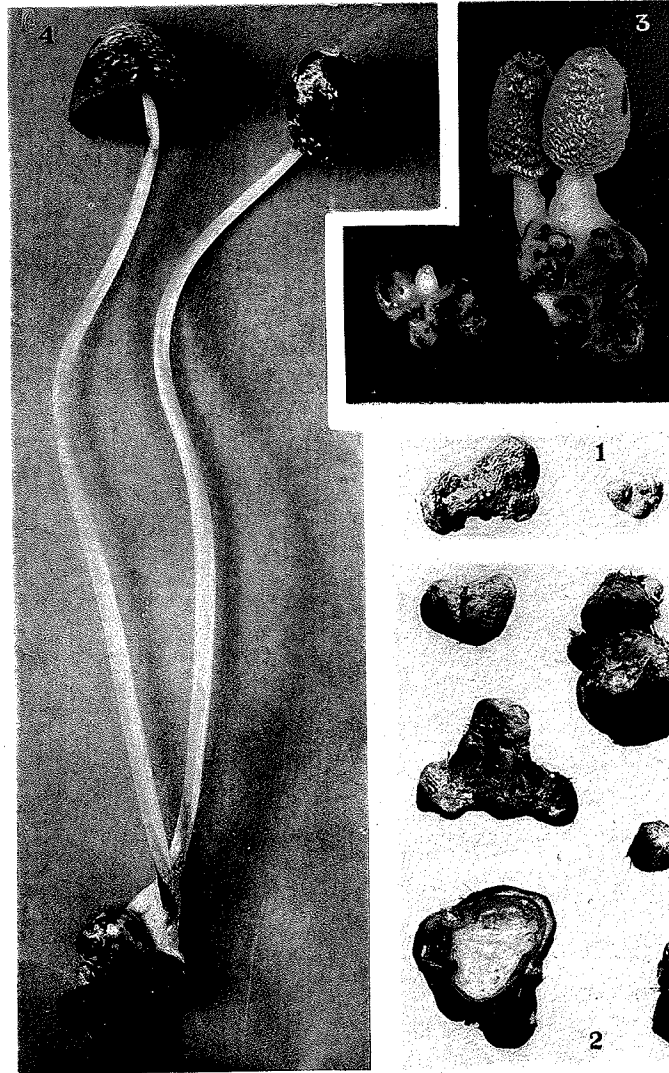
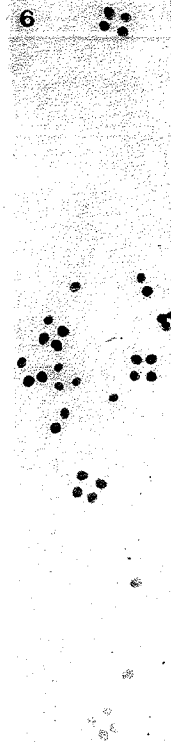
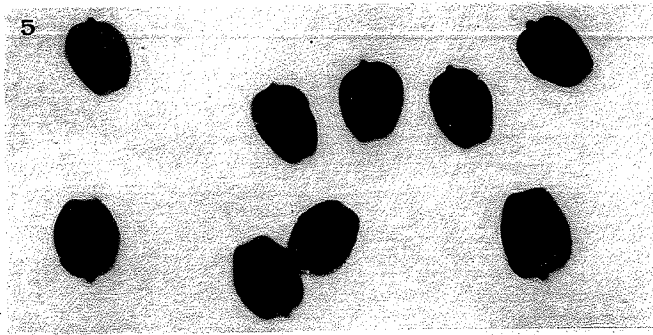
Fig. 2. A group of nine moist and fully swollen sclerotia showing variation in form and size, grown from polysporous (diploid) mycelia on sterilized horse-dung in the laboratory. Natural size.

Fig. 3. The production of fruit-bodies from sclerotia found in old cow-dung. Photograph taken three weeks after the sclerotia had been placed on moist sand in the laboratory. On the left, a single sclerotium bearing the rudiment of a fruit-body. On the right, two sclerotia clinging together, each bearing a fruit-body which is about to elongate its stipe and expand its pileus. Natural size.

Fig. 4. The same two sclerotia and two fruit-bodies as those shown in Fig. 3, one day older. The stipes are now fully elongated. The pilei, which have become campanulate and are now shedding spores, still have their gills interlocked by long cystidia which could be seen with the naked eye. Natural size.

Fig. 5. Spores which settled on a glass slide placed beneath a pileus, photographed dry. $\times 700$.

Fig. 6. A spore-deposit on a cover-glass, obtained by the coverglass-contact method, showing five spore-tetrads, each tetrad being made up of the four spores of a single basidium. $\times 100$.



Huth coll.

NEWTON-COPRINUS.

2

NEWTON, DOROTHY E.

THE DISTRIBUTION OF SPORES OF DIVERSE SEX
ON THE HYMENIUM OF COPRINUS LAGOPUS.

The Distribution of Spores of Diverse Sex on the Hymenium of *Coprinus lagopus*.

BY

DOROTHY E. NEWTON, M.Sc. (McGILL).

With four Diagrams in the Text.

I. INTRODUCTION.

THE observations of Mlle Bensaude, Kniep (6, 7), Miss Mounce (9), Vandendries (11, 12), Brunswik (1), Hanna (4), the writer (10), and others, made from 1918 onwards, have shown that, while certain species of Hymenomycetes are homothallic, other species are heterothallic. The experiments so far made indicate that in the Hymenomycetes there are far more heterothallic species than homothallic.

In a homothallic species of Hymenomycetes each spore gives rise to a mycelium which is at first haploid, but which, in the course of a few days, spontaneously becomes diploid. The haplophase, in which the nuclei are isolated from one another, is associated with simple septa, while the diplophase, in which the nuclei are in pairs and undergo conjugate division, is associated with the presence of clamp-connexions at the cross-walls. Sooner or later the diploid mycelium produces a diploid fruit-body, the pair of nuclei of opposite sex in each basidium fuse together, the fusion nucleus undergoes two successive divisions during which segregation of sex factors takes place, four haploid nuclei are thus produced, and these nuclei pass up into the four spores. The haploid spores thus brought into existence are ready to initiate the life-history once more.

In a heterothallic species each spore on the hymenium of an individual fruit-body is unisexual and, normally, the mycelium produced by each spore, so long as it is grown in isolation, continues to be of one and the same sex as the parent spore. As in a homothallic species, each spore and its branching germ-tube are haploid; but, unlike the homothallic species, a monosporous mycelium of a heterothallic species does not pass spontaneously from the haplophase to the diplophase, but continues in the diplophase until conjugation with another mycelium of opposite sex takes place.

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Heterothallic Hymenomycetes are divisible into two groups: (1) species in which the spores on each fruit-body fall into two sexually opposite groups (haploid genotypes); and (2) species in which the spores on each fruit-body fall into four sexually different groups.

(1) In a heterothallic species with *two* sexually different kinds of spores on each fruit-body, e. g. *Coprinus radians* (11),¹ *C. Rostrupianus* (10), and *C. comatus* (1), normally the spores of one sex (after germination by means of their mycelia) react positively—forming pairs of nuclei, and giving rise to clamp-connexions—only with spores of the opposite sex. One Mendelian pair of factors may be considered as here involved. The diploid fusion nucleus in the basidium bears both factors and may be represented as (*Aa*). The haploid nucleus of each of the four spores which are produced on the basidium bears only one factor, so that sexually the two kinds of spores may be represented as (*A*) and (*a*). In a previous paper I have shown that in *C. Rostrupianus* all the basidia of any individual fruit-body are alike, in that they bear two spores of one sex and two of the other and opposite sex (10). Presumably this is true for other species resembling *C. Rostrupianus*, such as *C. radians* and *C. comatus*.

(2) In a heterothallic species with *four* sexually different kinds of spores on each fruit-body, e. g. *Aleurodiscus polygonius* (7), *Schizophyllum commune* (6), *Coprinus lagopus* (5), and *Hypholoma fasciculare* (3), normally one kind of spore (after germination by means of its mycelium) can react positively with only one of the other three kinds. Two Mendelian pairs of factors may be considered as here involved. The diploid fusion nucleus in the basidium bears both pairs of factors, and may be represented as (*AaBb*). The haploid nucleus of each of the four spores which are produced on the basidium bears only one of each pair of factors, so that, sexually, the four kinds of spores may be represented as (*AB*), (*ab*), (*Ab*), and (*aB*).

In species which on any individual fruit-body have four sexually different kinds of spores (*AB*), (*ab*), (*Ab*), (*aB*), it is of interest to determine the relative numbers and positions of the four kinds of spores on the hymenium which covers the gills. Towards the solution of this problem certain data have already been obtained by Kniep (7), Funke (3), Hanna (5), and myself (2, 10).

Kniep, in 1922, observed that in *Aleurodiscus polygonius*, under certain abnormal moisture conditions, the four spores of each basidium are shot

¹ Vandendries working with *Coprinus radians* (12) and the writer (10) working with *C. Rostrupianus* have observed that, while in these species all the monosporous mycelia are at first unisexual (haploid) and as such can be mated in the usual way, when these unisexual mycelia are kept in culture for several weeks or months a large percentage of them spontaneously become bisexual (diploid), as is indicated by the spontaneous development of clamp-connexions. This phenomenon, which is of considerable theoretical interest, requires further experimental elucidation. Species in which the monosporous mycelia are at first unisexual and subsequently bisexual have been called by Vandendries *hetero-homothallic*.

way together in a single mass. Taking advantage of this fact, he procured the spore-masses of thirty-five basidia. Each spore-mass was deposited in sterilized agar in a flask, and the four spores were then separated from one another by shaking the flask. After the spores had germinated, the four mycelia were isolated and then mated with one another in all possible ways. Kniep found that each of his thirty-five basidia had produced two kinds of spores, two of one sex and two of another and opposite sex. The four spores of some of the basidia contained the sex factors (AB) , (AB) , (ab) , (ab) , while the four spores of the other basidia contained the sex factors (Ab) , (Ab) , (aB) , (aB) . Kniep therefore concluded that the reduction process in the basidium takes place in the *first* division of the fusion nucleus and not in the second. Subsequently, he informed Professor A. H. R. Buller *in litt.* (March, 1925) that in further experiments with *Aleurodiscus polygonius* he had discovered a few basidia which had borne all four kinds of spores (AB) , (ab) , (Ab) , (aB) , so that he could no longer maintain that in this species the reduction process always takes place in the first nuclear division.

In October, 1924, Funke (3) announced that with the help of a micro-manipulator he had succeeded in removing the four spores of a few individual basidia of *Hypholoma fasciculare*, *H. capnoides*, and *Collybia velutipes*, in sowing the spores separately in culture media, and in analysing each set of four spores for their sex reactions. In *Hypholoma fasciculare* two basidia were of the type (AB) , (AB) , (ab) , (ab) , and four of the type (AB) , (ab) , (Ab) , (aB) . In *Collybia velutipes* one basidium was of the type (AB) , (AB) , (ab) , (ab) , and three others of the type (AB) , (ab) , (Ab) , (aB) . In *Hypholoma capnoides* similar results were obtained, but the number of basidia used was not stated. In none of these species, doubtless owing to the small numbers of basidia analysed, did Funke obtain a third type of basidium: (Ab) , (Ab) , (aB) , (aB) . He concluded from his results that, where a basidium had produced only two kinds of spores, the reduction process had taken place in the first division of the fusion nucleus and that, when a basidium had produced all four kinds of spores, the reduction process had taken place in the second division.

In November, 1924, Buller (2), without being aware of the publication of Funke's paper, gave a summary of some experiments on sex which had been made in his laboratory. He described the cover-glass-contact method for isolating the four spores of a single basidium; and he announced that Hanna had found two types of basidia on the gills of *Coprinus lagopus*, (AB) , (AB) , (ab) , (ab) , and (AB) , (ab) , (Ab) , (aB) , and that Newton had found that all the basidia on the hymenium of any fruit-body of *Coprinus Rostrupianus* were alike in that each of them bears two spores of one sex and two of another and opposite sex.

Hanna (4), in April, 1925, gave a detailed account of his experiments

on *Coprinus lagopus*. Of thirteen basidia analysed, seven bore two spores of one sex and two spores of another and opposite sex, while six bore spores of all four kinds (AB), (ab), (Ab), (aB). He stated that in the basidia with two spores of one sex and two of another and opposite sex there were in all probability two types present, namely (AB), (AB), (ab), (ab), and (Ab), (Ab), (aB), (aB), but he did not carry out any mating experiments to test his supposition (5).

In January, 1926, I stated (10) that I had analysed about twenty basidia of *Coprinus Rostrupianus* for the sexual reactions of their spores, and had found that every one of these basidia had borne two spores of one sex and two of another and opposite sex. There were no exceptions to this rule. Therefore I concluded that of the spores on any fruit-body of *C. Rostrupianus*, 50 per cent. are of one sex and 50 per cent. of another and opposite sex.

In the present paper, an attempt is made to extend our knowledge of the distribution of spores of diverse sex on the hymenium of *Coprinus lagopus*. Hanna, as we have seen, found that on a single fruit-body some of the basidia bore all four kinds of spores (AB), (ab), (Ab), (aB), and other basidia spores of two kinds only, but he did not demonstrate that of the latter some basidia were of the type (AB), (AB), (ab), (ab), and others of the type (Ab), (Ab), (aB), (aB), although he regarded this as probable. Analyses of the sexual reactions of the four spores of numerous individual basidia have been made for the following purposes: (1) to determine whether or not the three types of basidia (AB), (ab), (Ab), (aB), and (AB), (AB), (ab), (ab), and (Ab), (Ab), (aB), (aB), occur on the hymenium of any individual fruit-body, as supposed by Hanna; (2) to obtain data as to the relative numbers of the three types of basidia on any individual fruit-body; and (3) to determine in respect to sex the relative positions of the four spores on individual basidia, in the hope of throwing some light on the reduction process.

II. METHODS.

Some fruit-bodies of *Coprinus lagopus* came up spontaneously on fresh horse-dung balls contained in a large covered dish in the laboratory. A spore-deposit produced by the pileus of a single fruit-body was collected on a sterilized glass slide, and then many of the spores were sown together on sterilized horse-dung in a quart jar plugged with cotton-wool. After about fourteen days the mycelium began to yield fruit-bodies, and fruiting continued for several weeks. This culture, which was the product of a single sexual strain,¹ afforded material for most of the experiments, and

¹ Hanna (5) has shown that *Coprinus lagopus* is made up of many sexual strains, any two of which are perfectly fertile *inter se*, i. e. any monosporous mycelium of one strain will give a positive sexual reaction with any monosporous mycelium of the other strain.

was always employed where intercrossings between the spores of two or more basidia from the same or from diverse fruit-bodies were undertaken.

The four spores of single basidia were removed from the hymenium by the cover-glass-contact method (2, 5, 10), and then the individual spores of each tetrad were sown separately in dung-agar by the dry-needle method (4). A thin layer of dung-agar was poured into a Petri dish, and after it had set four circles, each about 1 cm. wide, were drawn in a row with a wax pencil on the under side of the dish, and numbered in succession 1, 2, 3, and 4. In order to keep a record of the relative positions of the four spores of a tetrad derived from a single basidium, the spores were removed by the needle in counter-clockwise succession: the first spore removed was set in the agar above the circle No. 1, the second spore above the circle No. 2, and so forth for spores No. 3 and No. 4.

In using the technique just described there are certain difficulties involved. (1) The four spores of a tetrad attached to the cover-glass, while separate from one another, are only a few microns apart, and to remove them one by one with the needle in counter-clockwise succession without allowing any one to touch its fellows requires steadiness of hand and some practice. If, after spores No. 1 and No. 2 had been successfully removed from a tetrad, spores Nos. 3 and 4 became stuck together accidentally, the original relative positions of the spores Nos. 3 and 4 were lost, and the whole tetrad had to be discarded so far as its value for determining the relative positions of the spores of diverse sex was concerned; but, after spores Nos. 3 and 4 had been separated by manipulations with the needle and had been sown separately, the tetrad could still be used for determining the nature of the sexes of the four spores, and thus for adding to the statistics concerning the relative numbers of the three types of basidia which occur on the hymenium. (2) It not infrequently happens that of the four spores of a tetrad which have been successfully isolated in counter-clockwise succession, and have been sown in their proper sequence above the four rings in the Petri dish, only one or two germinate. One is then obliged to discard the tetrad for all purposes. When three spores germinate, the tetrad can be further investigated advantageously; for, when the sexes of three of the four spores in any tetrad are known, one can readily infer to which of the three kinds of tetrads, namely (AB) , (ab) , (Ab) , (aB) ; (AB) , (AB) , (ab) , (ab) ; and (Ab) , (Ab) , (aB) , (aB) , the tetrad under investigation belongs.

When all four spores of a single tetrad, or three spores of the four germinated, the individual monosporous mycelia, when about two days old, were transferred to separate Petri dishes. The culture medium was always dung-agar, which was prepared in the usual manner (10). The method of pairing the monosporous mycelia on agar plates was the same as that described by Hanna (5).

The criteria of sex employed were those now generally in use in experimental work on hymenomycetous fungi (9, 10). The chief criterion was the presence or absence of clamp-connexions after two monosporous mycelia had been paired on dung-agar in a Petri dish. Normally, a monosporous mycelium, when grown for an indefinite period in isolation, never develops any clamp-connexions. When two monosporous mycelia have been paired on a plate, if, after union, they develop clamp-connexions, they are considered as being of opposite sex; whereas if, after union, they fail to produce clamp-connexions, they are considered as having one or both sex factors in common. Sexual union may be effected by the following pairings, $(AB) \times (ab)$, $(Ab) \times (aB)$, but not by the following pairings, $(AB) \times (AB)$, $(ab) \times (ab)$, $(Ab) \times (Ab)$, $(aB) \times (aB)$, $(AB) \times (aB)$, $(AB) \times (Ab)$, $(Ab) \times (ab)$, $(ab) \times (aB)$.

While the clamp-connexion criterion was employed in every experiment, another criterion, namely, the presence or absence of oidia, was also very generally used, although in a subsidiary manner. Haploid mycelia not only do not produce clamp-connexions, but they develop oidia freely, and the oidia give to the surface of the mycelium a more or less floury appearance. On the other hand, diploid mycelia, while producing clamp-connexions, never develop oidia, and the surface of such mycelia is never floury to the naked eye. When two monosporous mycelia have been paired, therefore, the cessation of oidia-production as the mycelia grow radially outwards indicates that the two mycelia are of opposite sex, whereas the continued production of oidia indicates that the two mycelia possess either one or both sex factors in common.

III. DISCUSSION OF THE THREE THEORETICAL TYPES OF BASIDIA.

Granted that a fruit-body of *Coprinus lagopus* bears spores of four sexually different kinds and that each spore bears two sex factors, one belonging to one pair of factors and the other to another pair of factors, the spores can be represented by the symbols (AB) , (ab) , (Ab) , and (aB) . Then there are three types of basidia of which the existence seems probable, and they may be represented by the sex factors of their spores: (AB) , (ab) , (Ab) , (aB) ; (AB) , (AB) , (ab) , (ab) ; and (Ab) , (Ab) , (aB) , (aB) .

When the four spores of a basidium of the type (AB) , (ab) , (Ab) , (aB) are crossed in all possible ways between themselves, we obtain a result like that shown in Table I. Each monosporous mycelium gives clamp-connexions with *one* only of the three other mycelia. The only pairs of mycelia which give clamp-connexions are those which have no sex factors in common. In all the tables a (+) sign indicates that, after pairing, clamp-connexions appeared in the culture, and a (-) sign that they did not.

When the four spores of a basidium of the type (AB) , (AB) , (ab) , (ab) are crossed in all possible ways between themselves, we obtain a result like that shown in Table II. Each monosporous mycelium gives clamp-connexions with *two* of the other three mycelia. Here, again, the only pairs of mycelia which give clamp-connexions are those which have no sex factors in common.

TABLE I.

	AB	ab	Ab	aB
AB	-	+	-	-
ab	+	-	-	-
Ab	-	-	-	+
aB	-	-	+	-

Bas. I.

TABLE I. All possible pairings of the four monosporous mycelia from a basidium of the type (AB) , (ab) , (Ab) , (aB) .

TABLE II.

	AB	AB	ab	ab
AB	-	-	+	+
AB	-	-	+	+
ab	+	+	-	-
ab	+	+	-	-

Bas. II.

TABLE II. All possible pairings of the four monosporous mycelia from a basidium of the type (AB) , (AB) , (ab) , (ab) .

TABLE III.

	Ab	Ab	aB	aB
Ab	-	-	+	+
Ab	-	-	+	+
aB	+	+	-	-
aB	+	+	-	-

Bas. III.

TABLE III. All possible pairings of the four monosporous mycelia from a basidium of the type (Ab) , (Ab) , (aB) , (aB) .

TABLE IV.

	AB	AB	ab	ab
Ab	-	-	-	-
Ab	-	-	-	-
aB	-	-	-	-
aB	-	-	-	-

Bas. II × Bas. III.

TABLE IV. All possible pairings of the four monosporous mycelia from a basidium of the type (AB) , (AB) , (ab) , (ab) with the four monosporous mycelia from a basidium of the type (Ab) , (Ab) , (aB) , (aB) .

When the four spores of a basidium of the type (Ab) , (Ab) , (aB) , (aB) are crossed in all possible ways between themselves, we obtain a result like that shown in Table III. Each monosporous mycelium gives clamp-connexions with *two* of the other three mycelia. Here, as in the other types of basidia, the only pairs of mycelia which give clamp-connexions are those which have no sex factors in common.

If now the four spores of a basidium of the type (AB) , (AB) , (ab) , (ab) are paired in all possible ways with the four spores of a basidium of the type (Ab) , (Ab) , (aB) , (aB) , the result to be expected should be that shown in Table IV, for in every pair of mycelia there will be one sex factor in common, and this will prevent the formation of conjugate pairs of nuclei and of clamp-connexions. Thus Table IV shows negative results only.

After pairing the four spores of one basidium with the four spores of another basidium: if (1) we obtain half-positive and half-negative results like those shown in Tables II and III, we may be quite sure that the two basidia crossed are of the same type, and either (AB) , (AB) , (ab) , (ab) , or (Ab) , (Ab) , (aB) , (aB) , although we cannot tell which; whereas, if (2) we obtain entirely negative results like those shown in Table IV, we may be

TABLE V.

		<i>Bas. II.</i>				<i>Bas. III.</i>			
		<i>AB</i>	<i>AB</i>	<i>ab</i>	<i>ab</i>	<i>Ab</i>	<i>Ab</i>	<i>aB</i>	<i>aB</i>
<i>Bas. I.</i>	<i>AB</i>	-	-	+	+	-	-	-	-
	<i>ab</i>	+	+	-	-	-	-	-	-
	<i>Ab</i>	-	-	-	-	-	-	+	+
	<i>aB</i>	-	-	-	-	+	+	-	-

All possible pairings of the four monosporous mycelia from each of the two types of basidia (AB) , (AB) , (ab) , (ab) and (Ab) , (Ab) , (aB) , (aB) with the four monosporous mycelia of a basidium of the type (AB) , (ab) , (Ab) , (aB) .

equally sure that the two basidia are of different types, one of them being of the type (AB) , (AB) , (ab) , (ab) and the other of the type (Ab) , (Ab) , (aB) , (aB) . If, arbitrarily, we give one set of symbols to one of the two groups of four monosporous mycelia, then we must give the other set of symbols to the other group.

If the four spores of a basidium of the type (AB) , (ab) , (Ab) , (aB) are paired with the four spores of a basidium of the type (AB) , (AB) , (ab) , (ab) , and also with the four spores of a basidium of the type (Ab) , (Ab) , (aB) , (aB) , theoretically we obtain results like those shown in Table V. Let us call the three basidia shown in the table Basidium I, Basidium II, and Basidium III, so that their numbers correspond to the three types just mentioned and in the same respective order. Then we perceive, by reference to Table V, that the four spores of Basidium II do not react with the spores (Ab) and (aB) of Basidium I, nor the four spores of Basidium III with the spores (AB) and (ab) of Basidium I, owing to the fact that in all these pairings one sex factor would be common to both mycelia. The positive

reactions between Basidia I and II are limited to pairings between (*AB*) and (*ab*) spores which have no sex factors in common; and, similarly, the positive reactions between Basidia I and III are limited to pairings between (*Ab*) and (*aB*) spores.

IV. AN EXPERIMENTAL PROOF OF THE EXISTENCE OF THE THREE TYPES OF BASIDIA.

(1) In the first series of experiments thirty basidia were investigated. Fifteen gave results like that shown in Table I, which indicates that they had produced spores of four different sexes, i. e. were quadrisexual, while fifteen gave half-positive and half-negative results like those shown in Tables II and III, which indicates that they had produced two spores of one sex and two spores of another and opposite sex, i. e. were bisexual. Without further investigation, however, as explained in the preceding section, it could not be known whether the fifteen bisexual basidia were all of the type shown in Table II, or were all of the type shown in Table III, or were some of one type and some of the other.

Of the fifteen basidia which bore spores of two kinds in pairs, eleven were selected for further analysis. In five of these all four spores had germinated, and in the remaining six, three spores only. Thus there were in hand a total of thirty-eight monosporous mycelia. These were then paired in all possible ways with a view to determining which were of the type shown in Table II and which of the type shown in Table III. The total number of pairings was 741, and the results were set out in a large table of which a representative section, embodying examples of all the kinds of reaction obtained, is shown in Table VI. In this table, the roman numerals attached to the basidia indicate the order in which the basidia were investigated by themselves before being paired with other basidia, while the arabic numerals indicate spores in the order in which they were removed from each tetrad, so that both kinds of numbers were chosen arbitrarily. The Mendelian symbols are those we seem compelled to adopt to explain the experimental results.

In Table VI it will be seen that the spores of three basidia (excepting one spore of Basidium VI which did not germinate) have been paired in all possible ways. The monosporous mycelia from Basidia VI and XIV gave no clamp-connexions with those of Basidium XIII. The monosporous mycelia from Basidia IX, XII, XIX, and XXVI behaved towards those from Basidium XIII exactly like those of Basidia VI and XIV. Hence we may conclude that Basidia VI, IX, XII, XIV, XIX, and XXVI are all of one and the same sexual type. The monosporous mycelia of Basidia XVIII, XXII, XXIV, and XXVII exactly resembled those of Basidium XIII in that they failed to react with the mycelia of Basidia VI, IX, XII, XIV,

XIX, and XXVI. Hence it is apparent that Basidia XIII, XVIII, XXII, XXIV, and XXVII belong to the same sexual type, but to one differing from that of Basidium VI and its like. If now to the spores of Basidia XIII, XVIII, XXII, XXIV, and XXVII we assign the symbols (AB) , (AB) , (ab) , (ab) , then to the spores of Basidia VI, IX, XII, XIV, XIX, and XXVI we must assign the symbols (Ab) , (Ab) , (aB) , (aB) . Thus of the eleven

TABLE VI.

		<i>Bas. VI</i>		<i>Bas. XIII</i>				<i>Bas. XIV</i>					
		<i>Ab</i>	<i>aB</i>	<i>AB</i>	<i>ab</i>			<i>Ab</i>	<i>aB</i>				
		1	3	2	1	2	3	4	1	4	2	3	
<i>Bas. VI</i>	<i>Ab</i>	1	-	-	+	-	-	-	-	-	+	+	
		3	-	-	+	-	-	-	-	-	+	+	
	<i>aB</i>	2	+	+	-	-	-	-	-	+	+	-	-
		4	+	+	-	-	-	-	-	+	+	-	-
<i>Bas. XIII</i>	<i>AB</i>	1	-	-	-	-	+	+	-	-	-	-	
		2	-	-	-	-	-	+	+	-	-	-	-
	<i>ab</i>	3	-	-	-	+	+	-	-	-	-	-	-
		4	-	-	-	+	+	-	-	-	-	-	-
<i>Bas. XIV</i>	<i>Ab</i>	1	-	-	+	-	-	-	-	-	+	+	
		4	-	-	+	-	-	-	-	-	+	+	
	<i>aB</i>	2	+	+	-	-	-	-	-	+	+	-	-
		3	+	+	-	-	-	-	-	+	+	-	-

Coprinus lagopus. All possible pairings of eleven monosporous mycelia from three different basidia, with a Mendelian interpretation of the results.

basidia which bore spores of two kinds in pairs, selected for further analysis, five were of the type shown in Table II and six of the type shown in Table III.

The above series of experiments clearly shows that, as supposed on theoretical grounds by Hanna, on the hymenium of any fruit-body of *Coprinus lagopus* there are three types of basidia the spores of which can be represented sexually as: (AB) , (ab) , (Ab) , (aB) ; (AB) , (AB) , (ab) , (ab) ; and (Ab) , (Ab) , (aB) , (aB) .

(2) In a second series of experiments the following basidia were procured: Basidium XL with four kinds of spores and Basidia XXXIX, XLIII,

XLV, XLVI, XLVII, and XLVIII, all of which, when analysed singly, were found to have produced only two kinds of spores in pairs. Of Basidia XXXIX, XL, XLIII, and XLV all the four spores had germinated, and of Basidia XLVI, XLVII, and XLVIII only three of the four. Thus there were in hand twenty-five monosporous mycelia. These were paired, not *inter se* as in the first series of experiments, but with the four mycelia of Basidium XXXV, which was of the four-sex type (AB) , (ab) , (Ab) , (aB) . The experimental results are embodied in Table VII. The roman and arabic numbers have the same significance as that already given for Table VI, and, in arranging the table, for clearness and making comparisons the like monosporous mycelia for each of the basidia have been placed together. The symbols (AB) , (ab) , (Ab) , (aB) for the monosporous mycelia of Basidium XXXV were chosen arbitrarily. The symbols for the

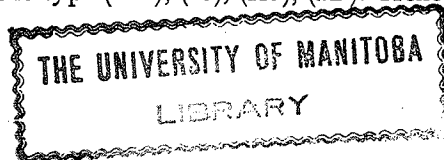
TABLE VII.

		Type I				Type II				Type III																	
		XL		XXXIX		XLIII		XLVIII		XLV		XLVI		XLVII													
		AB	ab	Ab	aB	AB	ab	AB	ab	AB	ab	Ab	aB	Ab	aB												
		1	4	3	2	2	3	1	4	2	4	1	3	1	2	4	3	4	1	2	2	3	1	1	4	2	
Type I XXXV	AB	-	+	-	-	-	-	+	+	-	-	+	+	-	-	+	-	-	-	-	-	-	-	-	-	-	-
	ab	+	-	-	-	+	+	-	-	+	+	-	-	+	+	-	-	-	-	-	-	-	-	-	-	-	-
	Ab	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	+
	aB	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	-	+

Coprinus lagopus. All possible pairings of twenty-five monosporous mycelia from seven different basidia with four monosporous mycelia from a basidium that produced spores of four different sexes. The results show that the seven basidia include representatives of all the three possible types.

monosporous mycelia of the other seven basidia are those which it was necessary to choose in order to explain the experimental results.

An examination of Table VII shows: (1) that Basidium XL gave with Basidium XXXV results like those shown in Table I, i. e. what we should expect theoretically when the four mycelia from each of two basidia both of the type (AB) , (ab) , (Ab) , (aB) are paired together in all possible ways; (2) that Basidia XXXIX, XLIII, and XLVIII gave with Basidium XXXV results like those shown in the left half of Table V, i. e. what we should expect theoretically when the four mycelia from a basidium of the type (AB) , (Ab) , (ab) , (aB) are paired with the four mycelia from a basidium of the type (AB) , (ab) , (Ab) , (aB) ; and (3) that Basidia XLV, XLVI, and XLVII gave with Basidium XXXV results like those shown in the right half of Table V, i. e. what we should expect theoretically when the four mycelia from a basidium of the type (Ab) , (Ab) , (aB) , (aB) are paired with the four mycelia from a basidium of the type (AB) , (ab) , (Ab) , (aB) . Hence



we may conclude from this second series of experiments that all the three types of basidia, which were postulated theoretically, were actually present together in one and the same hymenium.

V. THE RELATIVE NUMBERS OF THE THREE TYPES OF BASIDIA.

In the course of several months a total of forty-seven basidia were analysed for the sexual constitution of their spores. Four monosporous mycelia were obtained from the four spores of some of the basidia, but only three from others, owing to deficient germination. As there are only three types of basidia on the hymenium of *Coprinus lagopus* and as these types are so different from one another, when the sexual constitution of three of the four spores has been ascertained by experiment, one can say with certainty to which of the three types the basidium belongs. Hence, for our present inquiry, basidia of which only three spores germinated are just as useful as those of which all four spores germinated.

From the spores of the forty-seven basidia a total of one hundred and sixty-six monosporous mycelia was obtained. The mycelia from the spores of each basidium were first paired among themselves in all possible ways, as in Tables I, II, and III, so as to determine which of the basidia had borne spores of all four sexes (AB), (ab), (Ab), (aB), and which had borne spores of only two sexes in pairs; and then the mycelia from the basidia which had borne spores of only two sexes in pairs were paired in the manner illustrated in Tables VI and VII so as to determine which of them were of the type (AB), (AB), (ab), (ab), and which of the type (Ab), (Ab), (aB), (aB). The statistical results of the investigation may be summarized as follows:

1. Twenty-five basidia belonged to the (AB), (ab), (Ab), (aB), or four-sex type.
2. Twenty-two basidia belonged to the two-sex types, of which seventeen were further investigated in order to assign them to their respective types. Of these seventeen basidia:
 - (a) Nine belonged to the (AB), (AB), (ab), (ab) type, and
 - (b) Eight belonged to the (Ab), (Ab), (aB), (aB) type.

The data just recorded indicate: (1) that the number of basidia of the four-sex type (AB), (ab), (Ab), (aB) occurring upon the hymenium of any fruit-body is approximately equal to the number of basidia of the two two-sex types taken together; and (2) that the two two-sex types of basidia, namely, (AB), (AB), (ab), (ab), and (Ab), (Ab), (aB), (aB), are present on the hymenium in approximately equal numbers.

Expressed in percentages, the data given above indicate that of the three

types of basidia situated on the hymenium of any fruit-body of *Coprinus lagopus* :

- 50 per cent. are of the type (AB) , (ab) , (Ab) , (aB) ,
- 25 per cent. are of the type (AB) , (AB) , (ab) , (ab) , and
- 25 per cent. are of the type (Ab) , (Ab) , (aB) , (aB) .

These statistics will be further discussed in the next section.

VI. THE RELATIVE POSITIONS, IN RESPECT TO SEX, OF THE FOUR SPORES OF INDIVIDUAL BASIDIA.

A. *Experimental Results.*

The relative positions, in respect to sex, of the four spores of each of thirty-one basidia were determined by the methods already described in Section II. The results were as follows :

1. Twenty basidia bore spores of all four sexes. Of these there were :
 - (a) Eight with the (aB) spore in a diagonal position to the (AB) spore,
 - (b) Four with the (Ab) spore in a diagonal position to the (AB) spore,
 - (c) Eight with the (ab) spore in a diagonal position to the (AB) spore.
2. Eleven basidia bore spores of two sexes in pairs. Of these in the (Ab) , (Ab) , (aB) , (aB) basidia there were :
 - (d) Four with an (aB) spore in a diagonal position to an (Ab) spore,
 - (e) None with an (Ab) spore in a diagonal position to an (Ab) spore, while in the (AB) , (AB) , (ab) , (ab) basidia there were :
 - (f) Six with an (ab) spore in a diagonal position to an (AB) spore,
 - (g) One with an (AB) spore in a diagonal position to an (AB) spore.

In the accompanying diagram (see p. 904) are shown the seven possible arrangements of the spores of diverse sex on the basidia. The inner circle in each spore represents a nucleus, and the symbols within a pair of sex factors. The number in the centre of each basidium body gives the actual number of basidia found with the arrangement of spores represented.

B. *Theoretical Discussion.*

Theoretically, where there is only *one pair* of sex factors, the segregation of these factors¹ in the fusion nuclei of the basidia of any hymenium

¹ Reduction of the total number of chromosomes and the segregation of sex factors during the two divisions of the fusion nucleus are not identical phenomena. In this paper the phenomenon of segregation is of first interest, and hence the use of the term reduction will be avoided.

may take place in three possible ways: (1) in all the basidia in the first division of the nucleus; (2) in all the basidia in the second division; and (3) in some basidia in the first division and in other basidia in the second division.

In *Coprinus lagopus*, in any individual fruit-body, to explain the occurrence of four sexually different kinds of spores, it has been found

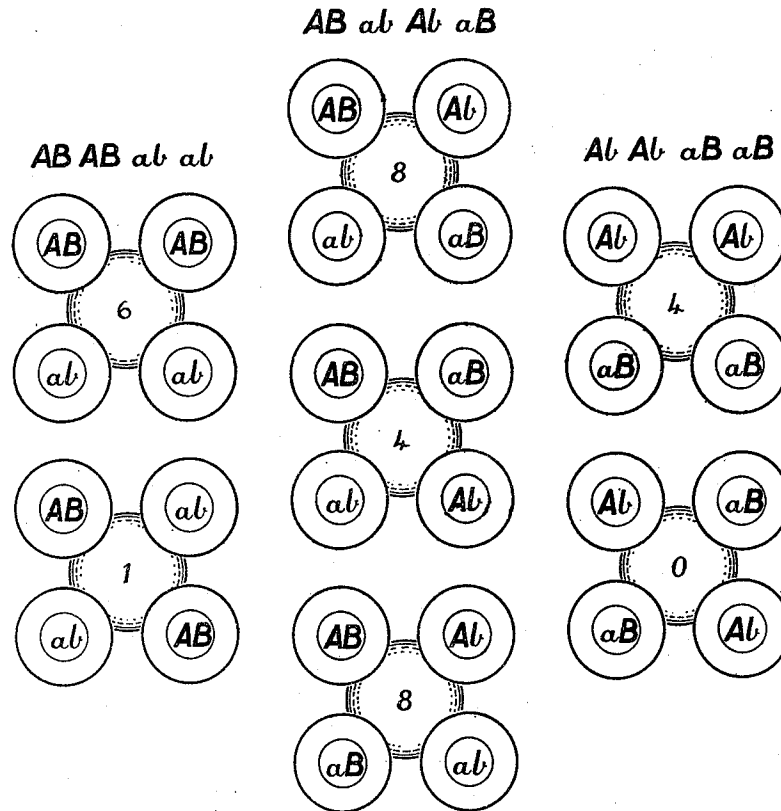


DIAGRAM 1. Showing the arrangement of the spores of diverse sex on thirty-one basidia of *Coprinus lagopus*.

necessary to assume that *two pairs of Mendelian factors are involved*, (*Aa*) and (*Bb*), and that each spore bears only one factor of each pair. The four kinds of spores possible on this assumption are: (*AB*), (*ab*), (*Ab*), and (*aB*).

In seeking for an explanation of the experimental data, it seems necessary to assume that each spore of *Coprinus lagopus* bears its two sex factors *not in the same chromosome but in two chromosomes*, one chromosome containing one factor and another chromosome the other factor.

Theoretically, where there are two pairs of sex factors, as in *Coprinus*

lagopus, the segregation of the sex factors in the fusion nuclei of the basidia of any hymenium may take place in seven possible ways. Let

A = segregation of both pairs of factors in the *first* division of the fusion nucleus,

B = segregation of both pairs of factors in the *second* division, and

C = segregation of one pair of factors in the *first* division, and of the other pair of factors in the *second* division.

Then the seven possible ways in which segregation may take place are as follows :

- (1) in all the basidia as in A ;
- (2) in all the basidia as in B ;
- (3) in all the basidia as in C ;
- (4) in some basidia as in A , in other basidia as in B ;
- (5) in some basidia as in A , in other basidia as in C ;
- (6) in some basidia as in B , in other basidia as in C ; and
- (7) in some basidia as in A , in other basidia as in B , and in yet other basidia as in C .

These various modes of segregation will now be discussed *seriatim* with a view to discovering which of them best fits the experimental facts.

(1) *In all the basidia segregation of both pairs of factors in the first division of the fusion nucleus.* Assuming that each of the two nuclei of opposite sex about to fuse in a young basidium contains two chromosomes bearing sex factors, and that segregation takes place in the manner just indicated, Scheme I represents what should happen during the process. Here, as in Schemes II and III which follow, the circles represent nuclei, the quadrilaterals chromosomes, and the nuclei are supposed to be viewed from the top of the young basidium, the observer looking down on the hymenium. The outlines of the spores and of the basidium wall are not shown. The five lines joining any two nuclei indicate that the two nuclei are daughter nuclei of a single parent nucleus.

At the top of Scheme I is shown a pair of nuclei of opposite sex, in a basidium, about to fuse, each having two chromosomes bearing sex factors. Two other nuclei, (Ab) and (aB), might have been substituted for the (AB) and (ab) nuclei actually here represented. The nuclei fuse (first large circle) with a pairing of (A) with (a) and of (B) with (b). Then in this fusion nucleus the chromosomes split (second large circle). Now segregation takes place in the first division of the fusion nucleus. There are two possibilities: the (AA) chromosomes may go with the (BB) chromosomes, and the (aa) with the (bb), as shown on the left, or the (AA) chromosomes may go with the (bb) chromosomes, and the (aa) with the (BB), as shown on the right. Finally, after the second nuclear division we get the arrangement of the nuclei shown at the base of the scheme.

It is known that the four nuclei resulting from the second division of the fusion nucleus make their way to the top of the basidium body and become attached there by their centrosomes. Above each centrosome the cell-wall grows upwards to form a sterigma and then a spore. The four nuclei then creep up their respective sterigmata, and so become introduced into the four spores (8). We shall assume that the four nuclei produced by divisions like those shown at the base of Scheme I are developed in a plane situated transversely to the basidium axis, as is usual in the higher Hymenomyces, and that they fix themselves to the top of the basidium in the same relative order as that in which they come into existence in the first place. On this assumption, which remains to be verified cytologically, the relative positions of the four spores of any basidium, from the sexual point of view, are identical with the relative positions which were occupied by the four nuclei in the basidium body. The four circles of each group at the base of Scheme I, and of Schemes II and III which follow, therefore represent in respect to sex the relative positions of the four nuclei produced from the fusion nucleus in (1) the basidium body where the nuclei were formed, and in (2) the four spores into which they moved.

Does Scheme I, in which the segregation of both pairs of sex factors takes place in all the basidia in the first division of the fusion nucleus, fit the experimental facts? Evidently not: it does not provide for the occurrence of any basidia of the four-sex type, yet of the forty-seven basidia analysed, twenty-five were four-sex and twenty-two two-sex. Scheme I, therefore, does not account for one-half of the experimental facts, and for this reason alone it must be discarded. Scheme I yields two kinds of basidia only: (AB) , (AB) , (ab) , (ab) , and (Ab) , (Ab) , (aB) , (aB) ; but, as we shall see, these two-sex basidia, theoretically, can come into existence by segregation taking place in the second nuclear division.

(2) *In all the basidia segregation of both pairs of factors in the second division of the fusion nucleus.* Assuming that each of the two nuclei of opposite sex about to fuse in a young basidium contains two chromosomes bearing sex factors, and that segregation takes place in the manner just indicated, Scheme II represents what should happen during the process.

Scheme II begins like Scheme I. At the top is shown a pair of nuclei of opposite sex, in a basidium, about to fuse, each having two chromosomes bearing sex factors. Two other nuclei, (Ab) and (aB) , might have been substituted for the (AB) and (ab) nuclei actually here represented. The nuclei fuse (first large circle) with a pairing of (A) with (a) and of (B) with (b) . Then in this fusion nucleus the chromosomes split (second large circle). There is no segregation in the first division of the fusion nucleus, so that each of the daughter nuclei, as shown, must contain all four sex factors (A) , (a) , (B) , and (b) . As a result of the second division of the fusion nucleus, as shown, there are six possible positions for the four nuclei. As in Scheme I,

the five lines joining any two nuclei indicate that the two nuclei are daughter nuclei of a single parent nucleus. The numbers placed in the centre of each group of four nuclei indicate how many of the thirty-

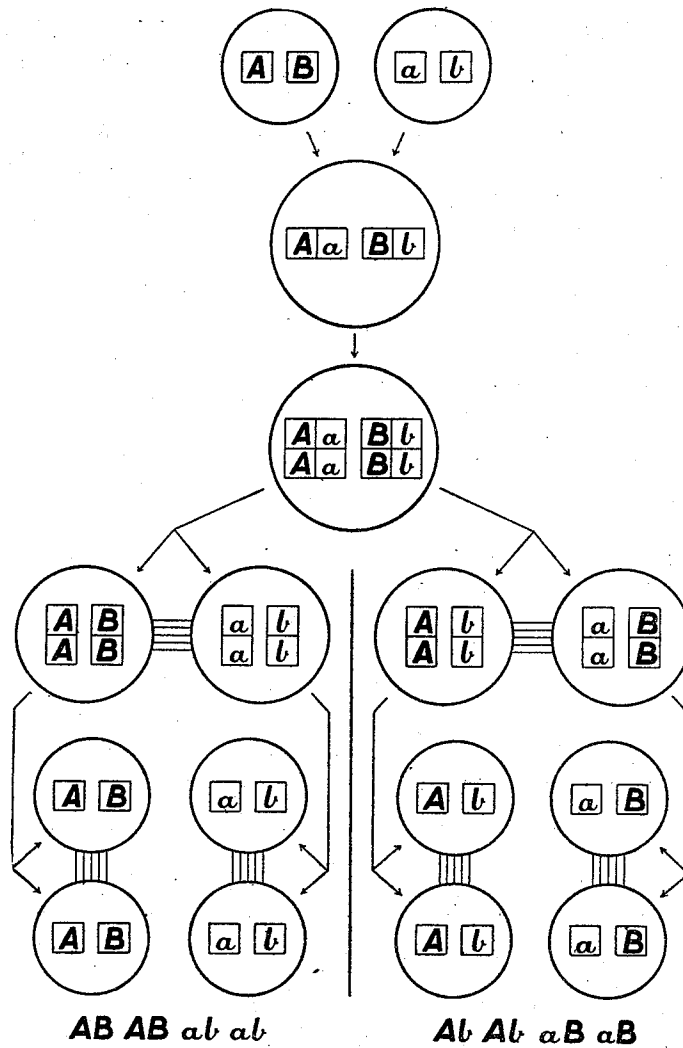


DIAGRAM 2. Scheme I, in which all the basidia show segregation of both pairs of sex factors in the first division of the fusion nucleus.

one basidia investigated had their spores arranged in the positions represented.

A survey of Scheme II shows that it provides: (1) for the occurrence of all three types of basidia (*AB*), (*AB*), (*ab*), (*ab*); (*Ab*), (*Ab*), (*aB*), (*aB*); and (*AB*), (*ab*), (*Ab*), (*aB*); and (2) for the arrangement of the spores.

of each type of basidium in two different ways, as may be seen by comparing the spores in diagonal positions.

It is not difficult to perceive that: (1) a basidium of the four-sex type (AB) , (ab) , (Ab) , (aB) should be formed just as often as a two-sex basidium of the type (AB) , (ab) , (AB) , (ab) ; and that (2) a basidium of the four-sex type (AB) , (ab) , (Ab) , (aB) should be formed just as often as a two-sex basidium of the type (Ab) , (aB) , (Ab) , (aB) . In other words, according to the law of chance, other things being equal, Scheme II with segregation wholly in the second division should give us 50 per cent. of basidia of the four-sex type (AB) , (ab) , (Ab) , (aB) , 25 per cent. of two-sex basidia of the type (AB) , (AB) , (ab) , (ab) , and 25 per cent. of two-sex basidia of the type (Ab) , (Ab) , (aB) , (aB) .

Another theoretical deduction from Scheme II is that the two arrangements of the spores of each type of basidium should occur with equal frequency. Thus in the (AB) , (AB) , (ab) , (ab) type we should have (AB) and (ab) in diagonal positions just as often as (AB) and (AB) ; in the (AB) , (ab) , (Ab) , (aB) type we should have (AB) and (aB) in the diagonal position just as often as (AB) and (Ab) ; and in the (Ab) , (Ab) , (aB) , (aB) type we should have (Ab) and (aB) in the diagonal position just as often as (Ab) and (Ab) .

Does Scheme II, in which the segregation of both pairs of sex factors takes place in all the basidia in the second division of the fusion nucleus, fit the experimental facts? The answer is: To a large extent, but by no means completely.

Firstly, Scheme II, unlike Scheme I, provides for all the three types of basidia. Secondly, it provides for the three types occurring in the right numerical ratio; for, as we have seen, it was found by experiment with forty-seven basidia that there are 50 per cent. of basidia of the (AB) , (ab) , (Ab) , (aB) type, 25 per cent. of the (AB) , (AB) , (ab) , (ab) type, and 25 per cent. of the (Ab) , (Ab) , (aB) , (aB) type.

With regard to the theoretical and actual positions of spores of diverse sex of the thirty-one basidia investigated, our Scheme II to a large extent is unsatisfactory. The actual number of basidia found with spores in the positions shown in each of the six sets of four in the lower half of the scheme is indicated by a central number. In the centre column we have 8 and 4 instead of the theoretical 6 and 6. The agreement between experiment and theory here is perhaps as great as might be expected with such small numbers; but, in the first column, we have 6 and 1 instead of 3 in one arrangement and 4 in the other; while in the third column we have 4 and 0 instead of 2 and 2. Theory and actuality, therefore, are very divergent in the first and third columns. The scheme does not provide at all for basidia of the four-sex type in which (AB) and (ab) are in diagonal positions, as may be seen by looking down the central column; yet 8 such

basidia were found in the 31 investigated. For this reason, in particular, Scheme II does not sufficiently explain the experimental data.

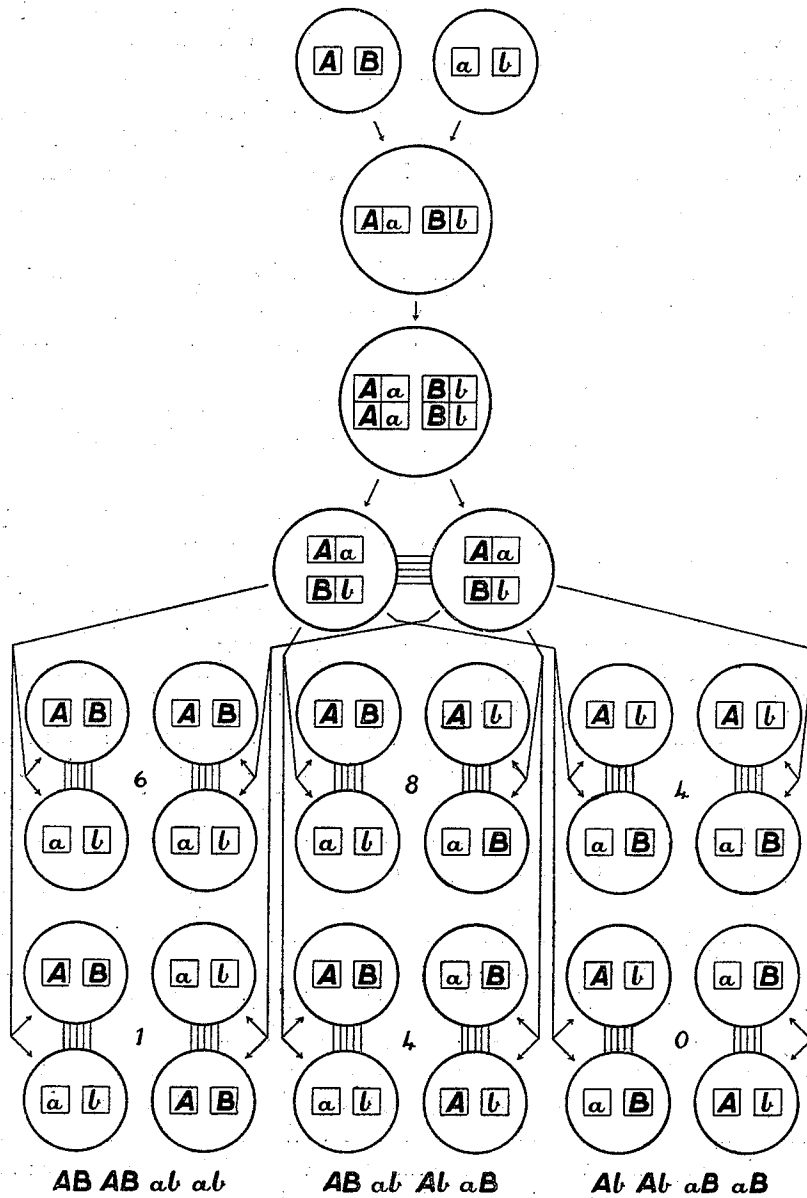


DIAGRAM 3. Scheme II, in which all the basidia show segregation of both pairs of sex factors in the second division of the fusion nucleus.

(3) *In all the basidia segregation of one pair of factors in the first division of the fusion nucleus and segregation of the other pair in the second*

division. Assuming this type of segregation to take place, it might be represented by a scheme like Scheme III, but with the outer (AB), (AB), (ab), (ab), and (Ab), (Ab), (aB), (aB) columns removed. It would yield exclusively four-sex basidia of the type (AB), (ab), (Ab), (aB).

It was found by experiment with forty-seven basidia that 50 per cent. of the basidia on the hymenium of a fruit-body belong to the two-sex types collectively, and 50 per cent. to the four-sex type. It is evident that the kind of segregation here under discussion, since it fails to yield any two-sex basidia, does not account for one-half of the experimental facts, and must therefore be discarded.

(4) *In some basidia segregation of both pairs of factors in the first division of the fusion nucleus, and in other basidia segregation of one pair of factors in the first division, and of the other pair in the second division.* Assuming that each of the two nuclei of opposite sex about to fuse in a young basidium contains two chromosomes bearing sex factors, and that segregation takes place in the manner just indicated, the following Scheme III represents what should happen during the process.

Scheme III begins with the two alternative possibilities for the formation of the fusion nucleus. Right and left we have a pair of nuclei of opposite sex, in a basidium, about to fuse, each nucleus having two chromosomes bearing sex factors. The nuclei of each pair fuse (first large circle) in such a manner that (A) joins (a) and (B) joins (b). Then in this fusion nucleus the chromosomes split (second large circle). In the first division of the fusion nucleus there are, as shown, four possibilities. In the two outer columns the two daughter nuclei show complete segregation, so that each nucleus contains only two kinds of sex factors, and not all four kinds. In the two central columns, the two daughter-nuclei show partial segregation, and this is of such a nature that each nucleus contains three of the four kinds of sex factors; and the process of segregation thus begun in the first division of the fusion nucleus is completed only in the second division. As a result of the second division of the fusion nucleus, as shown, there is only one possible position for the four nuclei in each of the two outer columns, but two possible positions for the four nuclei in each of the two central columns. However, since the top set of four in the left-hand central column is really nothing more than the looking-glass picture of the top set of four in the right-hand central column, the positions of the nuclei in these two sets may be considered as the same. As in Schemes I and II, the five lines joining any two nuclei indicate that the two nuclei are daughter nuclei of a single parent nucleus. The numbers placed in the centre of each group indicate how many of the thirty-one basidia investigated had their spores arranged in the positions represented. Since the top fours of the two central columns have essentially the same arrangement, and there were eight basidia with this arrangement, the number eight has been placed between them.

A survey of Scheme III shows that it provides: (1) like Scheme II, for the occurrence of all three types of basidia (AB), (AB), (ab), (ab); (Ab), (Ab), (aB), (aB); and (AB), (ab), (Ab), (aB); (2) for the arrangement of the (AB), (AB), (ab), (ab), and (Ab), (Ab), (aB), (aB) basidial types in one way only, and not in two ways as in Scheme II; and (3) for the arrange-

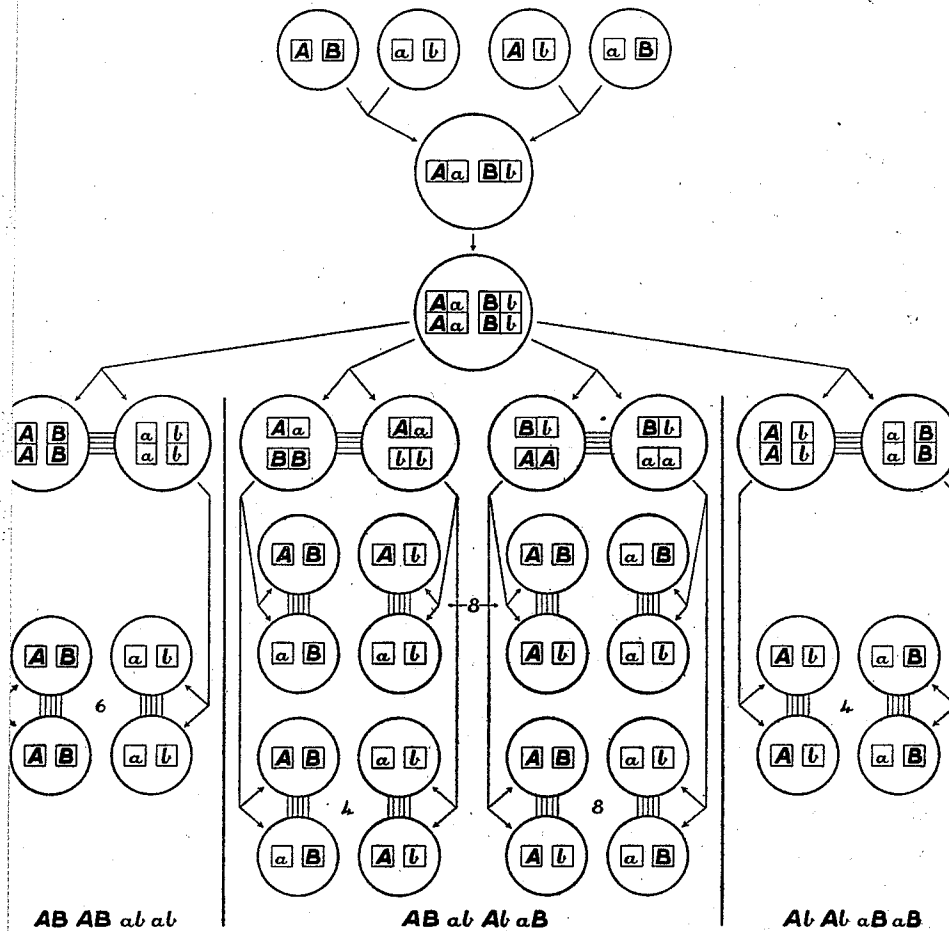


DIAGRAM 4. Scheme III, in which some basidia show segregation of both pairs of sex factors in the first division of the fusion nucleus, and other basidia show segregation of one pair of factors in the first division and of the other pair in the second division.

ment of the (AB), (ab), (Ab), (aB) basidial type in three ways, the diagonal to (AB) being (ab) or (Ab) or (aB), whereas in Scheme II the only possible diagonals to (AB) are (Ab) and (aB).

Here, as in Scheme II, it is not difficult to perceive that: (1) a basidium of the four-sex type (AB), (ab), (Ab), (aB) should be formed just as often as a two-sex basidium of the type (AB), (ab), (AB), (ab); and that a basidium

of the four-sex type (AB) , (ab) , (Ab) , (aB) should be formed just as often as a two-sex basidium of the type (Ab) , (aB) , (Ab) , (aB) . In other words, according to the law of chance, other things being equal, Scheme III should give us 50 per cent. of basidia of the four-sex type (AB) , (ab) , (Ab) , (aB) , 25 per cent. of two-sex basidia of the type (AB) , (AB) , (ab) , (ab) , and 25 per cent. of two-sex basidia of the type (Ab) , (Ab) , (aB) , (aB) .

In Scheme III, unlike Scheme II, there is only one and not two possible arrangements of the spores on each of the two types of two-sex basidia (AB) , (AB) , (ab) , (ab) and (Ab) , (Ab) , (aB) , (aB) .

In Scheme III the two upper sets of four in the middle columns, where (ab) is diagonal to (AB) , although different in origin, are, as already pointed out, simply mirror pictures of one another. Theoretically, therefore, in the four-sex basidia, we should expect the arrangement where (ab) is diagonal to (AB) to occur twice as often as the arrangement where (Ab) is diagonal to (AB) , and also twice as often as the arrangement in which (aB) is diagonal to (AB) .

Does Scheme III, in which in some basidia the segregation of both pairs of sex factors takes place in the first division of the fusion nucleus, while in other basidia segregation of one pair takes place in the first division and of the other pair in the second division fit the experimental facts? The answer is: Very well indeed except for a single basidium.

Firstly, Scheme III, like Scheme II, provides for all the three sexual types of basidia. Secondly, it provides for the three types occurring in the right numerical ratio; for, as we have seen, the data obtained from an investigation of forty-seven basidia indicate that on any hymenium there are 50 per cent. of basidia of the (AB) , (ab) , (Ab) , (aB) type, 25 per cent. of the (AB) , (AB) , (ab) , (ab) type, and 25 per cent. of the (Ab) , (Ab) , (aB) , (aB) type.

Thirdly, with regard to the theoretical and actual positions of the spores of diverse sex, our Scheme III is very fairly satisfactory. (1) According to it, in the two-sex type (Ab) , (Ab) , (aB) , (aB) there is only one possible arrangement, namely, where (aB) is diagonal to (Ab) . Observation showed that there were four basidia of this type and none with an arrangement such as we find in Scheme II, where (Ab) is diagonal to (Ab) . (2) Again, according to Scheme III, in the four-sex type (AB) , (ab) , (Ab) , (aB) there are three possible ways in which the spores may be arranged, and not two only as shown in Scheme II, and the arrangement in which (ab) is diagonal to (AB) should occur twice as often as the arrangement in which (Ab) is diagonal to (AB) , and also twice as often as the arrangement in which (aB) is diagonal to (AB) . Of twenty basidia there were eight with (ab) diagonal to (AB) , four with (Ab) diagonal to (AB) , and eight with (aB) diagonal to (AB) . The theoretical numbers instead of being 8:4:8 should have been 10:5:5. The agreement, in view of the small numbers involved, is sufficiently satis-

factory. (3) Finally, according to Scheme III, in the two-sex type (AB), (AB), (ab), (ab) there is only one possible arrangement of the spores, namely, where (ab) is diagonal to (AB), and there should be no basidia where (AB) is diagonal to (AB). Observations showed that there were six with the expected arrangement with (ab) diagonal to (AB), and one with the unexpected arrangement with (AB) diagonal to (AB). For this last basidium Scheme III has no place.

We thus perceive that Scheme III accounts sufficiently well for all the experimental data obtained with thirty-one basidia, except for the one basidium last mentioned.

It is just possible, but not likely, that the spore tetrad of the exceptional basidium was not a true tetrad, but that two of the spores belonged to one basidium and two to another, the other four spores not having adhered to the cover-glass. It is also possible that in this particular basidium segregation of both pairs of sex factors, instead of taking place in the first division of the fusion nucleus as demanded by Scheme III, took place in the second division in the manner shown on the left-hand side of Scheme II.

Of the four possible modes of segregation which have been set forth above there can be no doubt that No. 4, illustrated by Scheme III, fits the observed facts far better than any of the others.

(5) *In some basidia segregation of both pairs of factors in the second division of the fusion nucleus, and in other basidia segregation of one pair of factors in the first division and of the other pair in the second division.* Assuming this type of segregation to take place, it might be represented by a scheme which would include the two outer columns of Scheme II and the two central columns of Scheme III.

The experimental facts fit the outer columns of Scheme III much better than the outer columns of Scheme II for reasons already set forth. Therefore our new scheme, as a whole, is not as good an explanation for the data obtained with the thirty-one basidia as Scheme III.

(6) *In some basidia segregation of both pairs of factors in the first division of the fusion nucleus, and in other basidia segregation of both pairs of factors in the second division.* Assuming this type of segregation to take place, it might be represented by a scheme which would be a combination of Schemes I and II.

The objections to Scheme II have already been discussed. In our new combination scheme the basidia of the four-sex type would be like those shown in the central column of Scheme II. This column has no place for four-sex basidia in which (AB) and (ab) are in diagonal positions, yet eight of the thirty-one basidia investigated were of this type. On this ground, in particular, our new scheme fits the experimental facts much less successfully than Scheme III.

(7) *In some basidia segregation of both pairs of factors in the first*

division of the fusion nucleus, in other basidia segregation of both pairs of factors in the second division, and in yet other basidia segregation of one pair of factors in the first division and of the other pair of factors in the second division. Assuming this type of segregation to take place, it might be represented by a scheme which would be a combination of Scheme I, Scheme II, and the central part of Scheme III, or, since Scheme III includes Scheme I, it might be represented by a combination of Scheme II and Scheme III.

Scheme III, as already pointed out, except for one basidium, fits the experimental data very closely. The objections to Scheme II have already been discussed. A combination of Schemes II and III does not fit the experimental data as well as Scheme III by itself.

The experimental data can only be interpreted successfully on the supposition that the segregation of both pairs of factors does not take place in all the basidia in the first division of the fusion nucleus. If we accept Scheme III as the scheme most in accordance with the experimental data, we admit that segregation of one of the two pairs of factors frequently takes place in the second division of the fusion nucleus. Now, if one pair of factors frequently segregates in the second division, there seems no good reason why we should not admit that *both* pairs of factors may segregate in the second division. As may be seen by comparing the central columns of Schemes II and III, basidia of the four-sex type may arise in either of two ways: (1) by segregation of both pairs of factors in the second division of the fusion nucleus, and (2) by segregation of one pair of factors in the first division and of the other pair of factors in the second division. It will be remembered that of the forty-seven basidia investigated for basidial types twenty-five were four-sex and twenty-two two-sex. It is possible that not all of the twenty-five four-sex basidia became four-sex by the step-wise segregation shown in the centre columns of Scheme III, but that at least some of them became four-sex by segregation of both pairs of factors in the second division as shown in Scheme II. It is therefore possible that, although of all the schemes suggested Scheme III seems to fit the experimental data best, further investigation with a greater number of basidia might result in favour of a scheme which would represent segregation of the type No. 7. This scheme has this advantage over all the other schemes: it allows for all possible types of segregation, i.e. for segregation of both pairs of factors either at the first or at the second division, and for segregation of one pair of factors at the first division and of the other pair at the second division.

The occurrence of segregation of both pairs of sex factors in the second division of the fusion nucleus would explain the existence of the basidium of the (AB) , (AB) , (ab) , (ab) type in which the two (AB) spores were found to have diagonal positions (cf. Scheme II). Segregation of both pairs of

sex factors in the second division of the fusion nucleus is not provided for in Scheme III, but it is to be expected in a combination of Schemes II and III such as that here under discussion. Thus the existence of the exceptional basidium may be taken as evidence which definitely favours the combination scheme. However, if segregation of both pairs of factors at the second division of the fusion nucleus takes place at all, the evidence so far obtained seems to show that such a mode of segregation is comparatively rare.

Finally, it may be remarked that, whatever the exact steps in the segregation of the two pairs of sex factors in *Coprinus lagopus* may be, it seems impossible to explain the experimental data unless one accepts the view that the disjunction of homologous chromosomes may take place either at the first or at the second of the two divisions of the fusion nucleus.

Coprinus Rostrupianus.

Coprinus Rostrupianus is a species in which the spores on any individual fruit-body fall into two opposite sexual groups. We may suppose that the bisexuality depends on the presence in the fusion nucleus of a single Mendelian pair of factors (Aa), and that the nucleus of each spore bears either the (A) or the (a) factor, but not both. Every basidium bears two (A) spores and two (a) spores (10).

If segregation of the sex factors takes place always in the first division of the fusion nucleus of *C. Rostrupianus*, then the two (A) spores should have adjacent positions in every spore-tetrad. If, on the other hand, segregation takes place always in the second division, the two (A) spores should occupy adjacent positions in some basidia and diagonal positions in others.

Up to the present, as regards sex, the positions of the spores have been determined for six basidia of *C. Rostrupianus*. The two (A) spores had adjacent positions in four of the six basidia and diagonal positions in the other two. This evidence points to the segregation process taking place in *C. Rostrupianus* at least sometimes during the second division of the fusion nucleus. Further work on this species is in progress.

VII. SUMMARY.

1. As found by Hanna (5), on the hymenium of any individual fruit-body of *Coprinus lagopus* there are four sexually different types of spores, each of which bears two sex factors, one belonging to one Mendelian pair of factors and the other belonging to another Mendelian pair of factors, so that the spores may be represented by the symbols (AB), (ab), (Ab), and (aB).

2. Three types of basidia, and three only, are present in the hymenium

of any fruit-body of *Coprinus lagopus*, and they may be represented by the sex factors of their spores as follows: a four-sex type (AB), (ab), (Ab), (aB); a two-sex type (AB), (AB), (ab), (ab); and another two-sex type (Ab), (Ab), (aB), (aB).

3. Of the three types of basidia in the hymenium of any fruit-body of *Coprinus lagopus* 50 per cent. are of the type (AB), (ab), (Ab), (aB), 25 per cent. of the type (AB), (AB), (ab), (ab), and 25 per cent. of the type (Ab), (Ab), (aB), (aB).

4. By means of the cover-glass-contact and dry-needle methods the spores were removed from thirty-one basidia and sown singly in culture media, a record being kept of the actual positions the spores had had on their respective basidia. Then, by mating the monosporous mycelia in Petri dishes and employing the clamp-connexion criterion, the sexes of the spores were determined. The actual positions of the spores of diverse sex on each one of the thirty-one basidia thus became known. These basidia were all derived from fruit-bodies which appeared on a mycelium developed from spores liberated by a single wild fruit-body.

5. For any individual fruit-body of *Coprinus lagopus* the existence of four-sex basidia as well as two types of two-sex basidia, the numerical ratio in which the three types of basidia occur, and the actual positions of the spores of diverse sex on the individual basidia, are best explained on two assumptions: (1) that the two sex factors in the nucleus of each spore are carried by two separate chromosomes, one factor being carried by one chromosome and the other factor by the other chromosome; and (2) that in some basidia the segregation of both pairs of sex factors takes place in the first division of the fusion nucleus of the basidium, while in other basidia segregation of one pair takes place in the first division and of the other pair in the second division. If in some basidia segregation of both pairs of sex factors takes place in the second division of the fusion nucleus, this mode of segregation is relatively infrequent.

6. In *Coprinus lagopus* it is inferred from the experimental data that the disjunction of homologous chromosomes may take place either at the first or at the second of the two divisions of the fusion nucleus.

7. In *Coprinus Rostrupianns*, in which the spores on any fruit-body fall into two opposite sexual groups, and where each basidium produces two spores with an (A) sex factor and two with an (a) sex factor, the positions of the spores as determined for six basidia indicate that in this species segregation of the sex factors takes place at least sometimes during the second division of the fusion nucleus.

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LITERATURE CITED.

1. BRUNSWIK, H. : Untersuchungen über die Geschlechts- und Kernverhältnisse bei der Hymenomyzetengattung *Coprinus*. K. Goebel's Botanische Abhandlungen, Heft v, pp. 1-152, 1924.
2. BULLER, A. H. R. : Experiments on Sex. *Nature*, cxiv, p. 826, 1924.
3. FUNKE, G. L. : Über die Isolierung von Basidiosporen mit dem Micromanipulator nach Janse und Péterfi. *Zeit. f. Bot.*, Jahrg. xvi, pp. 619-23, 1924.
4. HANNA, W. F. : The Dry-needle Method of making Monosporous Cultures of Hymenomyces and other Fungi. *Ann. Bot.*, xxxviii, pp. 791-5, 1924.
5. ———— : The Problem of Sex in *Coprinus lagopus*. *Ibid.*, xxxix, pp. 431-57, 1925.
6. KNIEP, H. : Über morphologische und physiologische Geschlechtsdifferenzierung. *Verhandl. der Physikal.-Med. Gesellschaft zu Würzburg*, Bd. xlvi, pp. 1-18, 1920.
7. ———— : Über Geschlechtsbestimmung und Reduktionsteilung. *Ibid.*, Bd. xlvii, pp. 1-29, 1922.
8. LEVINE, M. : Studies in the Cytology of the Hymenomyces, especially the Boleti. *Bull. Torrey Bot. Club*, xl, pp. 137-81, 1913.
9. MOUNCE, I. : Homothallism and Heterothallism in the Genus *Coprinus*. *Trans. Brit. Mycolog. Soc.*, viii, pp. 256-69, 1922.
10. NEWTON, D. E. : The Bisexuality of Individual Strains of *Coprinus Rostrupianus*. *Ann. Bot.*, xl, pp. 1-24, 1926.
11. VANDENDRIES, R. : Contribution nouvelle à l'étude de la sexualité des Basidiomycètes. *La Cellule*, tom. xxxv, pp. 129-55, 1924.
12. ———— : L'Hétéro-homothallisme dans le Genre *Coprinus*. *Soc. Roy. de Bot. de Belgique*, tom. lvii, pp. 1-8, 1925.

BULLER, A. H. REGINALD, AND
NEWTON, DOROTHY E.

THE MATING METHOD OF IDENTIFICATION OF
A COPRINUS GROWING ON GERMINATING SEEDS OF
MANGEL AND SUGAR-BEET.

The Mating Method of Identification of a *Coprinus* growing on Germinating Seeds of Mangel and Sugar-beet.

BY

A. H. REGINALD BULLER

AND

DOROTHY E. NEWTON.

With Plate XXVIII and six Tables in the Text.

I. INTRODUCTION.

IN 1914 Mr. Raymond Finlayson, of the Seed Testing Laboratory, Wood Green, London, observed a *Coprinus* coming up on germinating seeds¹ of Mangel, Beet, and Sainfoin, and he sent some of the material to Mr. Carleton Rea. Mr. Rea regarded the fungus as *Coprinus pilosus*, Beck,² but sent some of the seeds to the senior author (A. H. R. B.) with a request for his opinion. The *Coprinus* duly came up on some of the moistened seeds and from its morphological characters it was identified by Buller as *Coprinus lagopus*, Fr., and it was considered by him as identical with the form of *C. lagopus* which occurs very commonly on horse-dung both in England and Canada.³

In 1919 Pape⁴ published a note on the occurrence of a *Coprinus* on germinating Mangel and Beet seeds. His attention had been called to the matter by Dr. Zade, of Jena. Pape observed that the fungus grew almost invariably on the seed-balls (pericarps) themselves, and he observed nothing to suggest that the fungus is a parasite. He says: 'The question of the significance of the fungus in respect to the Beet seed upon which it grows

¹ Mangel and Beet seeds, when sown, are enclosed by a rough pericarp. The *Coprinus* under discussion grows on the pericarp.

² Carleton Rea, *in litt.*

³ A. H. R. Buller: *Researches on Fungi*, London, vol. iii, p. 308, 1924. The *Coprinus* obtained from the Mangel seeds is illustrated in Fig. 141, D, p. 320.

⁴ Pape: *Coprinus* auf Rübensamen. *Mitteilungen a. d. Biol. Reichsanst. f. Land- und Forstwirtschaft*, Heft 17, pp. 13-16, 1919, with two illustrations.

must be left open. A lowering of the germinating power of the seed due to the fungus or damage to the young seedling from it has not been observed. The fact that the fungus is most abundant upon seed which we found to have a low germinating power may be due to the fungus finding more nourishment in seeds which have failed to germinate than in those which produce seedlings.' Pape identified the fungus as *Coprinus nycthemerus*, Fr.

In 1926 the *Coprinus* was observed by Mr. W. A. Dillon Weston, of the School of Agriculture at Cambridge, England, coming up on Mangel seeds, and by Mr. M. W. Gardner, of the Agricultural Experiment Station at Purdue University, Lafayette, U.S.A., coming up on Sugar-beet seeds; and each of these investigators sent a packet of his infected seeds to the senior author of this paper (A. H. R. B.) with a request for an identification of the fungus. Thereupon the work about to be described was undertaken.

New *Coprinus* fruit-bodies were obtained by the writers from (1) the Mangel seeds, and (2) the Sugar-beet seeds, by sowing the seeds on sheets of wet filter-paper in a crystallizing dish. Some of the seeds germinated normally, whilst others did not germinate at all. On the latter, in both cultures, after a lapse of about three weeks, several *Coprinus* fruit-bodies appeared (Pl. XXVIII). The *Coprinus* on the Mangel seeds was identical in appearance with the *Coprinus* on the Sugar-beet seeds, and both Coprini had all the morphological and growth characteristics of *Coprinus lagopus*, Fr., as described and illustrated in vol. iii of Buller's 'Researches on Fungi'.¹

Coprinus lagopus is of very common occurrence on horse-dung balls both in England and in Canada, and it can almost always be procured by putting fresh horse-dung balls in a covered crystallizing dish and leaving them there for two or three weeks at room temperatures.

The recent advance in our knowledge of the sexual process in the Coprini permits of an experimental verification of such an identification as has just been made. The experimental method of identification may be called the *mating method*; and, in what follows, it will be treated of both in theory and in practice.

II. THE MATING METHOD.

When monosporous mycelia derived from spores of a *single* fruit-body of *Coprinus lagopus* are paired in all possible ways on dung-agar plates and the subsequent development or non-development of clamp-connexions is taken as a criterion of a positive or of a negative sexual reaction respectively, it is found that the mycelia fall into *four* sexual groups, which may be expressed in Mendelian symbols as follows: (AB), (ab), (Ab), and (aB).

¹ A. H. R. Buller: loc. cit., pp. 299-327, Figs. 130-47.

Thus Hanna¹ with ten monosporous mycelia from as many spores of a single wild fruit-body, which he paired in all possible ways, obtained the results embodied in Table I. In this table a (+) sign indicates that clamp-connexions appeared in the compound mycelium and a (-) sign that they did not. The numbers 50-59 were arbitrary numbers given to particular mycelia. After the results of pairing had been obtained, the table was rearranged so as to bring like mycelia together, and the Mendelian symbols required to explain the reactions were then added.

	AB			ab			Ab		aB	
	51	52	54	55	57	58	59	50	56	53
AB	51	-	-	+	+	+	+	-	-	-
	52	-	-	+	+	+	+	-	-	-
	54	-	-	+	+	+	+	-	-	-
ab	55	+	+	+	-	-	-	-	-	-
	57	+	+	+	-	-	-	-	-	-
	58	+	+	+	-	-	-	-	-	-
Ab	59	+	+	+	-	-	-	-	-	-
	50	-	-	-	-	-	-	-	-	+
aB	56	-	-	-	-	-	-	-	-	+
	53	-	-	-	-	-	-	+	+	-

TABLE I. *Coprinus lagopus*. All possible pairings of ten monosporous mycelia obtained from the spores of a single Vancouver fruit-body. A similar table is obtained when ten monosporous mycelia derived from ten spores of one fruit-body are paired in all possible ways with ten monosporous mycelia derived from ten spores of another fruit-body, provided the two fruit-bodies belong to one and the same geographical strain.

In Table I it will be observed that the ten monosporous mycelia fall into *four* groups.

When monosporous mycelia derived from spores of a fruit-body of *one* so-called geographical strain of *Coprinus lagopus* are paired in all possible ways with monosporous mycelia derived from spores of another fruit-body of the *same* geographical strain, it is found that, as before, the mycelia fall into four sexual groups to which may be given the Mendelian symbols (AB), (ab), (Ab), and (aB). This was proved by Hanna,² whose table of results for matings of the kind under discussion resembles Table I.

When monosporous mycelia derived from a fruit-body of *one* so-called geographical strain of *Coprinus lagopus* are paired in all possible ways with the monosporous mycelia of *another* geographical strain, perfect fertility

¹ W. F. Hanna: The Problem of Sex in *Coprinus lagopus*. Ann. Bot., vol. xxxix, p. 436, 1925.

² Ibid., p. 443.

results, i. e. clamp-connexions appear in every plate. Thus Hanna¹ mated eleven monosporous mycelia, derived from as many spores taken from an Edmonton (Province of Alberta) fruit-body, in all possible ways with eleven monosporous mycelia derived from as many spores taken from a Winnipeg (Province of Manitoba) fruit-body, with the results shown in Table II.²

		A ⁴ B ⁴			a ⁴ b ⁴			A ⁴ b ⁴			a ⁴ B ⁴		
		4	7	8	5	2	6	10	11	1	3	9	
A ² B ²	25	+	+	+	+	+	+	+	+	+	+	+	
	26	+	+	+	+	+	+	+	+	+	+	+	
	27	+	+	+	+	+	+	+	+	+	+	+	
	28	+	+	+	+	+	+	+	+	+	+	+	
a ² b ²	20	+	+	+	+	+	+	+	+	+	+	+	
	23	+	+	+	+	+	+	+	+	+	+	+	
	24	+	+	+	+	+	+	+	+	+	+	+	
a ² B ²	21	+	+	+	+	+	+	+	+	+	+	+	
	29	+	+	+	+	+	+	+	+	+	+	+	
A ² b ²	30	+	+	+	+	+	+	+	+	+	+	+	
	16	+	+	+	+	+	+	+	+	+	+	+	

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

Table II contains nothing but (+) signs, which indicates that clamp-connexions appeared in every pairing.

When monosporous mycelia derived from the spores of a fruit-body of *one species* of *Coprinus* are paired with monosporous mycelia derived from the spores of *another* morphologically distinct species of *Coprinus*, there is complete infertility. Thus the authors made pairings between *Coprinus lagopus* and *C. macrorhizus*, between *C. lagopus* and *C. Rostrupianus*, and between *C. Rostrupianus* and *C. macrorhizus*, with the results shown in Tables III, IV, and V respectively.

Tables III, IV, and V contain no (+) signs, thus showing that no clamp-connexions were developed in any of the pairings. The absence of

¹ Loc. cit., p. 441.

² Similar results have been recorded for *Schizophyllum commune* (Kniep), *Coprinus Rostrupianus* (Newton), *C. comatus* (Brunswik), *C. radians* (Vandendries), and *Panaeolus campanulatus* (Vandendries); but, recently, Vandendries has found an exception to this rule in some strains of *Coprinus micaceus* which were sterile with one another (R. Vandendries, L'hétérohomothallisme et la stérilité entre races géographiques de *Coprinus micaceus*, in Mém. de l'Acad. roy. de Belgique, tom. ix, pp. 42-50, 1927).

clamp-connexions clearly indicates a failure in the attempt to produce *Coprinus* hybrids.

The difference in the results obtained by mating (1) monosporous mycelia of *Coprinus lagopus* derived from a single fruit-body or from two fruit-bodies of one and the same geographical strain, (2) monosporous mycelia of *Coprinus lagopus* derived from two geographical strains, and

		<i>C. macrorhizus.</i>			
		1	2	3	4
<i>C. lagopus.</i>	I	-	-	-	-
	II	.	-	-	-
	III	-	-	-	-
	IV	-	-	-	-

TABLE III. The pairing of four monosporous mycelia of *Coprinus lagopus*, Fr., with four monosporous mycelia of *C. macrorhizus*, (Pers.) Rea.

		<i>C. Rostrup.</i>		
		1	2	3
<i>C. lagopus.</i>	I	-	-	-
	II	-	-	-
	III	-	-	-
	IV	-	-	-

TABLE IV. The pairing of four monosporous mycelia of *Coprinus lagopus*, Fr., with three monosporous mycelia of *C. Rostrupianus*, Hansen.

		<i>C. macrorhizus</i>			
		1	2	3	4
<i>C. Rostrupianus</i>	I	-	-	-	-
	II	-	-	-	-
	III	-	-	-	-
	IV	-	-	-	-

TABLE V. The pairing of four monosporous mycelia of *Coprinus Rostrupianus*, Hansen, with four monosporous mycelia of *C. macrorhizus*, (Pers.) Rea.

(3) monosporous mycelia derived from two morphologically distinct species of *Coprinus* has now been set forth. Using this difference as a guide, an attempt was made to determine whether or not the *Coprinus* which occurs on Mangel seeds at Cambridge, England, and on Sugar-beet seeds at Lafayette, U.S.A., are geographical strains of one and the same species, and, in particular, of *Coprinus lagopus* as it occurs on horse-dung at Winnipeg, Canada.

III. EXPERIMENTAL RESULTS.

Three monosporous mycelia were obtained from three spores derived from each of the following: (1) a *Coprinus* fruit-body which grew on Cambridge Mangel seeds; (2) a *Coprinus* fruit-body which grew on Lafayette Sugar-beet seeds; and (3) a *Coprinus lagopus* fruit-body which

came up spontaneously on horse-dung gathered at Winnipeg. These nine monosporous mycelia were then paired on dung-agar plates in all possible ways, with the results embodied in Table VI.

		<i>C. lagopus</i>			Mangel			Sugar-beet		
		1	2	3	1	2	3	1	2	3
<i>C. lagopus</i>	1	-	-	+	+	+	+	+	+	+
	2	-	-	-	+	+	+	+	+	+
	3	+	-	-	+	+	+	+	+	+
Mangel	1	+	+	+	-	-	-	+	+	+
	2	+	+	+	-	-	-	+	+	+
	3	+	+	+	-	-	-	+	+	+
Sugar-beet	1	+	+	+	+	+	+	-	-	-
	2	+	+	+	+	+	+	-	-	+
	3	+	+	+	+	+	+	-	+	-

TABLE VI. The pairing of nine monosporous mycelia, three derived from a Winnipeg *Coprinus lagopus* fruit-body, three derived from a Cambridge Mangel-seed *Coprinus* fruit-body, and three derived from a Lafayette Sugar-beet-seed *Coprinus* fruit-body.

In Table VI, in the pairings *Coprinus lagopus* × *Coprinus lagopus*, Mangel × Mangel, and Sugar-beet × Sugar-beet, the results, as was to be expected, are essentially like those shown in Table I; but the pairings were too few to give all the four possible groups of mycelia (AB), (ab), (Ab), (aB). However, in the pairings of *C. lagopus* × *C. lagopus* and Sugar-beet × Sugar-beet, it is obvious that three of the four possible groups of mycelia are represented (cf. Table I). In the pairings Mangel × Mangel, since no clamp-connexions appeared in any pairing, the three mycelia may have all belonged to one of the four possible groups, or one of the mycelia may have belonged to one group, e. g. (AB), and the two other mycelia to another group, e. g. (Ab) or (aB).

In Table VI complete fertility is shown in all of the following pairings: *Coprinus lagopus* × Mangel, *Coprinus lagopus* × Sugar-beet, and Mangel × Sugar-beet. These results, which resemble those of Table II, indicate that the *Coprinus* occurring at Cambridge, England, on Mangel seeds, the *Coprinus* occurring at Lafayette, U.S.A., on Sugar-beet seeds, and *Coprinus lagopus* occurring on horse-dung at Winnipeg, Canada, are merely three so-called geographical strains of one and the same species, namely, *Coprinus lagopus*, as described in vol. iii of Buller's 'Researches on Fungi'.

IV. DISCUSSION.

As we have seen, the *Coprinus* on Mangel seeds was regarded by Mr. Carleton Rea as *C. pilosus*, Beck, and by Pape as *C. nycthemerus*, Fr., whereas we identify it as *Coprinus lagopus*, Fr., as very fully described and illustrated with numerous photographs and drawings in Buller's 'Researches on Fungi'.¹

It seems highly probable that *Coprinus pilosus* and *C. nycthemerus*, as understood by Rea and Pape respectively, are but synonyms for *C. lagopus*, as understood by us. Pape² gives not only some illustrations but also a description of his fungus which enables us to perceive that his fungus and ours are in all probability one and the same species, and he adds: 'The foregoing characters of the fungus point to *Coprinus nycthemerus*, Fr., as being nearest to it. At the same time, a comparison between the present fungus and the figures given by Brefeld and Cooke of *C. nycthemerus* leave it uncertain whether the two are actually identical.' The diversity of opinion as to what the Mangel-seed fungus should be called is doubtless due to the fact that the original descriptions of many of the smaller Coprini are so brief and incomplete as to be little more than useless for identification purposes. Exactly what species of *Coprinus* are to be identified with *C. pilosus* and *C. nycthemerus* is not clear to the writers. *Coprinus pilosus* may well be a synonym for *C. lagopus*. The illustrations of *C. nycthemerus* as given by Brefeld,³ Cooke,⁴ and Ricken⁵ do not resemble one another and do not suggest the *Coprinus* shown in our photographic illustration. Fries⁶ and Rea⁷ state that *C. nycthemerus* is uncommon, and Lange⁸ in his monograph of the Danish Coprini does not even mention it. On the other hand, *Coprinus lagopus*, as understood by us, is an exceedingly common fungus in both Europe and North America. One of us (A. H. R. B.) has observed it many times on the under side of horse-dung plats in fields in England, and the ease with which the fungus may be obtained in fresh horse-dung cultures in both England and Canada goes to show that its spores must be widely dispersed in the pastures of both these countries.

As we have seen, the mating method has enabled the writers to determine with certainty that three Coprini obtained from as many different countries belong to one and the same species. There seems no reason why

¹ A. H. R. Buller: loc. cit.

² Pape: loc. cit., pp. 14-15.

³ O. Brefeld: Untersuchungen über Pilze, Leipzig, Heft viii, 1909, Pl. XII, Figs. 10, 11.

⁴ M. C. Cooke: Illustrations of British Fungi, London, Pl. 682, B.

⁵ A. Ricken: Die Blätterpilze, Leipzig, 1915, Taf. xxi, Fig. 5.

⁶ E. Fries: Hymenomyces Sueciae, Upsala, 1857, p. 466.

⁷ C. Rea: British Basidiomycetae, Cambridge, 1922, p. 511.

⁸ J. E. Lange: Studies in the Agarics of Denmark. Dansk Botanisk Forening, 1915, Part II, *Coprinus*, pp. 32-50.

the same method should not be used to establish the identity of other heterothallic Hymenomyces, physiological experiment thus directly helping the systematist.

V. SUMMARY.

1. As shown by comparative morphology and by mating experiments, the *Coprinus* which comes up on germinating Mangel seeds in England and on germinating Sugar-beet seeds in the United States of America is identical with *Coprinus lagopus*, Fr., as it occurs on horse-dung in Canada.

2. *Coprinus lagopus* is not confined to a substratum of seeds, for it is a fungus of wide occurrence on horse-dung in pastures both in Europe and North America.

3. The mating method may be used to assist the systematist in deciding specific identity in the heterothallic Hymenomyces in general.

EXPLANATION OF PLATE XXVII.

Illustrating the paper of Professor A. H. R. Buller and Miss D. E. Newton on the Mating Method of Identification of a *Coprinus*.

Photograph of fruit-bodies of *Coprinus lagopus* growing on Sugar-beet seeds (fruits) obtained from Lafayette, Indiana, U.S.A., about three weeks after the seeds had been placed on wet filter-paper in a crystallizing dish. Some of the seedlings have developed normally. Natural size.



Huth coll.

BULLER & NEWTON—IDENTIFICATION OF COPRINUS.