

Botany. — *The sexuality of Coprinus fimetarius.* (Preliminary note.)
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Introduction.

The haploid mycelia developed from the spores of a single fruiting-body of quadrisexual Hymenomycetes can be divided into four sex-groups. The discrimination of these four groups depends upon the fact that in certain combinations copulation occurs. As a consequence of this copulation a diploid mycelium is formed, which cytologically is characterized by conjugated nuclei, morphologically by clamp-connections. Following the nomenclature of KNIEP we shall designate these sex-groups by the characters AB , Ab , aB and ab , assuming that there are two pairs of sex-factors: $A—a$ and $B—b$. Copulation and the ensuing diploid mycelium occur exclusively in those combinations in which the components have no common factor. Consequently the diploid mycelium is always heterozygous in both factors ($AaBb$).

My experiments have shown that there are distinctly visible differences in habit between the various haploid combinations. Considering all possible combinations (including the copulating ones) we can with the aid of the above mentioned differences distinguish four combination-groups.

I. There is no difference between the components. This group contains the combinations of mycelia belonging to the same sex-group: $AB \times AB$, $Ab \times Ab$, $aB \times aB$ and $ab \times ab$. In the following these will be called O combinations.

II. The components differ in the A factor: $AB \times aB$ and $Ab \times ab$. We shall designate these combinations by A .

III. The components differ in the B factor: $AB \times Ab$ and $aB \times ab$. These combinations will be called B combinations.

IV. The components differ in both factors: $AB \times ab$ and $Ab \times aB$. In these combinations copulation takes place and a diploid mycelium is formed. They will be called AB combinations.

The distinction of these combination-groups rests upon:

1. Differences in habit.
2. Formation or non-formation of fructifications.

Before going into these differences, it is necessary to say a few words about the circumstances under which the experiments were made.

Method.

I worked with a strain of *Coprinus fimetarius* isolated from germinating seeds of Beta. For studying the habit I used Petri dishes with sterilized horse dung. In order to get a more or less level nutrient medium the dung was loosened and distributed as equally as possible over the dish. With a dish fitting well into the Petri dish (e.g. the cover of a smaller dish) the dung was pressed together and after this a little water was added. The combinations were made by putting together two strips of agar covered with the mycelia in the form of a cross. It is necessary to make certain that the ends touch the nutrient medium, in order to prevent desiccation and death of the mycelium of the strip. The temperature for the cultivation was kept approximately between 23°—25°. In order to get good results (that is, to get evident differences), it is advisable to pay special attention to the following points :

The mycelia which are to be combined, must have been transferred recently (5—10 days ago) and thus be in a state of rapid growth. It is also desirable that the two mycelia have been transferred at about the same time. As any small impurity has an influence on the formation of air-mycelium, it is necessary to work with absolutely pure cultures. For the same reason the humidity of the medium must not be too great. In order to eliminate differences of humidity and constitution of the medium, cultures which are to be compared should be made on the same Petri dish.

In order to study the fruiting-bodies I used tubes with sterilized horse dung. The cultures were kept in daylight, the temperature varying from 15°—25°.

Habit.

We now pass to the discussion of the results, first of all to the differences in habit.

The *O* combinations form a smooth white air-mycelium with downy edge, the habit of which generally agrees completely with that of the haploid mycelium. To some extent this was to be expected. For the haploid mycelium can also be considered as a combination, if it be a combination of two parts of the same mycelium, whereas a *O* combination consists of two different mycelia of the same sex-group. In the following the haploid mycelia will be designated by *OO*, to discriminate them from the real *O* combinations. All the *OO* combinations show the same appearance: there is no morphological sex-differentiation. Thus an *AB* has the same habit as for instance *Ab* or *ab*. The same applies to the real *O* combinations. As far as outward appearance is concerned, *AB* × *AB* cannot be distinguished from *Ab* × *Ab* or *ab* × *ab*. In a few cases a faint line of separation can be seen where the two mycelia are growing together. (Fig. 1: *g* and 3: *h*.)

The components of *the A combinations* ($AB \times aB$ and $Ab \times ab$) seem to check each other, which is evidenced by:

1. retarded growth. (compared with a *O* combination.)
2. formation of fewer air-mycelium; for this reason the culture is more grey in appearance than a *O* combination. Further evidence has been obtained that the two mycelia penetrate each other and thus form a mixed mycelium. The differences between the *A* and *O* combination are sometimes very distinct, sometimes less so. I do not know whether this is caused by individual variations or by differences in constitution or humidity of the medium. (Fig. 1: *a* and *d*, 2: *a*, *f* and *h*, 3: *c*.)

The components of *the B combinations* ($AB \times Ab$ and $aB \times ab$) repel each other; consequently there is a distinct line of separation between the mycelia. With our method of combining, these lines of separation form an easily observable cross. A mixed mycelium is not formed. For the rest the habit is the same as that of the *O* combinations as regards rapidity of growth and quantity of air-mycelium (white colour). (Fig. 1: *c* and *f*, 2: *c*, *d* and *g*, 3: *a*.)

The components of *the AB combinations* ($AB \times ab$ and $Ab \times aB$) form the diploid mycelium, which differs from the *O* combination in greater rapidity of growth and in a contour of apparently radial structure. The greater rapidity of growth cannot or hardly be seen on the pictures because it takes some days before diploid mycelium begins to develop, whereas the haploid mycelium starts at once. (Fig. 1: *b* and *e*, 2: *b* and *e*, 3: *e* and *g*.)

The differences described were obtained with a great number of mycelia.

We can discuss the results from two points of view. Comparing for instance a *B* combination $AB \times Ab$ with the *OO* combination $AB \times AB$ we can say that the difference is caused by the inequality of the *B* factor. If however we compare it with the diploid mycelium $AB \times ab$ we can say that the copulation is prevented by the equality of the *A* factor. The reason of the peculiar habit can thus be sought in the equality of the *A* factors or in the inequality of the *B*'s. It has not been possible to decide which of the two is the principal cause, but the first point of view seems to be more probable. In any case it is now certain that the *A* and the *B* factor do not act in the same way, though we cannot decide to which of the two the observed properties belong.

In order to ascertain that the observed peculiarities were not limited to the strain used, I have made similar experiments with the mycelia of another strain. These were found to lead to the same results.

Other quadrisexual species have not yet been investigated. There are indications that *Coprinus micaceus* behaves at least partly in the same way as *C. fimetarius*. I derived these indications from a table of VANDENDRIES (1927, p. 16, table 7).

Differences in habit (except *C. micaceus*) have not previously been found. However there have been found some other differences between

the *A* and the *B* factor. KNIEP (1928, p. 416) says that with *Schizophyllum commune* the *A* factor is more inclined to mutations than the *B*. BRUNSWIK (1924, see also KNIEP 1928, p. 410) has found something similar with *Coprinus picaceus*. The so-called "Durchbrechungskopulationen" occur in certain combinations (viz. equality of *B* or *b* factor), not in the other ones (viz. equality of *A* or *a* factor).

Fructification.

We shall now consider some other differences between the various combinations, namely concerning the fructification.

Fruiting-bodies occur only very rarely with the strain investigated in the *OO*, *O* and *A* combinations. The fructifications of the *B* combinations occur fairly regularly. However it should be noted that some mycelia are more inclined to form pilei than others. If for the present we limit ourselves to the fructifications of the *B* combinations it may be remarked that the fruiting-bodies of the *B* combinations differ in several respects from the diploid ones. For this reason we have tentatively called them haploid pilei. The differences are so pronounced that one can easily distinguish the two kinds of fruiting-bodies. So far as we have studied them, all haploid pilei formed well germinating spores, though in much smaller quantity than the diploid ones. By an analysis of the spores it should be possible to prove whether we are dealing with real haploid fruiting-bodies, perhaps with a kind of illegitimate diploid fructifications.

When we investigate the spores of a pileus grown from a *B* combination it appears that two kinds of spores have been formed and that these belong to the sex-groups of which the combination was composed. In one case the four spores of one basidium could be isolated. They appeared to belong to one sex-group. Thus it has been shown that we are dealing with haploid fruiting-bodies, though of a peculiar kind, which we must consider as built up of two kinds of hyphae. Both kinds of hyphae form basidia of which, accordingly, we get two kinds. For instance the combination $AB \times Ab$ forms some basidia with the spores *AB*, *AB*, *AB* and *AB* and other basidia with the spores *Ab*, *Ab*, *Ab*, and *Ab*. We might, therefore, regard these pilei as a sort of haploid chimaerae.

The single *A* combination of which a fruiting-body was examined also appeared to form two kinds of spores.

Clamp-connections and mutations.

A closer inspection showed that there were some complications. If we make polysporous cultures of the fruiting-bodies of a *B* combination, these polysporous mycelia (consisting of two kinds of mycelia which together form a *B* combination) make clamp-connections to some degree. Often the clamp-connections are so-called "Pseudo-Schnallen", but many others are quite normal in appearance. The whole phenomenon shows considerable

resemblance to the "Durchbrechungskopulationen" found by BRUNSWIK i.c. in the case of *Coprinus picaceus*. Now a polysporous culture from a fruiting-body of a *B* combination can in itself be considered as a *B* combination, in which the combination took place before the germination. But a disadvantage of these cultures is that we start from a mixture of so many spores (there might perhaps be mutated spores). In order to avoid this I made the spores of a *B* fruiting-body germinate in drops of dung agar in groups of two. The spores were cultivated in moist rooms. In this way I could observe under a microscope that both spores had been germinated. In this case one has to expect 50 % *O* combinations and 50 % *B* combinations. Through re-combination of these bisporous mycelia with the four test-mycelia it is possible to decide whether one has a *O* or a *B* combination. Nearly half of these bisporous mycelia, which later on appeared to be the *B* combinations, showed the following peculiarities:

1. The mycelia did not repel each other so that there were no lines of separation (as in a normal *B* combination).
2. There were spots in the apparently normal haploid mycelium which attracted the attention by a strong formation of air-mycelium, that is by whiter colour.

In some cultures these spots formed sectors, in others they were found near the centre. On studying these whiter spots under the microscope it appeared that in these spots the mycelia formed clamp-connections to some degree, just like the mycelia of the polysporous cultures. The normal mycelium did not show any clamp-connections. Upon transferring parts of the normal mycelium another normal mycelium was formed. By transferring parts of the white sectors a mycelium was formed which at the edges grew normally but at the centre of which a whiter spot with clamp-connections was produced.

In no case did these bisporous or polysporous cultures form diploid fructifications.

Like BRUNSWIK we have thus found "Durchbrechungskopulationen" which in my opinion must be understood in this way: Copulation occurs illegitimately, but *no* normal diploid mycelium is formed and therefore we do not find any diploid fruiting-bodies. This is not the place for an extensive discussion of the literature, but I want to draw attention to the fact that these experiments have shown most clearly that it is *not* justifiable to speak of mutations when clamp-connections occur in combinations in which, according to the scheme, clamp-connections ought to be absent. As a criterion for diploid mycelia, the occurrence of clamp-connections can only be of a restricted use. Used in connection with the characteristic diploid habit and the diploid fruiting-bodies it remains, of course, a valuable criterion. Presumably many cases which in the literature were described as mutations will appear to be nothing but "Durchbrechungskopulationen". Real mutations probably are much rarer than has been hitherto assumed, whereas illegitimate copulations would seem to occur frequently.

LITERATURE.

BRUNSWIK, H. 1924. Untersuchungen über die Geschlechts- und Kernverhältnisse bei der Hymenomyzetengattung *Coprinus*. Botan. Abhandl. 5.

KNIEP, H. 1928. Die Sexualität der niederen Pflanzen.

VANDENDRIES, R. 1927. Nouvelles recherches expérimentales sur le comportement sexuel du *Coprinus micaceus*. Mém. de l'Acad. Roy. Belg. 2e série, 9 : 128.

EXPLANATION OF THE FIGURES.

The photographs were taken 4–6 days after transferring.

- Fig. 1: a. A combination $AB \times aB$.
 b. AB combination $AB \times ab$.
 c. B combination $aB \times ab$. (One of the components somewhat impure).
 d. A combination $Ab \times ab$.
 e. AB combination $Ab \times aB$.
 f. B combination $AB \times Ab$.
 g. OO combination $AB \times AB$.
- Fig. 2: a. A combination $AB \times aB$.
 b. AB combination $AB \times ab$.
 c. B combination $AB \times Ab$.
 d. B combination $aB \times ab$.
 e. AB combination $Ab \times aB$.
 f. A combination $Ab \times ab$.
 g. B combination $AB \times ab$. (One of the components grows faster; it has been transferred more recently).
 h. A combination $AB \times aB$.
- Fig. 3: a. B combination $aB \times ab$.
 c. A combination $aB \times AB$.
 e. AB combination $AB \times ab$. (Partially impure).
 g. AB combination $Ab \times aB$.
 h. O combination $aB \times aB$. (With a faint line of separation; cf the B comb. fig. 3: d).

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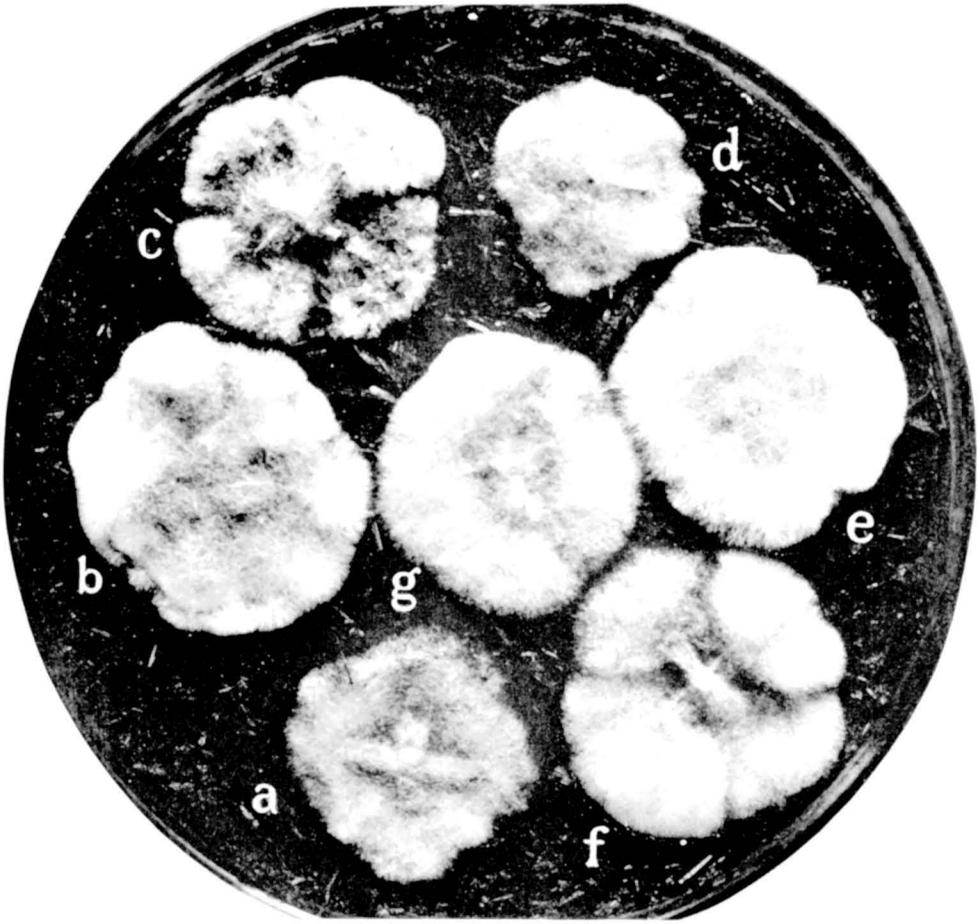


FIG. 1

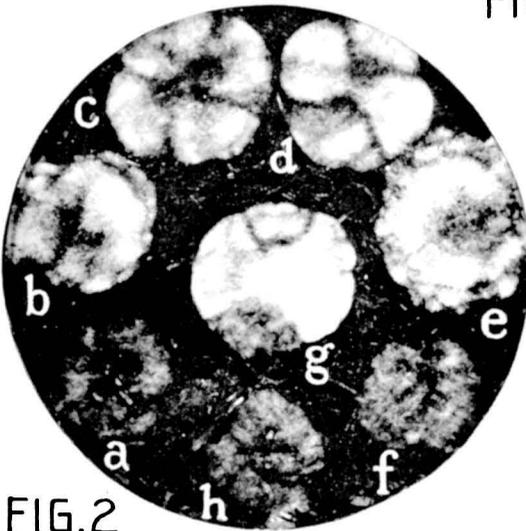


FIG. 2

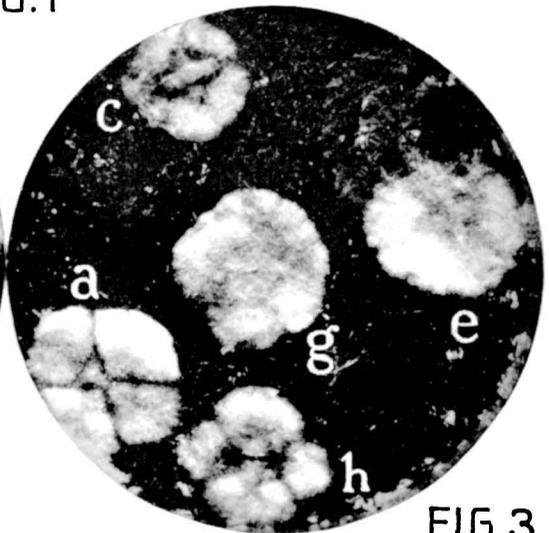


FIG. 3