NOTES.

SEXUAL SPORE-FORMATION AMONG THE SACCHAROMYCETES¹.—In a paper read before the Royal Society on June 6 of this year the author has described a Yeast-form, the vegetative propagation of which takes place by budding in a manner exactly similar to that of *Saccharomyces cerevisiae* (Hans.) and other budding Yeasts, but which is distinguished from them by a curious process of spore-formation. The usual process among the Saccharomycetes is apparently a very simple one, the spores being formed under appropriate conditions in any cell by the division of its contents into a number of bodies, each of which develops into a spore. In this particular case, however, spore-formation is preceded by a kind of conjugation between two cells. Considering the case of any pair of cells which are about to take part in the process, the first stage consists in the development by each cell of a small protuberance. These protuberances are developed from neighbouring parts of the two cells, and grow towards each other, evidently under the influence of some stimulus acting between them. Growth continues until their tips are in contact, and then a solution of the walls at that point occurs, so that a continuous canal of communication is opened between the cells through the bridge or neck formed by the junction and fusion of the protuberances. Spore-formation then takes place, usually in each of the cells, although in some cases spores appear in one of the cells only, the contents of the other disappearing and leaving merely an empty sac. The spores make their appearance in a manner similar to that occurring in the other Saccharomycetes. No cases have been observed in which spore-formation was not preceded by conjugation. The behaviour of the nuclei during the

¹ Read before the Botanical Section of the British Association, at the Glasgow Meeting, September, 1901.

process was studied in specimens hardened and stained at different stages. In all independent cells, before the protuberances are developed, there was found a deeply stained body placed more or less in the centre of the cell. This moved to the side of the cell to the point where the protuberance was put out, as soon as the development of the latter began. It took up a position at the tip of this, and remained there during the growth, until contact with the corresponding protuberance of the other cell taking part in the conjugation was reached. At the tip of this also a similar deeply stained body was situated, and on the fusion of the tips of the protuberances the two deeply stained bodies also fused, forming one large body filling the greater part of the junction between the two cells. This body subsequently divided into two portions, which were withdrawn from the neck into the bodies of the cells, and there again they underwent, as a rule, another division, each of the bodies so produced becoming the basis of a spore. In spite of the controversy as to the nature of the Yeast nucleus, there can scarcely be any doubt that the deeply stained bodies represent, in part at any rate, the nucleus, that consequently a nuclear fusion actually does take place, and that the whole process is a kind of isogamous sexual act.

It was pointed out in the paper that in the case of spore-formation in the fission-yeast, *Schizosaccharomyces octosporus* (Beijerinck), a process, somewhat approaching this in nature, was already known to occur. Schiönning ('95) described the details as follows:—Across the middle of a cell a partition-wall was formed: this splits, and the two new cells so formed assumed a round shape, remaining attached at one point. They then again coalesce, and form an hour-glass shaped cell, which increases in size and swells up, until it becomes of an oval shape. Spores, usually eight in number, are then formed within this. He gave, however, no account of the nuclear behaviour. Hoffmeister ('00), in a paper on the nucleus of Yeasts, gave figures showing that nuclear fusion took place, when the two cells coalesced, before the subsequent divisions of spore-formation occurred. Hence this process also must be regarded as a sexual act.

Since the publication of the above, Guilliermond has described a third case of a sexual act preceding spore-formation among the Saccharomycetes. He has not only verified and amplified the results of Schiönning and Hoffmeister for *Schizosaccharomyces octosporus*, but has also observed a somewhat similar process in *Schizosaccharo-*

myces Pombe. The details of the process in the latter case seem to be identical with those just described for *Zygosaccharomyces*. A pair of cells situated near together put out protuberances, which meet at their tips and fuse, spore-formation then following in the cells. The fused compound-cell usually retains the form of two cells united by a neck. In *Schizosaccharomyces octosporus* he finds that the method of fusion described by Schiönning is not the most common. Usually a cell divides into two daughter-cells by a transverse wall. A small protuberance is formed at the extremity of each of these daughter-cells. These protuberances fuse and form a canal of communication. The separating wall disappears and the canal enlarges, the single cell so formed becoming gradually oval, and spores are rapidly formed within. Very often the fusion of the cells is not complete, while in exceptional cases the cells become converted into asci directly without any fusion taking place.

Since no mention is made of Hoffmeister's work nor of the case of conjugation in *Zygosaccharomyces*, Guilliermond has probably not yet seen the papers on those subjects.

He has gone into the question of the behaviour of the nuclei very fully, and has published earlier this year two papers, which contain the results of his work on the Yeast nucleus, and which must therefore be considered before giving details of the nuclear actions which he observed during the process of conjugation. He found in *Saccharomyces cerevisiae* and other species a nucleus of definite structure, consisting of nucleoplasm surrounded by a membrane and containing several granules, of which one, larger and more regular, is the nucleolus. The 'nuclear vacuole with chromatin granules' of Wager is, according to him, not nuclear in nature. He has studied the structure and development of these granules and the vacuoles in connexion with them in numerous species of *Saccharomyces* and particularly in a *Dematium*, sp. The granules are not fatty in nature, nor apparently are they proteid, since they resist the action of pepsin. Nuclein solvents leave them untouched. They are, however, likely to be overlooked after such treatment, since their staining is rendered more difficult. They possess the characteristics of the 'red grains' of Bütschli. They are usually situated in the interior of vacuoles, and have their origin in connexion with that of the latter. At their first appearance they are very small, and are found in small hyaline spaces, probably the precursors of the vacuoles. These increase in size, and

fuse together to form a large vacuole or vacuoles, while the granules also increase in size by fusion. The fine granules of Wager, which are contained in vacuoles, and which the latter author considered as nuclear, are shown to be identical in nature with the larger granules, which he considered as proteid. Dealing more particularly with the spore-formation in *S. Ludwigii*, a small nucleus with no observable structure is found in young vigorous cells in connexion with a vacuole containing numerous small granules, the 'red grains' of Bütschli. Later, one or two more vacuoles containing glycogen appear at the poles of the cell. In the first stages of sporulation each of these divides, until the protoplasm appears to be entirely filled with small vacuoles separated by very fine meshes of protoplasm. The glycogen vacuoles and the vacuoles containing 'red grains' can be distinguished. Later the vacuoles containing 'red grains' change in appearance and the granules diminish in number and size, the vacuoles themselves then staining a uniform pale-red colour with those stains which give the 'red grains' their characteristic red tint (haematoxylin, gentian violet, &c.). This colouration of the vacuoles is explained by the solution of the granules, since it never occurs at other times. At this stage the nucleus divides into two daughter-nuclei, which travel to either end of the cell and divide again. The division is intermediate between direct division and karyokinesis. Each of these nuclei becomes the basis of a spore, which is formed by the condensation of the protoplasm around a nucleus. The epiplasm contains a substance that stains uniformly red, when the spores are very young, but this gradually disappears as the spores ripen. On account of these facts Guilliermond regards the 'red grains' as reserve material, which is used up in spore-formation.

Returning now to his account of the nuclear behaviour of *S. octosporus* during spore-formation, he states that the nucleus of this form consists of a deeply staining nucleolus surrounded by non-staining nucleoplasm with traces of a limiting membrane. It is in the neighbourhood of small vacuoles or one large vacuole containing 'red grains.' The nuclei in the cells about to conjugate are situated close to the point of fusion. When the wall disappears the two nuclei fuse. The fused nucleus soon divides into two daughter-nuclei, which travel into the cells and take part in spore-formation divisions. The 'red grains' disappear at the moment of fusion. Similar phenomena are observed in *S. Pombe*.

There is thus in these two cases an exact parallel to the behaviour of *Zygosaccharomyces* during spore-formation, and there can be very little doubt that a sexual act takes place here, as in the case already described by the author of the present communication. Having regard to the irregular forms of spore-containing cells of such species as *Schizosaccharomyces mellacei* and *S. Comestii*, which closely resemble the spore-containing cells of *S. Pombe* and *Zygosaccharomyces*, it seems very probable that in these cases also conjugation takes place as a preliminary to spore-formation.

In the three cases of conjugation which have now been observed, there seem to be suggestive modifications of the process. In *Zygosaccharomyces* the ultimate shape of the compound cell remains the same as it is found immediately after conjugation; i. e. two more or less ovoid cells joined together into one compound cell by a conspicuous neck or bridge, which under certain conditions can be made to grow out to some considerable length. In *S. Pombe*, while the compound cell has a similar form to that in *Zygosaccharomyces*, in some cases it loses this structure, being modified so as to appear as a rather enlarged ovoid cell. In *S. octosporus* the latter is the usual occurrence, all traces of the two individual cells being lost. Moreover, in this species conjugation does not always seem to occur, a single cell becoming an ascus without any conjugation with another cell. In the light of these facts, Janssen and Leblanc's observation on the division of the nucleus of the spore mother-cell into two nuclei, and the subsequent fusion of these previous to the series of divisions which yield the nuclei of the spores, becomes very interesting. If Guilliermond's statement, that Wager's 'chromatin-containing vacuole' is not a part of the nuclear apparatus, be correct, then the latter's interpretation of the fusion described by Janssen and Leblanc cannot suffice. It is true that Guilliermond has not observed such fusions, but he evidently does not consider that that fact proves that they do not occur, for he proposes to investigate the point specially.

A confirmation of Janssen's and Leblanc's observations would throw considerable light on the question of spore-formation among the *Saccharomycetes*, strongly supporting the hypothesis that it is a very much reduced sexual act. The gradual reduction or degradation of this act is traceable through the forms described above. In *Zygosaccharomyces*, independent cells fuse, retaining their individuality after fusion. In *S. Pombe* this is also usually the case, but

is less pronounced. In *S. octosporus* two cells, the products of a single mother-cell, fuse, usually completely, so as to reproduce practically the mother-cell, spore-formation then occurring. In occasional cases, however, the mother-cell does not divide into two daughter-cells which fuse again, but proceeds at once to the formation of spores. Such cases would represent a still further suppression of the individuality of the conjugating cells, if it be shown that a nuclear division, followed by re-fusion of the two daughter-nuclei, does occur previous to spore-formation, the two nuclei representing the two daughter-cells of the usual process. If no such process takes place, it may be regarded as the extreme case of reduction of the sexual act, the spores being produced parthenogenetically or, as it appears, asexually.

There is, then, a fair amount of evidence to support the view that the *Saccharomycetes* are derived from Fungi, which possessed a complete sexual act, and that this act has suffered in most cases so great a reduction as to be represented merely by the fusion of the two nuclei in the spore mother-cell, if Janssen's and Leblanc's observations be correct, or for all traces of it to have disappeared, if these authors be wrong.

REFERENCES.

- BARKER, B. T. P., A Conjugating Yeast. Proc. Roy. Soc., vol. lxxviii, p. 345.
 SCHIÖNNING, H. ('95): Nouvelle et singulière formation de l'ascus dans une levure. Compt. rend. des Meddelels. fra Carlsb. Lab. IV. 1. 1895.
 HOFFMEISTER, C. ('00): Zum Nachweis des Zellkerns bei *Saccharomyces*. Sitzungsber. d. deutsch. naturw.-med. Ver. Lotos. 1900, No. 5.
 GUILLIERMOND, A., Recherches sur la structure de quelques champignons inférieurs. Compt. rend., tom. cxxxii, No. 3, p. 175.
 ————— Recherches histologiques sur la sporulation des levures. Compt. rend., tom. cxxxii, No. 19, p. 1194.
 ————— Recherches histologiques sur la sporulation des Schizosaccharomycètes. Compt. rend., tom. cxxxiii, No. 4, p. 242.
 WAGER, H. ('98): The nucleus of the yeast-plant. Ann. Bot., 1898, p. 499.
 JANSSEN and LEBLANC, Recherches cytologiques sur la cellule de levure. La Cellule, tom. xiv, fasc. 1, pp. 203-243.

B. T. P. BARKER.

CAMBRIDGE.

Barker, B. T. P. 1901. "Sexual spore-formation among the Saocaromycetes."
Annals of botany 15, 759–764.

<https://doi.org/10.1093/oxfordjournals.aob.a088848>.

View This Item Online: <https://www.biodiversitylibrary.org/item/236929>

DOI: <https://doi.org/10.1093/oxfordjournals.aob.a088848>

Permalink: <https://www.biodiversitylibrary.org/partpdf/318652>

Holding Institution

Smithsonian Libraries

Sponsored by

Biodiversity Heritage Library

Copyright & Reuse

Copyright Status: Not in copyright. The BHL knows of no copyright restrictions on this item.

This document was created from content at the **Biodiversity Heritage Library**, the world's largest open access digital library for biodiversity literature and archives. Visit BHL at <https://www.biodiversitylibrary.org>.