Fig. 1, which corresponds to known areas of limestone outcrop. Robertson (*4*) reports *P. obeliscus* from a similar habitat in the Rio Hondo of northern British Honduras.

Go-To-Hell Creek flows through a typical tropical rain forest environment and is not sufficiently large to develop a foliage-free corridor where sunlight can penetrate with ease. Nevertheless, sufficient available light is present to stimulate a subaqueous growth of blue-green algae on rock surfaces near the air-water interface. These plants proliferate in the relatively protected microhabitat of the horizontal solution grooves where they accumulate as a thick, palpable slime.

This concentrated algal growth affords a rich pasturage for the snails, which always align their shells parallel to the grooves. Ingestion of algae was observed to occur as a result of rasping action by radula strokes directly perpendicular to the longitudinal axis of the grooves. Closely spaced scratches made by the feeding snails can be seen with the unaided eye and are clearly defined in Fig. 2. These scratches extend through a thin, partially decomposed surface layer of chalky consistency to expose unweathered limestone. Continued rasping action thus enlarges grooves initiated by solution activity, deepens the trough, and presents a fresh rock surface for carbonate solution as well as possible softening by the algae.

In describing a remarkably similar instance involving the tidewater snail *Nerita plicata*, Weins (*5*) cites Doty and Morrison (*6*) who suggest that blue-green algae have a very significant role in the decomposition of limestone. Their data indicate that the process involves local changes in *p*H brought about through metabolic activities of the algae.

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**References and Notes**

1. For example, the *Pholadidae* such as *Parapholas concamerita*, *Pholadidea melanura*, and *Pholadidea tridens*.
2. B. T. Bunting, *The Geography of Soil* (Aldine, Chicago, 1965), p. 43.
3. C. G. Dixon, *Geology of Southern British Honduras with Notes on Adjacent Areas* (The Government Printer, Belize, no date, about 1955), p. 23.
4. R. Robertson, *Phila. Shell Club Proc.* **1**, 20 (1963).
5. H. J. Weins, *Atoll Environment and Ecology* (Yale Univ. Press, New Haven, Conn., 1962), p. 264.
6. Herold J. Weins (*5*) cites Maxwell Doty and J. P. E. Morrison, "Interrelationships of the Organisms on Raroia Aside from Man," *Atoll Res. Bull. No. 35*.

2 October 1967

## "Twin Meiosis" and Other Ambivalences in the Life Cycle of Schizosaccharomyces pombe

Abstract. *Diploid cells of the yeast* Schizosaccharomyces pombe *carrying the mating-type allele* $h^{90}$ *are capable of sporulation and copulation. After copulation karyogamy does not always occur. In this case both nuclei will undergo meioses separately (twin meiosis). Asci with eight haploid spores derive from this event. Diploid cells homozygous for the mating-type alleles* $h^+$ *or* $h^-$ *do not sporulate. However, their nuclei can perform meiosis when they are in a common cytoplasm with a diploid nucleus of compatible mating type.*

In the life cycle of the fission yeast *Schizosaccharomyces pombe* Lindner a key role is played by the mating-type alleles. They control the abilities for copulation as well as for sporulation (*1, 2*). Below I report experiments with diploid *S. pombe* strains, which show some ambivalences in the course of events controlled by the mating-type alleles. For instance, after cell fusion nuclear fusion does not occur in part of the zygotes, and both nuclei undergo separate meioses (twin meiosis).

The vegetative cells of *S. pombe* are normally haploid. At the end of vegetative growth, cells of compatible mating type fuse pairwise (*1, 2*). In the resulting zygotes, fusion of the nuclei (karyogamy) and meiosis take place, and four ascospores are formed within the cell wall of the original zygote (zygotic asci). Three alleles ($h^{90}$, $h^+$, $h^-$) are known at the mating-type locus. They determine three different mating types (*1–3*): haploid strains having the allele $h^{90}$ are homothallic, whereas strains with $h^+$ or $h^-$ are heterothallic (*4*). The following lists show which mating types are compatible and incompatible, respectively:

1) compatible (copulation): $h^{90} \times h^{90}$, $h^{90} \times h^+$, $h^{90} \times h^-$, $h^+ \times h^-$;

2) incompatible (no copulation): $h^+ \times h^+$, $h^- \times h^-$.

Although the cells of *S. pombe* are normally haploid, it is possible to select diploid strains. An analysis of these strains has shown that the mating-type alleles not only determine the capability to copulate but also the capability to sporulate (*2*). Cells of the constitution $h^{90}/h^{90}$, $h^{90}/h^+$, $h^{90}/h^-$, and $h^+/h^-$ undergo meiosis at the end of the period of vegetative growth and form directly four ascospores (azygotic asci). Diploid cells which are $h^+/h^+$ or $h^-/h^-$ do not sporulate but will copulate with either haploid or diploid cells of compatible mating type (*2, 5*). After copulation of diploid cells, in general four diploid ascospores are produced (*6*).

Two different complete culture media are used in experiments with *S. pombe*: YEA (*7*) and an incubation temperature of 30°C are suitable for vegetative growth, whereas MEA (*7*) and 25°C are favorable for copulation and sporulation. The results with diploid strains cited above (*2, 6*) were obtained with MEA (or beer wort) at 25°C.

In experiments using YEA at 30°C, I observed that $h^{90}/h^{90}$ cells not only sporulate, but also frequently copulate with each other. After 2 to 4 days of incubation, $h^{90}/h^{90}$ cultures show mainly azygotic asci, but a considerable number of giant zygotic asci are present too. The latter have either four large, apparently diploid spores, or they have six, seven, or, more frequently, eight spores of normal size (*8*). Spores from eight-spored asci were isolated by micromanipulation. Upon cultivation they gave rise to haploid cultures. This preliminary finding seemed to be consistent with the idea of "brachymeiosis" discussed in the earlier literature on Ascomycetes (*9*). It was therefore of interest to perform a more detailed analysis of the multispored asci by means of strains with different genetic markers. In addition, the possible formation of eight-spored asci in mating-type combinations other than $h^{90}/h^{90} \times h^{90}/h^{90}$ was examined.

I made nine different crosses with diploid strains on YEA and incubated them 2 to 4 days at 30°C. To avoid sporulation of the $h^{90}/h^{90}$ and $h^{90}/h^-$ strains before mating, the freshly selected diplonts were grown in liquid yeast-extract medium on a shaker. In liquid medium only very few azygotic asci are formed, in contrast to cultures on agar. With respect to the mating-type combinations the crosses were of five different types (Table 1).

In type I, both parents were able to sporulate; in types II, III, and IV, sporulating strains were mated with nonsporulating diplonts ($h^+/h^+$ or $h^-/h^-$); and in type V, both diplonts were unable to sporulate. In all crosses one strain was auxotrophic for uracil, the other for lysine (*10*). Furthermore, each strain was heterozygous for two nonidentical *ade-6* alleles giving strong complementation (*11*). Due to the allelic complementation, the diplonts did not form the red pigment characteristic of *ade-6* mutants. As an example, the full genotypes are given in Table 2 for one cross of type IV. All markers used (*ade-6, ura-1, lys-2*, mating-type) are unlinked.

In the crosses of types I to IV, both four-spored azygotic asci (due to direct sporulation of $h^{90}/h^{90}$ and $h^{90}/h^-$ cells, respectively) and zygotic asci were present. As expected, in the cross of type V only zygotic asci were present. In all crosses (even in type V!) about 30 to 50 percent of the zygotic asci showed six to eight spores and the rest had four large, apparently diploid spores (*12*; see also *8*). Figure 1 shows asci from a cross of type III.

Altogether 124 eight-spored asci were dissected. In 23 asci only four or fewer spores germinated; they were not analyzed further. The other 101 asci yielded between five and eight single-spore colonies. These colonies were examined further. In Table 1 are shown the numbers of asci with five to eight germinated spores obtained in the various crosses. A total of 641 single-spore colonies were analyzed. All colonies were red—no white ones were found. All sporulating colonies showed zygotic asci of normal size; azygotic asci were not found. This strongly indicates that all spores were haploid. If diploid spores had occurred, part of their colonies would have been white owing to complementation between the *ade-6* alleles, and/or would have sporulated with azygotic asci. Intragenic recombination between the *ade-6* heteroalleles, which gives rise to spores prototrophic for adenine, is not to be expected in the relatively small sample of asci analyzed (see *11*).

The unlinked markers *ura-1* and *lys-2* did not recombine in the eight-spored asci; the spore cultures were either *ura-1* + or + *lys-2*. As an example, the segregation in the asci with complete spore germination is shown in Table 2 for a cross of type IV. It is seen that recombination has further not occurred between the mating-type locus and either auxotrophic markers *ura-1* or *lys-2*. Furthermore, within both groups *ura-1* + and + *lys-2* the *ade-6* heteroalleles showed a 2 : 2 segregation. Corresponding results were obtained in all other crosses. The segregations in the asci with incomplete spore germinations were similar except that one to three random spore colonies were missing.

In the cross type Ib a lethal mutation (*let*) had occurred in the $h^{90}/h^-$ parent which is closely linked with the $h^-$ allele. In that cross a maximum of six spores germinated, and all $h^-$ alleles were lost. In the crosses of types I, II, and IV some selfings had obviously occurred within the $h^{90}/h^{90}$ parents. Twelve asci (listed in the bottom row of Table 1) were obtained, which yielded five to eight haploid $h^{90}$ colonies possessing only the markers of the $h^{90}/h^{90}$ strain involved in the corresponding cross.

The observed segregations strongly suggest that the giant eight-spored asci originate from zygotes in which, after plasmogamy, fusion of the diploid nuclei does not occur, but both nuclei undergo separate meioses. The extraordinary fact is that the diploid nuclei stay genetically distinct and meiosis goes on separately in the two nuclei in the fused cells. I suggest calling this phenomenon "twin meiosis."

The results show that diploid cells are ambivalent in respect to meiosis, copulation, and karyogamy if grown on YEA at 30°C. On MEA at 25°C this ambivalence is only slightly indi-

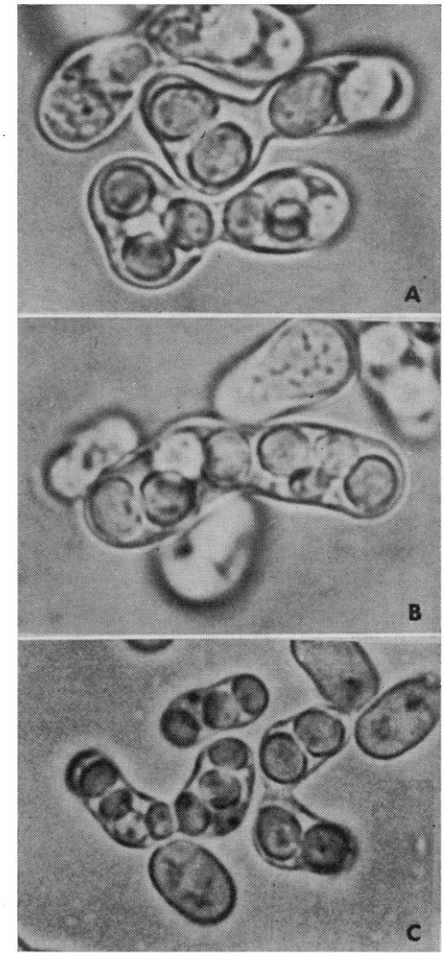


Fig. 1. Asci from a cross of type III. In each picture one zygotic ascus with eight spores is shown. In (A) and (C) zygotic asci with four large spores are to be seen also. Furthermore, (C) shows one azygotic ascus. Not all spores are in focus. Magnification: (A) and (B) × 2270; (C) × 1500.

Table 1. Types of crosses made between diploid strains, and number of eight-spored asci analyzed. Only those asci are listed in which at least five spores germinated.

| Type of cross | No. of asci analyzed | No. of asci with 8, 7, 6, or 5 germinated spores | | | |
|---|---|---|---|---|---|
| | | 8 | 7 | 6 | 5 |
| Ia) $h^{90}/h^{90} \times h^{90}/h^-$ | 5 | 5 | 0 | 0 | 0 |
| Ib) $h^{90}/h^{90} \times h^{90} + /h^-$ *let* | 9 | 0 | 0 | 8 | 1 |
| II) $h^{90}/h^{90} \times h^+/h^+$ | 9 | 2 | 1 | 4 | 2 |
| III) $h^{90}/h^- \times h^+/h^+$ | 21 | 0 | 2 | 8 | 11 |
| IV) $h^{90}/h^{90} \times h^-/h^-$ | 24 | 10 | 3 | 7 | 4 |
| V) $h^+/h^+ \times h^-/h^-$ | 21 | 2 | 5 | 8 | 6 |
| Selfings in $h^{90}/h^{90}$ strains | 12 | 7 | 3 | 1 | 1 |

Table 2. Genotypes of the strains used in one cross of type IV, and segregation in the asci with completely germinated spores.

| Cross (type IV) | Segregation in the eight-spored asci | |
|---|---|---|
| | ura-1 + | + lys-2 |
| $\frac{h^{90}}{h^{90}}$ $\frac{ade\text{-}6\text{-}M216}{ade\text{-}6\text{-}M210}$ $\frac{ura\text{-}1}{ura\text{-}1}$ $\frac{+}{+}$ | Four $h^{90}$ spores: | Four $h^-$ spores: |
| $\times$ $\frac{h^-}{h^-}$ $\frac{ade\ 6\text{-}M210}{ade\text{-}6\text{-}L702}$ $\frac{+}{+}$ $\frac{lys\text{-}2}{lys\text{-}2}$ | Two *ade-6-M216*<br>Two *ade-6-M210* | Two *ade-6-M210*<br>Two *ade-6-L702* |

cated. If we consider the behavior on MEA at 25°C as normal, the following instabilities can be stated.

Diploid homothallic cells (either homozygous or heterozygous for the $h^{90}$ allele) normally sporulate directly (azygotic asci). On YEA at 30°C they also copulate frequently. In the latter case a further ambivalence is obvious: the nuclei either fuse (zygotic asci with four large spores, see *6*) or do not fuse but undergo separate meioses (twin meiosis, zygotic asci with eight haploid spores). Thus two conclusions can be drawn for homothallic diploid cells: although having a nucleus able to perform meiosis, the cells still have a tendency for copulation. Also, their nuclei still have an ability to fuse. In about 50 percent of the zygotes karyogamy occurs, whereas the rest of the copulations result in twin meiosis.

The diploid cells homozygous for incompatible mating-type alleles ($h^+/h^+$ or $h^-/h^-$) are not able to sporulate but can copulate with cells of compatible mating type. Normally asci with four diploid spores are formed (*2, 6*). However, on YEA at 30°C twin meioses also occur frequently (see *12*). The occurrence of twin meiosis in crosses with $h^+/h^+$ and $h^-/h^-$ cells demonstrates an interesting complementation mechanism since their nuclei individually do not have the complete genetic information for meiosis. In each of the crosses of types II to IV, one of the diploid nuclei involved in the twin meioses was able to perform meiosis. In these cases, it is therefore sufficient to assume that due to the diploid homothallic nucleus all substances necessary for meiosis are present in the common cytoplasm, and that these substances enable the $h^+/h^+$ or $h^-/h^-$ nucleus to undergo meiosis too. In the cross of type V, however, neither nucleus was capable of independent meiosis. Here one has to conclude that complementing gene products are formed by the $h^+/h^+$ and $h^-/h^-$ nuclei, and that these substances, when combined, enable the nuclei to undergo meiosis regardless of whether they are fused or not.

The latter situation is surprising: after copulation of the diploid cells ($h^+/h^+ \times h^-/h^-$), their nuclei have a tendency to fuse, but are obviously producing at the same time the (incomplete) gene products necessary for meiosis. Whether twin meiosis or karyogamy will occur seems to depend merely on whether the "meiotic" gene products complement each other to a sufficient level before the nuclei have fused.

The observed ambivalences might offer a possibility of evaluating in further experiments the regulatory mechanisms involved in copulation, karyogamy, and meiosis.

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### References and Notes

1. U. Leupold, *Compt. Rend. Lab. Carlsberg, Sér. Physiol.* **24**, 381 (1950).
2. ———, *Cold Spring Harbor Symp. Quant. Biol.* **23**, 161 (1958).
3. The three mating types of *S. pombe* are determined by a short region (1.1 map units) on the genetic map (*2*). Whether this region consists of one or more cistrons cannot be decided at present. In the present report the terms mating-type "alleles" and "locus" are used for simplicity.
4. "Homothallic" means that the descendants of a single cell are compatible with each other (copulation), whereas the descendants of a heterothallic cell are incompatible with each other but can copulate with cells of a compatible mating type.
5. For the selection of diplonts (*2*) strains with complementary growth requirements are mated, and young zygotes are transferred to minimal agar. A small number of the zygotes grow up to diploid colonies. In $h^+/h^-$ diplonts nonsporulating cells ($h^+/h^+$ or $h^-/h^-$) occur by mitotic crossing over between the centromere and the mating-type locus.
6. U. Leupold, *Compt. Rend. Lab. Carlsberg, Sér. Physiol.* **26**, 221 (1956).
7. ———, *Arch. Julius Klaus-Stift.* **30**, 506 (1955). YEA, yeast-extract agar (0.5 percent Bacto-yeast extract, 3 percent glucose, 2 percent agar); MEA, malt-extract agar (3 percent Bacto-malt extract, 2 percent agar).
8. Zygotic asci with only two or three large spores also occur rarely. A few of the six-spored asci have two large spores and four of normal size. Leupold (*6*) has already observed zygotic asci with seven to eight spores in a homothallic diploid strain. They occurred only rarely under his experimental conditions (beer wort, 25°C), and he did not carry out a genetic analysis of these asci. In agreement with Leupold's observation I found that zygotic asci (especially with six to eight spores) are much less frequently formed on MEA at 25°C than on YEA at 30°C.
9. In the postulate of "brachymeiosis" it was assumed that a tetraploid nucleus is reduced to eight haploid nuclei in three consecutive meiotic divisions. For references see E. Gäumann, *Die Pilze* (Birkhäuser, Basel and Stuttgart, ed. 2, 1964), p. 198.
10. Notation: *ura-1, lys-2, ade-6*: genes involved in the synthesis of uracil, lysine, and adenine, respectively; *M216, M210*, and *L702* are nonidentical *ade-6* mutations; *let*: lethal factor.
11. H. Gutz, *Habilitationsschrift* (Technische Universität, Berlin, 1963); U. Leupold and H. Gutz, *Proc. Intern. Congr. Genet. 11th* **2**, 31 (1964). The complementing *ade-6* alleles were used to select the diploid strains.
12. The frequencies of zygotic asci with four and six to eight spores, respectively, fluctuated from cross to cross. In the crosses with diploid homothallic strains (types I to IV) four-spored zygotic asci were approximately as frequent as six- to eight-spored. In the cross $h^+/h^+ \times h^-/h^-$ (type V) about 30 percent six- to eight-spored asci were found. When plated on MEA (25°C), the latter cross showed approximately 96 percent four-spored and only about 4 percent six- to eight-spored asci.
13. I thank Mrs. Lois Wilson for excellent technical assistance. Supported by NSF grant GB-4286 and NIH grant GM 13234.

21 August 1967

## Boron in Plants: A Biochemical Role

Abstract. *Boron, as borate, appears to have a role in partitioning metabolism between the glycolytic and pentose-shunt pathways. This effect results from the association of borate with 6-phosphogluconic acid, forming a virtual substrate that inhibits the action of 6-phosphogluconate dehydrogenase. In the absence of borate, the inhibition of the enzyme is released, and excess phenolic acids are formed. These acids also associate strongly with borate and thus develop an autocatalytic system for production of excess phenolic acids which cause necrosis of tissue and eventual death of the plant.*

A biochemical syndrome of boron deficiency in plants (*1, 2*) is the accumulation of phenolic acids; their excessive concentration appears to be the immediate cause of necrosis and ultimate death from this nutritional deficiency (*1*). One may therefore presume that boron plays a role in the regulation of phenol synthesis, either directly or indirectly—for example, by control of substrate permeability through membranes, or by interaction with enzymes of the glycolytic or pentose-shunt pathway, or of both. The metabolism of

Table 1. Radioactivities of selected compounds from B+ and B− sunflower fed $^{14}CO_2$ for 30 minutes under light.

| Compound | Time after feeding with $^{14}CO_2$ (hr) | | | |
|---|---|---|---|---|
| | 0 (count/min) | | 30 (count/min) | |
| | B+ | B− | B+ | B− |
| Citric acid | 614 ± 11 | 421 ± 10 | 54,010 ± 73 | 16,620 ± 41 |
| Glyoxylic acid | | | 19,540 ± 44 | 38,462 ± 62 |
| Phenylalanine | 633 ± 12 | 576 ± 10 | 10,950 ± 33 | 25,080 ± 50 |