Stability of Hybrids in *Saccharomyces*¹

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It was first shown by Powell (1951) that supposed hybrids often produce haploid buds without undergoing sporulation. This is interpreted to indicate that the zygote is a dikaryon instead of a true hybrid with a diploid nucleus. Zygotes of *Saccharomyces* begin their life cycles as dikaryons (fig. 1a). Plasmogamy is followed by nuclear division rather than by karyogamy (fig. 1b). The two daughter nuclei pass into a bud produced at the point of union of the two gametes and the daughter nuclei fuse in the bud to produce a diploid nucleus (fig. 1c); the original dikaryotic zygote continues as a dikaryon and produces a second bud (fig. 1d). This bud also receives a pair of non sister nuclei produced by the mitotic division of the two nuclei of the original dikaryon. The diploid nucleus simultaneously produces a diploid bud. This process may be deduced by studying the fusion of yeast gametes shown in the film "The Life Cycle of a Yeast Cell" (Lindegren, 1952). Since the original dikaryon persists indefinitely, it may occasionally produce buds identical to the original gametes.

Fig. 1. Diagrammatic representation of the steps in the production of a hybrid from fused yeast gametes. The nuclei are represented schematically as circles. In b the daughters of the original gametic nuclei have migrated into the bud. In c the nuclei in the hybrid bud have fused. In d the fused hybrid nucleus has divided, the original gametic nuclei have divided again, and their daughter nuclei have migrated into the bud.

The continued maintenance of a dikaryon (or the protracted delay of karyogamy) is a mechanism widely distributed among the fungi which serves (1) to maintain flexibility with regard to the capacity for producing other

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dikaryons (Buller, 1931) and (2) to maintain the structural stability necessary for the construction of complex organs (Lindegren, 1942). In the hymenomycetes each cell contains a dikaryon which multiplies by synchronous conjugate division. The fact that each cell of the mushroom contains two nuclei insures structural stability; if each cell contained only a single haploid nucleus, mutation might readily distort the structure. The structurally stable dikaryon is, nevertheless, capable of copulation with any other cell of appropriate mating type without undergoing the formality of a reduction division.

The disintegration of a supposed hybrid into its parental components without a reduction division has been encountered frequently in this laboratory, especially in attempts to produce stable triploids. The recommended procedure for producing a triploid is to plate out colonies from a mass mating and retain the most stable which contains large cells. If the original zygote were selected, as suggested by Fowell, the possibility of obtaining haploid and diploid buds would still obtain. Pomper (1952) has proposed the following method for the production of triploid *Saccharomyces*: Mutually deficient haploid and diploid gametes are mated on minimal medium under conditions in which neither is capable of independent growth. This permits the survival only of zygotes. It is clear, however, that this method cannot insure the production of true hybrids since the zygotes may either dikaryons or true hybrids. Although Pomper's method could serve to keep dikaryons together which might otherwise separate, there are no indications that it could insure the copulation of gametes otherwise incapable of copulating. The presumptive hybrids would certainly have to be tested for stability on complete medium in any event.

There have been many proposals for modifications of the mass mating technique (Lindegren and Lindegren, 1943) because the original gametes are mixed with the hybrid cells. This difficulty can be circumvented by using gametes sufficiently well-marked genetically to insure the identity of a hybrid by the analysis of the ascospores which it produces. Since any single cell selected from a mating may be a persistent dikaryon rather than a true hybrid, there is no certain guarantee that a vegetative cell is a true hybrid, except the analysis of its ascospores. Mass mating has the further advantage that each tetrad analyzed comprises a test of a separate individual mating. If the matings are heterogeneous, due to the presence of some diploid gametes, some of the tetrads will give aberrant results and the event can be detected by genetical analysis of a variety of tetrads. In the experience of this laboratory, approximately one tetrad in 25 (of those in which all four spores survive) has been produced by the reduction of a tetraploid zygote. Segregants of triploids are probably only poorly viable and for this reason would not be readily detected in an analysis in which only four spored asci were utilized. Under Fowell's or Ephrussi's method all the tetrads could belong to an aberrant type.

References

- Buller, A. H. R. 1930. The biological significance of conjugate nuclei in *Coprinus lagopus* and other hymenomycetes. *Nature*, 126, 686-689.
- Ephrussi, B. 1950. Sur une nouvelle technique de croisement des levures. *C. R. Acad. Sc.*, 230, 1897-99.
- Fowell, R. R. 1951. Hybridization of yeasts by Lindegren's technique. *Jour. Inst. Brewing*, 57, 180-195.
- Lindegren, Carl C. 1942. The use of the fungi in modern genetical analysis. *Iowa State College Jour. of Sci.*, 45, 371-390.
- Lindegren, Carl C. and Lindegren, Gertrude 1943. A new method of hybridizing yeast. *Proc. Nat'l. Acad. Sci.*, 29, 306-08.
- Lindegren, Carl C. 1952. Life Cycle of a Yeast Cell. 16 mm sound motion picture produced by Southern Illinois University, Carbondale, Ill.
- Seymour, Seymour 1952. Isolation of triploid *Saccharomyces cerevisiae*, *Nature*, 170, 892-93.
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