GENETICS

Mitotic Haploidization by Treatment of *Aspergillus niger* Diploids with *para*-Fluoro-phenylalanine

In genetic analysis based on mitotic segregation[1–4] an important step is the recovery of haploid segregants from heterozygous diploids. This has been done until recently by laborious or complicated techniques[4,5]. I am indebted to Dr. G. Morpurgo, of the Istituto Superiore di Sanità, Rome, for informing me and permitting me to make use of his discovery that treatment with *para*-fluoro-phenylalanine of diploids of *Aspergillus nidulans* leads to wholesale segregation (this discovery was made known in the privately circulated *Aspergillus News Letter*, p. 10, No. 2, Spring 1961). In an extensive work expanding the formal genetical analysis—via mitotic segregation—of the fungus *A. niger*[6], I have applied this treatment to diploids of this asexual species.

The amino-acid analogue DL-*para*-fluoro-phenylalanine was incorporated in standard complete medium[7] to a final concentration of 1/10,000 w/v and conidia from diploid strains heterozygous for a number of colour and nutritional markers were plated on the surface. At this concentration of the analogue, the viable counts remain the same as on standard complete medium, but the rate of growth is much slower (diameter of colonies after 5 days: 0·5–1 cm. instead of about 10 cm.) and sporulation is very poor. These colonies produced sectors, the rate of growth and sporulation of which was about the same as on normal complete medium. After 10 days, conidia of these sectors were streaked on complete medium, classified for ploidy on the basis of the diameter of their conidia and tested for requirements. All of them turned out to be haploid and of all possible recombinant types between independent markers.

As an example, a heterozygous diploid with black heads (wild-type) and prototrophic was synthesized from two haploid strains: one fawn (*a*) and the other olive (*o*) (two recessive non-allelic markers belonging to the same linkage group). The two strains also differed in four recessive nutritional requirements and the genotype of the diploid formed from them was:

$$\frac{a \quad\quad +}{+ \quad\quad o} \quad \frac{+}{hist} \quad \frac{+ \quad\quad gua_7}{lys_4 \quad\quad +} \quad \frac{meth_7}{+}$$

The vigorously growing sectors produced by this diploid were either fawn or olive. To minimize clonal effects, only one sector of each colour (fawn or olive) was picked from a colony. The 74 sectors so isolated, 38 fawn and 36 olive, fell into the 8 possible classes of segregants and recombinants in respect of the four other markers belonging to three different linkage groups.

That haploidization, as a consequence of *para*-fluoro-phenylalanine treatment, occurs by successive losses of chromosomes—as found by Käfer[4] for *Aspergillus nidulans* in the case of 'spontaneous' haploidization—is shown by the following evidence: The results just mentioned are obtained by isolating vigorously and well-sporulating sectors from diploid colonies grown in the presence of the analogue for ten days. If small groups of hyphal tips are taken from diploid colonies only after 48 hr. of growth in the presence of the analogue and transferred to normal medium, they produce sectors in which more and more nutritional markers segregate as growth proceeds.

On *para*-fluoro-phenylalanine the amount of growth intervening between the original diploid mycelium and the completely haploid sectors is relatively small, indicating a selective effect against diploids and disomics. I interpret this to mean that loss of individual chromosomes in a haploid leads to no further divisions of that nuclear lineage, while in a diploid or disomic stunted growth may go on.

The availability of a treatment which produces wholesale haploidization is a very valuable addition to the techniques for genetical analysis via mitotic segregation.

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[1] Pontecorvo, G., *Ann. Rev. Microbiol.*, **10**, 393 (1956).
[2] Pontecorvo, G., and Käfer, E., *Proc. Roy. Phys. Soc. Edin.*, **25**, 16 (1956).
[3] Pontecorvo, G., and Käfer, E., *Advances in Genetics*, **9**, 71 (1958).
[4] Käfer, E., *Nature*, **186**, 619 (1960).
[5] Forbes, E., *Heredity*, **13**, 67 (1959).
[6] Pontecorvo, G., Roper, J. A., and Forbes, E., *J. Gen. Microbiol.*, **8**, 198 (1953).
[7] Pontecorvo, G., Roper, J. A., Hemmons, C. M., MacDonald, K. D., and Bufton, A. W. J., *Advances in Genetics*, **5**, 142 (1953).

Hybrids between *Solanum bulbocastanum* and *Solanum cardiophyllum*

The Mexican diploid tuberous *Solanum* series Bulbocastana and Cardiophylla are important potential sources of genes for resistance to late blight of potatoes. Niederhauser and Mills[1] noted field immunity in certain clones of *S. bulbocastanum* in their field trials in Mexico. No investigator has hitherto reported hybrids between these series and the Tuberosa. A knowledge of the crossability relationships of the Mexican diploid species may be useful in overcoming their apparent isolation.

Extensive collections were made of *S. bulbocastanum* Dun. and *S. cardiophyllum* Lindl. throughout their ranges in Mexico during 1956 and 1957. The collections of *S. cardiophyllum* were comprised of *S. cardiophyllum sensu stricto*, *S. ehrenbergii* (Bitt.) Rydb. and *S. cardiophyllum* var. *endoiodandrum* Bitt. Seed set occurred from crosses between *S. bulbocastanum* (C-238) and *S. cardiophyllum* (C-297), and in the reciprocal cross between *S. ehrenbergii* (C-370) and *S. bulbocastanum* (C-194).

The hybrid plants from both crosses showed in their seedling stages the unifoliate characteristic of the *S. bulbocastanum* parent. As they approached the flowering stage, they presented the aspect of a compound-leaved *S. bulbocastanum*.

Although the hybrids produced a high percentage of stainable pollen, it was not always possible to obtain fruit and seed set by sibling pollinations within the families *C*-238 × *C*-297 and *C*-370 × *C*-194. The inter-family cross-pollinations attempted were successful in both directions. This observation suggests a repetition of the intra-family cross-incompatibility noted by Graham *et al.*[2] in *S. bulbocastanum*.

K. M. Graham
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[1] Niederhauser, J. S., and Mills, W. R., *Phytopath.*, **43**, 456 (1953).
[2] Graham, K. M., Niederhauser, J. S., and Servin, L., *Canad. J. Bot.*, **37**, 41 (1959).

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