A system for increasing variability in filamentous fungi?

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The possibility of genetic analysis in the Fungi Imperfecti had its origin with the description of genetic recombination without sexual reproduction, later designated the parasexual cycle, in the ascomycete *Aspergillus nidulans* (Pontecorvo et al. 1953. Adv. Genet. 5:141-238; Pontecorvo 1956. Ann. Rev. Microbiol. 10:393-400). As the description occurred in a species with sexual reproduction, it obviously allowed comparisons between data obtained from sexual and parasexual analysis. Therefore it was possible to establish the basic parameters of genetic analysis far more easily that would have been possible if the parasexual cycle had been discovered in an imperfect species.

Typically, the parasexual cycle is a sequence of events as follows: Heterokaryosis occurs, followed by fusion of haploid nuclei in non-specialized structures producing relatively stable diploid nuclei. The resulting diploid colonies can be isolated as prototrophs using suitable selective procedures (Roper 1952. Experientia 8:14-15). Diploid nuclei can spontaneously generate haploid recombinants by non-disjunction, and/or diploid recombinants by mitotic crossing over, both events occurring at low frequencies (Pontecorvo and Käfer 1958. Adv. Genet. 9:71-104; Käfer 1961. Genetics 46:1581-1609). These low frequencies may well be of importance in imperfect species, or in heterothallic species in which compatible mating types get together infrequently. However it almost certainly means that, from a genetic diversity point of view, the parasexual cycle is a comparatively small effect in a species which is constantly producing spores by meiosis, as in *A. nidulans* (Fincham et al. 1979. Fungal Genetics. Blackwell Sci. Publications).

Following the description of the parasexual cycle in *A. nidulans*, the same recombination mechanism was found in several other species, and although variations were detected in some species, they were interpreted in light of the classical sequence of events. For instance, in Aspergillus niger the frequency of prototrophic colonies, presumed to be diploids, was higher than the frequency reported for *A. nidulans* (Pontecorvo et al. 1953. J. Gen. Microbiol. 8:198-210). Also in *A. niger* some prototrophic colonies were considered to be stable diploids as no spontaneous segregation was detected (Chang and Terry 1973. Appl. Microbiol. 25:890-895; Das and Ilczuk 1978. Folia Microbiol. 23:362-365). Lhoas (1967. Genet. Res. 10:45-61) and Bos et al. (1988. Curr. Genet. 14:437-443) pointed out that prototrophic colonies should be analyzed as soon as possible or even discarded, otherwise anomalous segregation patterns were likely to be detected. Lhoas (op. cit.) also reported a high frequency of mitotic recombination in *A. niger* when compared to *A. nidulans*. A more extreme variation, namely a transient diploid state, was proposed in *Acremonium chrysogenum* since recombinants (possibly haploids and hyperhaploids) were isolated directly from a heterokaryotic stage which was itself transient (Ball and Hamlyn 1978. Brazil. J. Genet. 1:83-96).

Working with a citric acid-producing strain of *A. niger*, Bonatelli Jr. et al. (1983. Brazil. J. Genet. 6:399-405) detected at least three types of prototrophic colonies arising from balanced heterokaryons. These were classified as haploid recombinants, diploids heterozygous for only some of the genetic markers and typical diploids as determined by the benlate test (Upshall et al. 1986. J. Gen. Microbiol. 100:413-418), conidial diameter, DNA content per nucleus and segregation analysis. Based on these data it was suggested by Bonatelli Jr. and Azevedo (1990. Abstracts 4th IMC, Regensburg, FRG. p. 143) that in *A. niger* besides the typical heterozygous diploid nuclei another diploid state might also occur originating from haploid and/or diploid recombinants in the heterokaryotic hyphae. These diploid nuclei are possibly committed to a process that could generate recombinants, which resembled meiosis but was not accompanied by differentiation of typical sexual structures, as is usual for sexual fungi, nor having the same complex genetic control. That process, which seems to be a variation of the parasexual cycle, was tentatively designated parameiosis (Bonatelli Jr. et al. 1983. Brazil. J. Genet. 6:399-405).

More recently, in a survey of prototrophic *A. niger* colonies from crosses involving multi-marked strains described by Masiero and Bonatelli Jr. (1989. Brazil. J. Genet. 12:707- 718) and Bos et al. (1988. Curr. Genet. 14:437-443), Calil and Bonatelli Jr. (data not published) also detected diploid colonies showing homozygosis for genetic markers. Furthermore, these workers also isolated diploid colonies which showed a frequency of mitotic crossing over two to eight fold higher than expected in two tested intervals. These data seem to corroborate the idea that different prototrophic colonies can arise from heterokaryons of that species and also that recombination can occur at relatively high levels at least in some diploid strains indicating that different types of diploid nuclei might exist.

Variations in the parasexual cycle have been described in other species besides *A. chrysogenum* and *A. niger*. In Metarhizium anisopliae (Bergeron et al. 1982. Can. J. Genet. Cytol. 24:643-651; Silveira and Azevedo 1987. Enz. Microb. Technol. 9:149-152; Bagagli et al. 1991. Brazil. J. Genet. 14:261-271); *Magnaporthe grisea* (Crawford et al. 1986 Genetics 114:1111-1129); *Beauveria bassiana* (Paccola-Meireles and Azevedo 1991. J. Inv. Pathol. 57:172-176); *Trichoderma sp*. (Stasz and Harman 1990. Exp. Mycol. 14:145-159; Furlaneto and Pizzirani-Kleiner 1992. FEMS Microb. Letters 90:191-196) and *Fusarium oxysporum* (Molnãr et al. 1990. Mycol. Res. 94:393-398) the emergence of mainly haploid and hyperhaploid recombinants directly from heterokaryons occurs at frequencies which considerably exceed the usual values of the parasexual cycle as in *A. chrysogenum*. That situation seems to indicate the occurrence of a high proportion of transient diploid nuclei, because very few if any diploid colonies were detected. For these cases mechanisms other than parameiosis have been proposed, e.g. a primitive meiosis (Bonatelli Jr. and Azevedo 1990. Abstracts 4th IMC, Regenburg, FRG p. 143; Bagagli et al. 1991. Brazil. J. Genet. 14:261-271) or even a mechanism of a natural intertransformation, as suggested in Trichoderma (Stasz and Harman 1990. Exp. Mycol. 14:145-159) and at this stage they cannot be conclusively ruled out. In *Fulvia fulva* (Talbot et al. 1988. Curr. Genet. 14:567- 572) and *Verticillium sp*. (Jackson and Heale 1987. J. Gen. Microbiol. 133:3537-3547) the situation is more similar to *A. niger* where haploid and/or diploid recombinants can occur in lower frequencies than in *A. chrysogenum* together with typical and non-typical diploid colonies. It seems to indicate that only some diploid nuclei in the heterokaryotic hyphae are transient and consequently committed to generate recombinants.

In all these cases, however, the existence of transient diploid nuclei can be suggested and that might be a process for obtaining variability in Fungi Imperfecti and also, as is the case of *M. grisea*, in heterothallic species where mating types occur apart from each other most of the time (Crawford et al. op cit.). It is interesting to note that in *A. nidulans*, where the parasexual cycle was first described, no evidence of a particularly unstable diploid state has been found, despite intensive genetic analysis over many years.

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