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Colourless mutants in *Ascobolus immersus* with alternative phenotypes

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Most of the genetic work with the ascomycete *Ascobolus immersus* was done on mutants with colourless ascospores, the wild-type being brown-violet (Rizet et al. 1960; Lissouba et al. 1962; Gajewski et al. 1963; Makarewicz 1964a, 1964b; Yu-Sun 1964; Surzycki and Paszewski 1964a, 1964b). Such mutants arise spontaneously in wild-type strains; they are isolated from 4:4 asci, containing four colourless ascospores besides four pigmented ones. Several thousands of colourless mutants, grouped in many compound loci, named series, are known. Crosses among them allow to distinguish which mutants belong to the same series and which to different ones. The former give asci with all ascospores colourless, 8:0, while in the latter three types of asci appear: PD — parental ditypes, 8:0, T — tetratypes, 6:2, and NPD — non parental ditypes, 4:4.

As in *Ascobolus immersus* tetrad analysis may be performed on a great scale, information is quite easily furnished on centromere distance as well as on linkage or independence. When at least one of the two tested independent mutants is far from its centromere, more than 33 units apart, the distribution PD:T:NPD is about 1:4:1 (Lindegren 1948, Shult and Lindegren 1956). When both mutants are located near their centromeres, the number of Ts decreases, the ratio T:NPD approaches to 0. Thus, when one is looking for centromere markers, crosses are performed between one of them and unlocated mutants.

In such crosses mutants were found giving unusual distribution of tetrads. Namely, on one side, the ratio T:NPD was low, suggesting the tested mutants are near to their centromeres, but, simultaneously, the PD asci, 8:0, were very rare if any. These mutants, denoted below as "leaky", originated in 4:4 asci from crosses between wild-type strains as other colourless mutants do. Non leaky mutants will be called below "common" mutants. When crossed with wild-type the leaky mutants have the same phenotype as the common ones. When, however, crosses between "+" and "—" of a leaky mutant are made, 0:8 asci appear instead of 8:0 ones. (Figs. 1 and 2).

Though the pigmentation of the leaky ascospores is, in general, less intense than of wild-type ascospores, they are easily distinguished from the colourless ones.

Asci from crosses involving leaky mutants were analysed. The results obtained for leaky and common mutants are shown in Table 1.

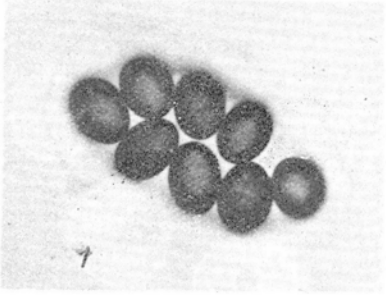

Fig. 1. A 0:8 ascus

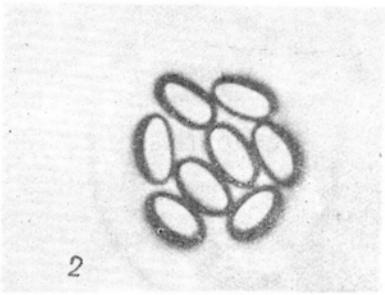

Fig. 2. A 8:0 ascus

It is seen, that only in crosses with the wild-type the results for common and leaky mutants are the same; in all other cases the results are different. As mentioned before, in crosses within a leaky mutant, "+" × "−", we observe 0:8 asci instead of 8:0; when a double mutant is crossed with a leaky single one, 4:4 asci instead of 8:0 are observed.

Table 1

Types of asci in crosses with colourless common and leaky mutants

	Mutant × mutant	"+" × "−" of the same mutant	Mutant × wild-type	Double mutant × wild-type	Double mutant × mutant
Common mutant	8:0, 6:2, 4:4	8:0	4:4	8:0, 6:2, 4:4	8:0
Leaky mutant	0:8, 6:2, 4:4	0:8	4:4	0:8, 6:2, 4:4	4:4

When one crosses two independent leaky mutants three types of asci are found, as it is the case with common ones, but, instead of 8:0 asci, 0:8 asci appear. The same holds for coupling, when a double leaky mutant is crossed with the wild-type.

Attention was paid to crosses between a common and a leaky mutant, where, as mentioned, only two types of asci appeared, namely 6:2 and 4:4, the latter in unusual high numbers (Fig. 3 and 4).

It turned out, that the 4:4 asci are a sum of PDs and NPDs. So in a half of such asci tested, instead of the expected recombinant ascospores, four double mutants and four wild-types, only parental ascospores were found. The four colourless ascospores represented the common parent, the four pigmented — the leaky one.

The above results show unambigously that mutants denoted as leaky have alternative phenotypic expression, as colourless mutants or as wild-types. Data of Table 1 furnish also information on the occurrence of both alternatives. It is evident, that, when in the ascus wild-type nuclei are present, regardless parental or recombinant, the phenotype of leaky mutants is colourless. When, however, wild-type nuclei are lacking, leaky mutants have the wild-type phenotype.

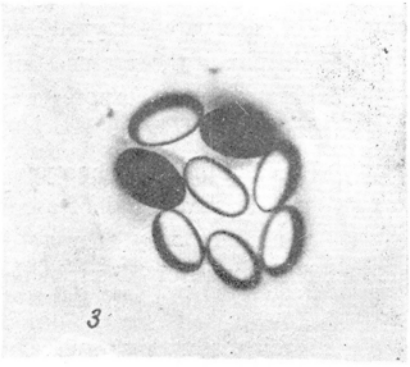

Fig. 3. A 6:2 ascus.

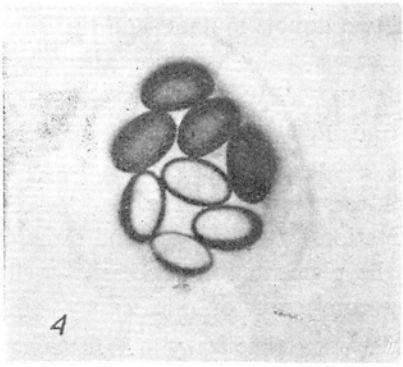

Fig. 4. A 4:4 ascus

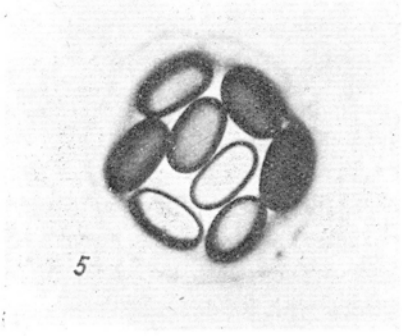

Fig. 5. A 2:6 ascus

Further studies are needed to be sure we have here to do with real dependence between the phenotypes of the leaky mutants and the genotypes of residual ascospores in the same ascus. If this dependence is assumed, it may be explained in the following way.

The unripe ascospores of *A. immersus* are colourless and become pigmented only short before reaching maturity. The pigment formation is probably complicated having different stages with precursor sustances. Wild-type ascospores do absorb pigment, while the common colourless mutants fail to perform this function. In leaky mutants the ability of pigment absorbtion is partially reduced; however, in the absence of wild-type ascospores the leaky ones become pigmented in spite of their reduced ability. In the presence of wild-type ascospores, the leaky ones are unable to compete for pigment and remain uncoloured.

Some additional observations confirm the assumption presented above. In crosses involving leaky mutants asci 8:0 as well as 2:6 incidentally appear. The first ones could not be analysed because they did not germinate. They were, obviously, unripe. However, some asci of the 2:6 type were analysed (Fig. 5). They

showed to be genotypically the same as the 6:2 asci, including two wild-type ascospores, two double mutants and two single mutants of each parental type. Evidently, in rare cases, leaky ascospores become pigmented in spite of the presence of wild-type ascospores. In all of these asci tested the leaky mutant ascospores were less intensively pigmented than the wild-type ones.

The existence of colourless leaky mutants with alternative phenotype offers some advantages in genetic work with *A. immersus*. As the period of ascospores pigmentation is well defined and rather short, leaky mutants may be useful in studying the pigmentation process as well as some other unresolved problems concerning conversion or odd-segregation.

At the same time leaky mutants suggest precaution in evaluating results obtained in tetrad analysis. Different unusual distribution patterns of PDs, Ts and NPDs may be due simply to leakiness. This may be of some importance, because, such mutants, arising quite frequently, are not easily distinguished. In our previous work, in spite of dealing with hundreds of colourless mutants, we have not found a single one: they were probably overlooked or discarded as "irregular", Recently, when adequate methods of testing were applied, already out of 83 tested, as much as 10 leaky mutants i.e. 12% were found.

Finally it may be mentioned, that the alternative phenotype of colourless leaky mutants ressembles, to some extent, the effect of incomplete penetrance. The phenomenon of penetrance, still poorly defined and understood, is among others, explained by the effect of different residual genotypes or "genetic background" of individuals. In our case we can also speak on the effect of the residual genotype considering an ascus with its different nuclei as one unit of phenotypic expression.

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Bezbarwne mutanty o alternatywnym fenotypie u Ascobolus immersus

Streszczenie

Wśród spontanicznych mutantów *Ascobolus immersus* o bezbarwnych askosporach wyróżniono specyficzny typ określany poniżej jako „leaky" w odróżnieniu od zwykłych. Mutanty te charakteryzuje alternatywny fenotyp: w jednych wypadkach dają askospory bezbarwne, w innych — typu dzikiego, brązowofioletowe. Przy krzyżowaniu z typem dzikim askospory ich są bezbarwne. Dwa mutanty leaky krzyżowane ze sobą dają T oraz NPD takie same, jak zwykłe dwa mutanty, ale PD zamiast worków o wszystkich askosporach bezbarwnych, 8:0, składa się z worków o askosporach zabarwionych, 0:8, Podwójne mutanty leaky mają fenotyp mutanta, krzyżowane z pojedynczym dają worki 4:4. Przejawianie się fenotypu można przewidzieć: gdy w worku znajdują się jądra typu dzikiego, bądź rodzicielskie, bądź rekombinacyjne, mutant leaky ma fenotyp bezbarwny. Gdy brak jąder typu dzikiego, askospory mutanta leaky są zabarwione. Można tu wskazywać na pewnego rodzaju współzawodnictwo o prekursor(y) pigmentu między jądrami tego samego worka. Zjawisko to w pewnym stopniu jest zbliżone do zjawiska penetracji genetycznej. Mutanty leaky mogą mieć znaczenie przy badaniach genetycznych nad *A. immersus*, w szczególności przy stosowaniu analizy tetrad.