tion strains, as far as the phage host specificity is concerned, was discussed by PILLICH et al.[9].

*Zusammenfassung.* Eine Reihe von Mutanten mit verschiedenen Phenotypen wurde nach einjähriger Lagerung des *Staphylococcus aureus* PS 80 isoliert. Sie unterscheiden sich in mehreren Merkmalen vom Wildtyp. Aus der Tetracyklin-empfindlichen Population der PS-80-Zellen wurden Tetracyklin-resistente Mutanten selektiert, die gleichzeitig resistent gegen Streptomycin, Chloramfenikol, Erythromycin, Cephaloridin, Lincomycin und Spiramycin sind.

J. SCHINDLER

*Department of Medical Microbiology and Immunology, Faculty of Medicine, Charles University, Praha–2 (Czechoslovakia), 22 December 1971.*

[9] J. PILLICH, M. KŘIVÁNKOVÁ, E. JANOVSKÁ, M. VIZDALOVÁ, Proc. IX. Congr. Czechoslovak Microbial Soc. (1971), p. 137.
[10] M. DEMEREC, E. A. ADELBERG, A. J. CLARK, P. E. HARTMAN, Genetics *54*, 61 (1966).

## A Quantitative Analysis of Cleistothecia Production in *Aspergillus nidulans*

Fungi are not frequently employed in studies of quantitative genetics inheritance. Being generally haploids they have a segregation pattern which is not amenable to the methods of quantitative genetics. However, during heterokaryon formation it is possible to suppose the existance of additive, dominant and heterotic effects of the genes. Such effects must bear similarities to those which occur in diploid organisms since in the heterokaryons there are 2 sets of genes, although in different nuclei. *Aspergillus nidulans* is a filamentous fungus which has been widely used in genetic research. It produces spherical fruiting bodies, the cleistothecia, 100 µm or more in diameter, which arise after 8–10 days incubation at 37°C. In the formation of cleistothecia, several cytoplasmic and nuclear factors are probably involved. Cleistothecia formation is irregular; certain strains produce only conidia, others are regularly sexual and finally others produce conidia and sporadically start to produce cleistothecia[1–5]. Since the variation in the number of cleistothecia produced in the same environmental conditions is due to hereditary factors, it is possible to estimate and to test through a diallel cross model, the general and specific combining abilities and to look for quantitative effects of the genes which take part in cleistothecia production in *A. nidulans*.

*Material and methods.* Minimal medium (MM) was Czapeck-Dox medium with 1% (w/v) glucose. Complete medium (CM) was a complex medium containing yeast extract, hydrolyzed casein, hydrolyzed nucleic acids, vitamins, etc[6]. Solid media contained 2% agar. The strains of *A. nidulans* all derived from Glasgow stocks, were kept at 5°C on CM slopes. They were purified at 6-month intervals by single colony isolation and auxanographic characterization. The following strains were used: Strain A: *y*, $nic_2$ $ribo_5$; strain B: *y*, $w_2$, $s_{12}$, $pyro_4$; strain C:

[1] M. MAHONEY and D. WILKIE, Proc. R. Soc. B. *148*, 359 (1958).
[2] F. ARLETT, Heredity *15*, 377 (1960).
[3] M. MAHONEY and D. WILKIE, Proc. R. Soc. B. *154*, 524 (1962).
[4] I. R. BARACHO, M. S. Thesis (University of São Paulo 1967), p. 57.
[5] I. R. BARACHO, Ph. D. Thesis (University of Campinas 1969), p. 63.
[6] G. PONTECORVO, J. A. ROPER, L. M. HEMMONS, K. D. MACDONALD and A. W. J. BUFTON, Adv. Genet. *5*, 141 (1953).

Table I. Mean frequencies of cleistothecia (per mm²) in the 15 crosses

| Strains | A | B | C | D | E | F |
|---|---|---|---|---|---|---|
| A | | 0.21 | 0.68 | 0.79 | 1.43 | 0.23 |
| B | | | 2.32 | 6.58 | 7.27 | 5.03 |
| C | | | | 2.21 | 1.47 | 2.19 |
| D | | | | | 2.43 | 1.12 |
| E | | | | | | 0.11 |

Table II. Analysis of variance for the general and specific combining abilities

| Source | D.F.[b] | M.S.[c] |
|---|---|---|
| General combining ability | 5 | 9.18[a] |
| Specific combining ability | 9 | 2.86[a] |
| Error | 14 | 0.41 |

[a] Significant at 1% level.
[b] Degrees of freedon
[c] Mean square

Table III. Estimates of the effects of the general combining ability for each strain

| Strain | Effects $(\hat{g}_i)$ |
|---|---|
| A | −2.00 |
| B | 2.51 |
| C | 0.62 |
| D | 0.44 |
| E | 0.33 |
| F | −0.67 |

Table IV. Estimates of the effects of the specific combining abilities for each strain

| Strains | Effects $\hat{s}_{ij}$ B | C | D | E | F |
|---|---|---|---|---|---|
| A | −2.57 | 1.03 | 0.08 | 0.82 | 0.63 |
| B | | −1.84 | 1.35 | 2.15 | 0.91 |
| C | | | 0.12 | −0.52 | 1.21 |
| D | | | | −0.62 | −0.92 |
| E | | | | | −1.83 |

*an*$_1$, *bi*$_1$; strain D: *bi*$_1$, *meth*$_1$, *fl*$_1$; strain E: *ad*$_{14}$, *y*, *co*; strain F: *su*$_1$*ad*$_{20}$, *paba*$_1$, *y*, *ad*$_{20}$, *Acr*, *lys*$_5$, *cha*. Mutant alleles in this study determined the phenotypes: *y*, yellow conidia; *w*$_2$ (epistatic to *y*/*y*+), white conidia; *cha*, chartreuse conidia; *co*, compact colonies; *fl*$_1$, fluffy mycelium; *ad*$_{14}$ and *ad*$_{20}$, *an*$_1$, *bi*$_1$, *lys*$_5$, *meth*$_1$, *nic*$_2$, *paba*$_1$, *pyro*$_4$, *ribo*$_5$, *s*$_{12}$, requirements, respectively, for adenine, aneurine, biotin, lysine, methionine, nicotinic acid, *p*-aminobenzoic acid, pyridoxine, riboflavine and thiosulfate; *Acr*$_1$, resistance to acriflavine; *su*$_1$*ad*$_{20}$, suppressor of adenine requirement caused by *ad*$_{20}$. Origin and location of the mutants can be found in BARRATT, JOHNSON and OGATA[7] and BALL and AZEVEDO[8]. General techniques were those of PONTECORVO et al[6]. The 6 strains were crossed in all possible combinations (15 crosses). After 2 days incubation the mycelial mat was transferred to dishes of solid MM (plus 0.02 µg/ml biotin for the cross C×D). After 28 days incubation at 37°C a counting of cleistothecia was carried out for each heterokaryon. The number of cleistothecia in 10 fields (7.2 mm diameter each) chosen at random in 4 heterokaryons, for each cross, was scored. For each cross 2 independent counts were performed. Statistical analysis of the data was carried out following the method suggested by GRIFFING[9]. The mathematical model in this is as follows: $x_{ij} = \mu + g_i + g_j + s_{ij} + \bar{e}_{ij}$ in which $\mu$ is the general mean, $g_i$ and $g_j$ the effects of the general combining ability, $s_{ij}$ the effect of the specific combining ability so that $s_{ij} = s_{ji}$ and $\bar{e}_{ij}$ is the exponential error from the observations of the order *ij*. The restrictions are $\Sigma g_i = 0$ and $\Sigma s_{ij} = 0$ (for each *j*).

*Results and discussion.* Table I gives the mean frequency of cleistothecia per mm² for each cross. From these data, the mean squares of the general and specific combining abilities and of the effects of the general combining ability for each cross were estimated (Tables II, III and IV). The application of the method of GRIFFING[9] to fungi seems viable in the case of heterokaryons since they present certain similarities with the diploid state[10]. The method assumes that when a set of inbred lines is used in a diallel crossing system, a genetic interpretation in terms of quantitative inheritance is made possible since the analysis is an analysis of the combining abilities of the gametes. Thus, the general properties of a diploid individual may be regarded as the combination of the genetic properties of the 2 gametes which united to form the individual. Therefore in the statistical analysis the genotypic effect of an individual may be considered as the summation of effects contributed by each gamete (that is, set of genes in the gamete) and the interaction of gametes (that is, interaction of the genes in one gamete with those in the other). Heterokaryons are not products of gamete fusion; they are, in fact, products of genes in each nucleus and, in the statistical analysis, the genotypic effect of a heterokaryon is the summation of effects given by each nucleus and interaction between such nuclei. The observed value of a given cross can be represented linearly by the estimates of combining abilities. The values of the general combining ability are an indication of the importance of the additive genic effects and the values of the specific combining abilities indicate the importance of the genic effects of dominance and epistasis[11]. The results obtained show that the genetic variation expressed by the amount of cleistothecia produced in different crosses is due, not only to the genic additive effects, but also to dominant and possibly epistatic effects (Table II). There is no doubt that dominance is a characteristic of some of the factors involved in the control of the character studied but other factors which do not show dominance are also present. The strains differed in their content of factors that increase the number of cleistothecia (Table III). Table IV gives the relative importance of the effects of the specific combining ability showing that certain pairs of strains have genetic constitutions that can complement each other.

Fungi present many advantages for genetic studies, including rapid growth, the large number of individuals which can be analyzed, and ease of handling and storage. Several characteristics presented by these organisms are suitable for quantitative genetic studies using the same process which was used in the present work. Many of the common industrial fungi readily form heterokaryons and diploids. The production of valuable substances such as antibiotics is directed by quantitative genes[12]. Thus quantitative genetic studies in industrial fungi can play an important role comparable with that achieved by these methods in plant and animal breeding.

*Resumen.* Cruzamiento dialélico entre seis cepas del hongo filamentoso *Aspergillus nidulans* fué analizado estadísticamente por el método sugerido por GRIFFING[9] visando establecer los valores de la capacidad general de combinacion y de la capacidad específica de combinación para la cantidad de cleistotécios. Los resultados obtenidos parecen indicar la participación de genes que exhiben effectos aditivos y dominantes. Es sugerido que el método puede ser aplicado en el analisis de otras características incluyendo aquellas de valor económico como producción de antibióticos.

I. R. BARACHO and J. L. AZEVEDO

*Department of Plants Genetics, University of Campinas, Campinas, São Paulo (Brazil) and Department of Genetics, University of São Paulo, Piracicaba, P.O. Box 83, São Paulo (Brazil),*
*24 December 1971.*

[7] R. W. BARRATT, G. B. JOHNSON and W. N. OGATA, Genetics *52*, 233 (1965).
[8] C. BALL and J. L. AZEVEDO, Aspergillus Newsl. *5*, 9 (1964).
[9] B. GRIFFING, Aust. J. biol. Sci. *9*, 463 (1956).
[10] I. R. BARACHO, R. VENCOVSKY and J. L. AZEVEDO, Trans. Br. mycol. Soc. *54*, 109 (1970).
[11] G. F. SPRAGUE and L. A. TATUM, J. Am. Soc. Agron. *34*, 923 (1942).
[12] G. SERMONTI, *Genetics of Antibiotic Producing Microorganisms* (Interscience, London 1969), p. 389.

## Lampbrush Chromosomes from Semi-Albino Crested Newts, *Triturus Cristatus Carnifex* (Laurenti)[1]

Semi-albinism, also known in amphibians as mutation 'yellow', does not affect the process of melanogenesis which takes place during embryonic and larval life, while it produces changes in melanin granules of pigmented cells at metamorphosis[2]. Some data concerning spermatogenesis of semi-albino crested newts were already available[2], whereas nothing was known so far on karyological aspects of oogenesis. For this reason we have carried out, on some of these mutants[3], a study of lampbrush chromosomes which, being 'high resolution chromosomes', had