# NOTES AND COMMENTS

## PHENOTYPIC LAG IN NEUROSPORA

G. W. GRIGG
*C.S.I.R.O. Animal Genetics Section, Zoology Department, Sydney University, N.S.W., Australia*

Received 21.ix.59

The study of heterocaryosis, where nuclei of more than one genotype share a common cytoplasm, has provided an insight into some of the mechanisms of nuclear and genic interaction (*e.g.* Beadle and Coonradt, 1944 ; Pontecorvo, 1946). A two component heterocaryon, represented as " *a*—*b* ", *a* and *b* being the genotypes of the component nuclear types, will usually have a wild type phenotype. The large, vegetative spores (macroconidia) will be of three types ; namely, those having only the " a " type of nucleus, those having only the " b " type of nucleus, and those having both types of nucleus. These latter heterocaryotic conidia will tend to resemble the parent culture. The microconidia, being uninucleate, will be of only the first two types. They will, however, receive their cytoplasm from the parental (heterocaryotic) culture so initially the cytoplasm of such microconidia may differ from that carried by plants of similar genotype derived from a homocaryotic culture.

This paper reports the aberrant behaviour of uninucleate microconidia derived from a heterocaryon which, having the nuclei of one component, behaved like the other component.

## 1. MATERIALS

The two strains from which the heterocaryon was synthesised were a histidine, macroconidial, non-colonial, albino one (*h* ; *col-1*$^+$ ; *m*$^+$*su-m*$^+$ ; *al-2*) and a prototrophic, macroconidial, colonial, non-albino one (*h*$^+$ ; *col-1* ; *m su-m* ; *al-2*$^+$). *m* is peach microconidial (*pe*$^m$) (Barratt and Garnjobst, 1949) ; *su-m* is a modifier of *m* such that although *col-1* ; *m su-m*$^+$ is microconidial *col-1* ; *m su-m* is macroconidial (Grigg, 1958*b*) ; *h* is the histidine gene *K26his-3* (Mathieson and Catcheside, 1955) ; *al-2* is an albino gene (Beadle and Tatum, 1945) ; and *col-1* is the Y8743 *colonial-1* gene of Barratt and Garnjobst (1949).

Despite the macroconidial nature of the two parental homocaryons the heterocaryon was microconidial and made a spreading growth on minimal medium. At the particular nuclear ratio obtaining, namely, 1 *h* to 15-30 *h*$^+$ nuclei, *h*$^+$ was " dominant " to *h*, *su-m*$^+$ was " dominant " to *su-m*, *al-2*$^+$ was " dominant " to *al-2*, but the mutant gene *m* was " dominant " to its wild type allele (*m*$^+$). Two phenotypic effects of *col-1*, namely, inhibition of macroconidia formation and restriction of growth rate, showed different dominance relations, the first being " dominant " to the wild type and the second being " recessive " (Grigg, 1958*b*).

The histidine strain will not grow on " complete " media presumably because histidine uptake is inhibited by other amino acids present (Mathieson and Catcheside, 1955). Heterocaryons grew well on rich conidiating complete medium (Horowitz, 1947), and produced approximately $10^8$ microconidia per slope.

## 2. RESULTS

In the course of an attempt to measure the nuclear ratio of the heterocaryon equal numbers of microconidia were plated (Grigg, 1958*a*, *b*) into a minimal medium and a similar medium supplemented with histidine (30γ/ml.). The method proved useless and gave an erroneous estimate of the nuclear ratio (Grigg, 1958*a*). Only one histidine colony was observed among over 6500 colonies which appeared on the supplemented medium. The others had the phenotype of the $h^+$ ; *col-1* ; *m su-m* ; *al-2*$^+$ strain. However, the numbers of these colonies which appeared on the supplemented medium was very different from those which appeared on the minimal medium. There was a considerable deficit on the minimal plates. Following this observation the plating experiment was repeated twelve times using fresh media each time. Similar results were obtained on each occasion. The data of a typical experiment are shown in table 1. Microconidia from a homocaryotic $h^+$ strain germinated equally well on the

TABLE 1

*Mean number of colonies per Petri plate of medium following the plating of equal numbers of microconidia on minimal and on histidine supplemented medium. The values are the means of four replicates*

| Source of microconidia | Media | |
|---|---|---|
| | Minimal | Histidine |
| $h^+$—$h$ heterocaryon . | 6·5±2·3 | 46·5±3·0 |
| $h^+$ homocaryon . . . | 100·0±7·0 | 104·3±2·6 |

minimal and supplemented media, but microconidia from the heterocaryon germinated much better on histidine medium. In the thirteen plating experiments the number of + ; *col-1* ; *m su-m* ; + colonies which appeared on minimal medium ranged from one half to one twenty-fifth of the number observed on the supplemented medium. In some experiments a layer of histidine medium was poured over the minimal medium in which conidia had been plated four days previously. Additional colonies appeared on these double layer plates so that the final numbers equalled those on the supplemented plates. Those colonies which were visible at the time when the second layer of medium was poured over them had not commenced to conidiate so the additional colonies cannot be ascribed to the presence of pre-existing colonies. We infer that they arose from previously ungerminated spores.

Germination of the microconidia from the heterocaryon was as good on complete (Horowitz, 1947, conidiating complete) as on histidine medium (table 2). The equivalence of conidiating complete to histidine medium in aiding germination of these conidia may be contrasted with its failure, although it contains histidine, to support growth of the K26 strain.

Addition of growth factors other than histidine did not increase the number of colonies which appeared on minimal medium. It is concluded that the effect is specific to histidine.

The colony counts on minimal medium have a higher coefficient of variation than those of the " histidine " and " complete " groups. On the other hand the colony counts on the minimal plates containing microconidia from an $h^+$ had a coefficient of variation comparable to those of the supplemented groups. The variance tended to be high in all cases where there was a deficiency of colonies.

The requirements for histidine which a proportion of the conidia exhibited was a transient phenomenon and was limited to their initial germination. Once germinated the conidia grew at the $h^+$ rate. The colonies grown from these microconidia produced normal $h^+$ conidia as judged by the way in which samples from 20 colonies germinated equally well on minimal and histidine supplemented medium.

TABLE 2

*Number of colonies per plate of medium following the addition of equal numbers of microconidia to minimal, histidine supplemented and complete media*

| Medium | | |
|---|---|---|
| Minimal | Histidine supplemented | Complete |
| 69 | 166 | 140 |
| 62 | 143 | 156 |
| 14 | 139 | 142 |
| 73 | 157 | 164 |
| $\bar{x}$ 54·5±13·7 | 151·25±6·3 | 150·5±5·7 |
| $v$ 562·25 | 117·5 | 99·0 |

The uninucleate nature of microconidia reported by Barratt and Garnjobst (1949) was confirmed by examination of microconidia stained according to Huebschman (1952).

## 3. DISCUSSION

The observation that germination of $h^+$ microconidia from $h^+$—$h$ heterocaryons is lower on minimal than on supplemented medium indicates that some factors are in low concentration or are entirely lacking in the cytoplasm of the freshly formed microconidium. To initiate synthesis of these factors by the $h^+$ nuclei, histidine must be supplied. We may conclude from this that somewhere along the synthetic pathway of the histidine factors there occurs an unbalanced or an irreversible reaction. If all the reactions along this pathway were completely reversible the depletion or absence of the histidine factors in the cytoplasm would change the relative reaction rates of reactants and products to result in increased histidine synthesis.

An alternative explanation is that some cytoplasmic factors, which require the presence of the $h$ nuclei for their continued production, are carried over into the freshly formed conidia. These factors might reduce the ability of the conidia to germinate on the minimal medium. This seems less likely than the first alternative.

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A phenomenon similar to this one in some respects involved the germination of macroconidia from some macroconidial heterocaryons (Grigg, 1958*b*). The histidine component in each of the macroconidial heterocaryons was the same (K26) as that in the microconidial heterocaryons. In a macroconidial heterocaryon in which the nuclear ratio in the culture of $h : +$ was 2 : 1 the heterocaryotic conidia germinated more slowly on minimal than on histidine medium. On the other hand when the nuclear ratio was 1 : 2 the macroconidia germinated equally well on both.

Both the microconidial and the macroconidial heterocaryons grew well on minimal or on complete medium. For reasons previously mentioned the histidine component of the heterocaryons does not grow on complete media despite the latter's content of histidine. It is perhaps puzzling then that complete medium aided the germination of the microconidia from the heterocaryon just as well as did histidine. Recently some partial back-mutants at the same histidineless locus have been found which show a similar pattern of growth requirements in that the growth rates are increased if either histidine or complete medium is added.

The observation that the percentage germination of partial revertants can be lower on minimal medium than on medium containing appropriate growth substances has importance in the planning and interpretation of mutation experiments. Any growth promoting substances present are released if conidia are killed (Grigg, 1958*a*). Mutagens may be often highly toxic and so the chance of pre-existing back-mutant nuclei germinating and being detected as colonies would be higher in the mutagen treated plates than in the control ones. As a result it might be difficult to decide whether the wild type colonies which arose following treatment of the conidia reflected the mutagenicity of the treatment or merely its toxicity.

## 4. REFERENCES

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