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A S P E R G I L L U S N E W S L E T T E R

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Dear Colleague,

This News Letter has now survived as far as the third number. Some deficiencies are already conspicuous. For instance, it would be useful, in the next number, to bring the list of publications up to date. Any other suggestions for improvement would be welcomed.

Once again I apologise for the inevitable errors. I should appreciate a note of gross errors or omissions which will be corrected in future numbers.

Yours sincerely,

J. A. ROPER.

PUBLICATIONS

E. Käfer-Boothroyd: The processes of spontaneous segregation in vegetative nuclei of Aspergillus nidulans (Genetics, in press).

A. Tector (1961): Genetic analysis of some radiation effects in diploid Aspergillus nidulans. (M.Sc. Thesis, McGill, Montreal).

PRELIMINARY NOTES ON CURRENT RESEARCH

C. TAKEICHI.

New heterokaryons containing haploid and diploid nuclei

Several unstable brown and albino strains were found among heterokaryons between yellow and albino auxothophs of Aspergillus sojae. The frequencies of occurrence of these strains differed according to the component strains employed. Their properties are as follows:

1) Growth on minimal medium is normal. Therefore, they are prototrophic.

2) On complete medium, they form brown or albino colonies with, occasionally, green and yellow sectors.

3) Opon conidiation, the brown strains segregate brown, yellow, and green progeny. On the other hand, the albino strains segregate albino and green progeny.

The green segregants are prototrophic diploid, as judged from the cytological study and the genetic stability. The yellow and albino segregants are haploid; their nutrient requirements are the same as the parental haploid strains.

On this basis, the brown and albino strains seem to be a kind of heterokaryon possessing haploid and diploid nuclei.

Z.A. KWIATKOWSKI.

Mycelial mutants in A. nidulans

Three strains of A. nidulans originating on complete medium showing mycelial morphology were isolated. All the variants failed to produce normal conidia. They are stable in culture. When crossed with normal type, the segregation 1 : 1 was observed. In diploids, mycelial character was recessive. Because of close resemblance to Roper's "mycelials" the mutant alleles were designated : m4, m5, and M6 respectively.

When heterokaryons between normal and mycelial strains were prepared, the mycelial determinant was transmitted from mycelial to normal. The recombinant association of m4 with the original markers of the normal partner was found in about 20% of the conidia isolated from the heterokaryon. It is postulated that in the described case, unlike in Roper's m1, m2 and m3 strains, a single nuclear gene is involved. On this gene some cytoplasmic determinant is dependent.

E. KÄFER-BOOTHROYD

Translocations: Several translocations have turned up in recombinant strains from crosses with Glasgow strains (publ. Adv. Genet. 1958). Two of these, a VI-VII and a I-VIII translocation, are being analysed and tracked down through the pedigree (in addition to T(I-VII) of the original cho-bi1-strain). T(I-VIII) involves the right arm of linkage group I: diploids T(I-VIII) /+ and y/+ do not produce y/y heads. It becomes evident that all multiple marker stocks of A. nidulans have translocations somewhere in the pedigree. Whether the "standard" chromosome complement is present in a strain, is being determined by checking against 8th generation back cross-strains.

The wildtype strain: The fluffyness of the original Glasgow wildtype strain

has turned out to be dominant in diploids and it appears likely that a recessive mutation occurred in one of the early strains, possibly the original bi1-strain. This mutant has been named "velvet", ve and is located in linkage group VIII. Since the majority of strains in use carry ve, it is suggested that the strains carrying the wildtype allele should be marked $+^{ve}$ (or Ve). The question of the origin of ve and its spread through the pedigree, especially the very first crosses, is being analysed.

G. A. VAN ARKEL

A new colour mutant: "pale".

A useful spontaneous conidial colour mutant ("pale") has been isolated from the Glasgow strain an 1 bi 1. Young p ; y and p ; Y colonies are light greyish-yellow (ecru) and light green respectively. On continued incubation colonies turn slightly brownish, making p ; w 3 and w 3 phenotypically distinguishable. Growing on CM (but not on MM) the cultures normally bear tiny brown droplets on top of the rather small and densely packed conidial heads.

The recessive mutation p has been located on linkage group V, but is not meiotically linked to lys 5, the RF's found in two crosses being 50.5 and 42.1 per cent. p can usefully replace lys 5 in those cases where poor sporulation and low viability of the latter marker is a disadvantage.

R. RITA ARDITTI and P. STRIGINI

On the origin of ascospores with diploid nuclei in Aspergillus Nidulans

The occurrence of rare diploid colonies after plating ascospores from crossed perithecia of Aspergillus nidulans has been reported by Pritchard (1954). He proposed three possible mechanisms by which these diploid colonies could arise: (1) failure of meiosis; (2) failure of separation of products of the a) first, or b) second meiotic division and (3) fusion of haploid products of meiosis.

The meiotic frequency of recombination in the chromosomes of the diploid ascospores and the non-complementarity of homologous chromosomes indicate conclusively that these chromosomes paired normally at a four strand stage. This rules out Pritchard's hypothesis (1) i.e., complete failure of meiosis. The diploid condition could result either from a failure of segregation of the centromeres in the first meiotic division or from a failure of separation of the sister chromatids in the second meiotic division (non-disjunction) (Pritchard's hypothesis (2)).

The fact that the fr-1 marker, closely linked to the centromere of chromosome IV, is always found in heterozygous condition is in favour of the first mechanism. Furthermore the markers linked to the centromere appear only occasionally in the homozygous condition.

Random fusion of the products of meiosis (Pritchard's hypothesis (3)) can be discarded essentially because it would result in a certain proportion (1/6) of diploids with fr-1 in homozygous condition.

The phenotypic analysis of the diploid ascospores may provide a method for the location of the centromeres in Aspergillus nidulans.

P. STRIGINI

On the effects of incorporated P^{32} in conidia of Aspergillus nidulans

The effects of P^{32} decay in conidia of A. nidulans were studied by the "suicide" technique, first applied by Hershey et al. (1951) to phage.

Survival curves proved logarithmic with haploid conidia. With diploid ones the logarithmic portion generally came after an interval in which no lethality was observed, as in multi-hit curves. Nuclear damage probably accumulated in the viable diploid conidia, as was shown by the appearance of dwarf colonies which spontaneously segregate normal haploid sectors. The frequency of these "aneuploids" among surviving nuclei increased with the decay.

The slope of the logarithmic portion of the survival curves was roughly proportional to the S.A. (specific activity of P); with diploids it was generally greater than with haploids.

Mutation frequency (resistance to 8-azaguanine with haploid conidia) increased as an exponential function of decay.

Lethal and mutagenic effects of external radiation (from non-assimilated P^{32} were also assayed. Lethal efficiency per eV absorved into conidia was calculated for external and internal P^{32}, on the hypothesis of no effect other than that of radiation: this would lead to the assumption that internal radiation should be 30 times more effective than the external. If one ascribes this difference to atomic transmutation $P^{32} \rightarrow S^{32}$ occurring in some vital structure - such as nucleic acids, or possibly DNA only - sensitive to P^{32} decay, the estimated lethal effeciency per disintegration further increases, by excluding some fraction of P.

Moreover, the mutagenic efficiency of incorporated P^{32} is many times higher than that of β or X rays, at the same lethality level.

C. F. ROBERTS

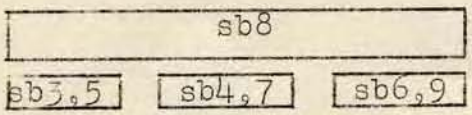
A difference in the complementarity of pairs of mutants when combined in heterokaryons or heterozygous diploids

The observations described in this article concern seven sorbitol mutants of *A. nidulans* isolated independently following U.V. irradiation, two (*sb3* and *sb5*) are total mutants which fail to grow with d-sorbitol as sole carbon source while the remaining five partial mutants (*sb4*, *sb6*, *sb7*, *sb8*, *sb9*) form characteristic sparse poorly conidiate colonies. Three of the mutants (*sb3*, *sb4*, *sb9*) have been located in linkage group VI by mitotic haploidisation but both *sb3* and *sb4* recombine freely at meiosis with all three group VI markers tested (*s3*, *lacI*, *niclO*). Reversion tests using conidia fail due to heavy background growth. In testing the mutants for their functional relationships the unusual result was observed that although certain pairs of mutants complement in heterozygous diploids all the mutants are non-complementary when tested in heterokaryons.

1. Qualitative observations.

Balanced heterokaryons were synthesized between the mutants taken in all possible pairs using strains of the type *biI;w3;sb* and *adl4pabaly;sb* and the corresponding series of heterozygous diploids selected. In both cases selection was done on MM containing glucose as carbon source and on which all the heterokaryons and diploids grow equally well. The test medium was MM in which glucose was replaced by sorbitol.

When the diploids are tested for their growth on sorbitol three pairs of mutants are found to be non-complementary (*sb3* and *sb5*, *sb4* and *sb7*, *sb6* and *sb9*) but complement in all other combinations, while *sb8* is non-complementary to all the other mutants. This result is described by a primitive complementation map



The heterokaryons were tested by transfering actively growing heterokaryotic mycelium from MM to the test medium. They all yield mutant phenotypes. Supplementing the sorbitol medium with nutrients required by the component strains, either altogether or in various combinations, did not affect the growth of the mycelium appreciably and only the mutant type was seen. Variation of the incubation temperature was also without effect. The mycelium maintained partial growth for three serial sub-cultures on unsupplemented medium without any apparent change but always immediately yielded vigorously growing heterokaryons on return to MM.

The last two results indicate that the mycelium remains heterokaryotic during its growth on unsupplemented sorbitol medium and that the nuclear balance suitable for nutritional independence persists. It may be argued that the mutants fail to complement because this balance cannot be modified to that required for complementary growth but this seems unlikely for two reasons. One is that addition of the nutrients required by the component strains does not alter the result although a change in nuclear balance could occur. The second is that precisely the same results have been obtained in a second series of experiments in which the mutants were combined with different nutritional markers (see Fig. 1).

2. Quantitative observations.

It was desirable to have a quantitative method to measure the growth of the heterokaryons and diploids. It is characteristic of Aspergillus growing on a limiting medium that the density of the mycelium is reduced and this suggested the following technique.

Measurement of the growth of colonies on solid media.

Colonies were grown on thick agar in large petri-dishes (15 cm. diameter) until growth was complete. Discs were then cut from the

plate with a cork borer and the agar dissolved by autoclaving them separately at 10 lbs./10 min. in about 20 ml. of distilled water. The discs of mycelium remained intact, they were washed in hot water and dried for at least 18 hr. at 105°C on microscope slides. The mass of each was determined by weighing the slide on a micro-balance and removing the discs with a razor blade. Generally eight discs were cut for each colony.

The validity of the technique is demonstrated in two ways. When different sized discs are cut from the same colony it is found that the mass is strictly proportional to the area of the disc. Duplicates are in close agreement and measuring one set of heterokaryons on different occasions yielded very similar results.

The technique was applied to two series of heterokaryons and diploids synthesized between strains of the type ribolprolbil;w3;sb and adl4pabaly;sb. The results are given in Fig. 1 and show that although the mutants yield less growth than the controls (sb^+/sb^+ etc.) the growth of all the heterokaryons is equivalent, whereas the diploids clearly separate into two groups which correspond to complementary and non-complementary mutants.

The method has also been applied to the same series of heterokaryons and diploids grown on standard MM. The controls yield about three times the weight of mycelium that they do on sorbitol and the combinations of mutants all yield values within the range of 80 - 120% of the controls.

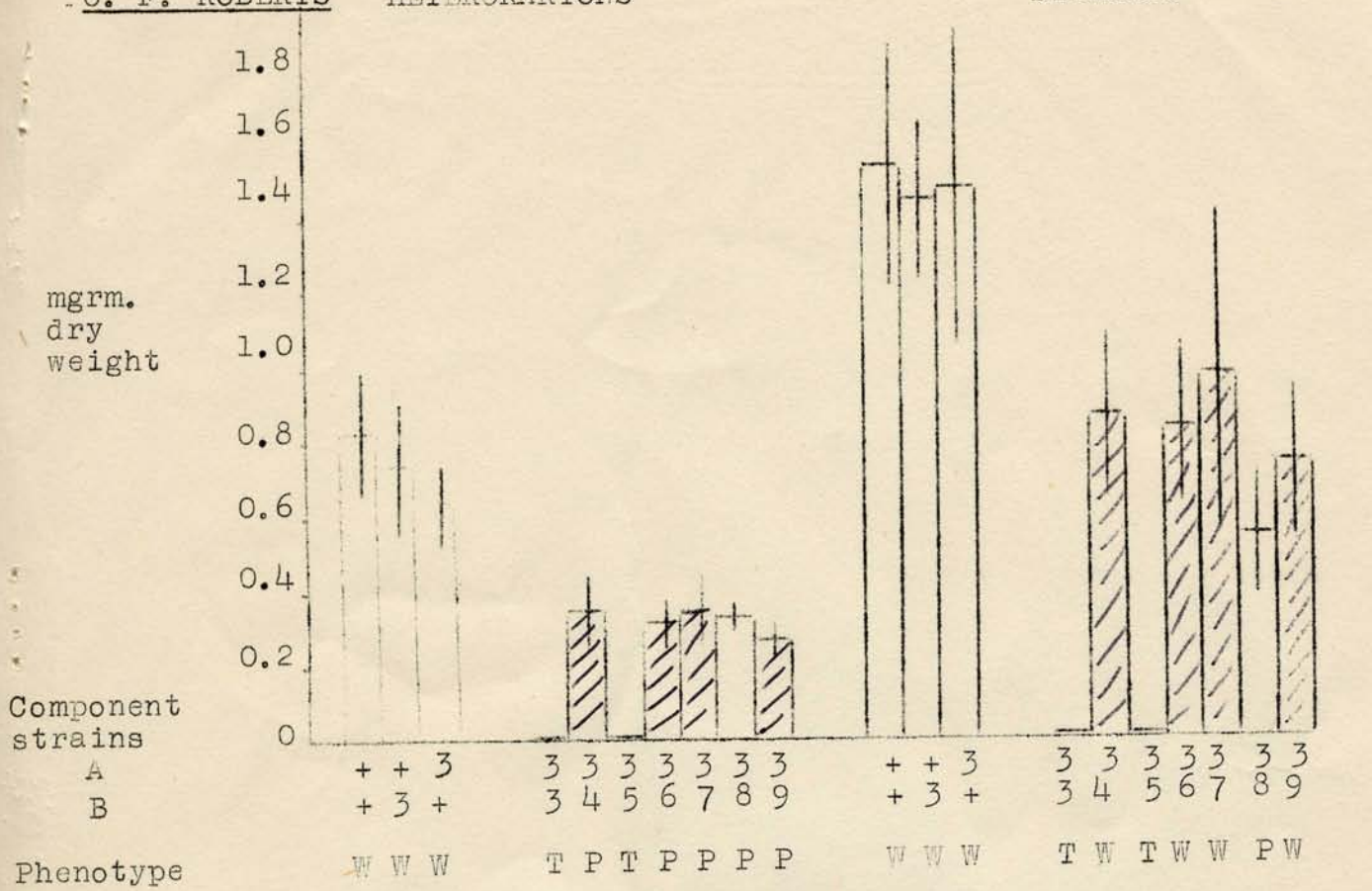
3. Comments.

The possibility that differences in function may occur when genes are combined in heterokaryotic or heterozygotic association was predicted by Pontecorvo in 1952. Differences have been described in Ustilago in the pathogenicity and nutritional independence of dikaryons and diploids (Holliday, 1961), and also between pan. 2 mutants of Neurospora combined in heterokaryons or pseudowilds (Case & Giles, 1960). In the present case the correspondence of the qualitative and quantitative data and the consistency with which the phenomenon can be repeated lead one to believe that a similar difference has also been found in Aspergillus.

The difference in growth of the heterokaryons and diploids is most readily intelligible as a result of a difference in enzyme formation in the two systems and this is the current working hypothesis. It has been found that the oxidation of sorbitol by whole cells involves an inducible enzyme system but to date all attempts to demonstrate the enzymes have been dismally unsuccessful and no dehydrogenation of either sorbitol or sorbitol-6-phosphate can be detected in cell-free extracts prepared by a variety of procedures.

On the genetic side it is clearly necessary to decide between the alternatives that the mutants represent either one locus or three linked loci spanned by a deletion. The lack of a linked marker is unfortunate (I will be very grateful for information on new markers in Group VI) but crosses are being analysed to test for recombination between the mutants. Further sorbitol mutants are also being isolated in the hope that the complemation map may be extended.

C. F. ROBERTS HETEROKARYONS DIPLOIDS



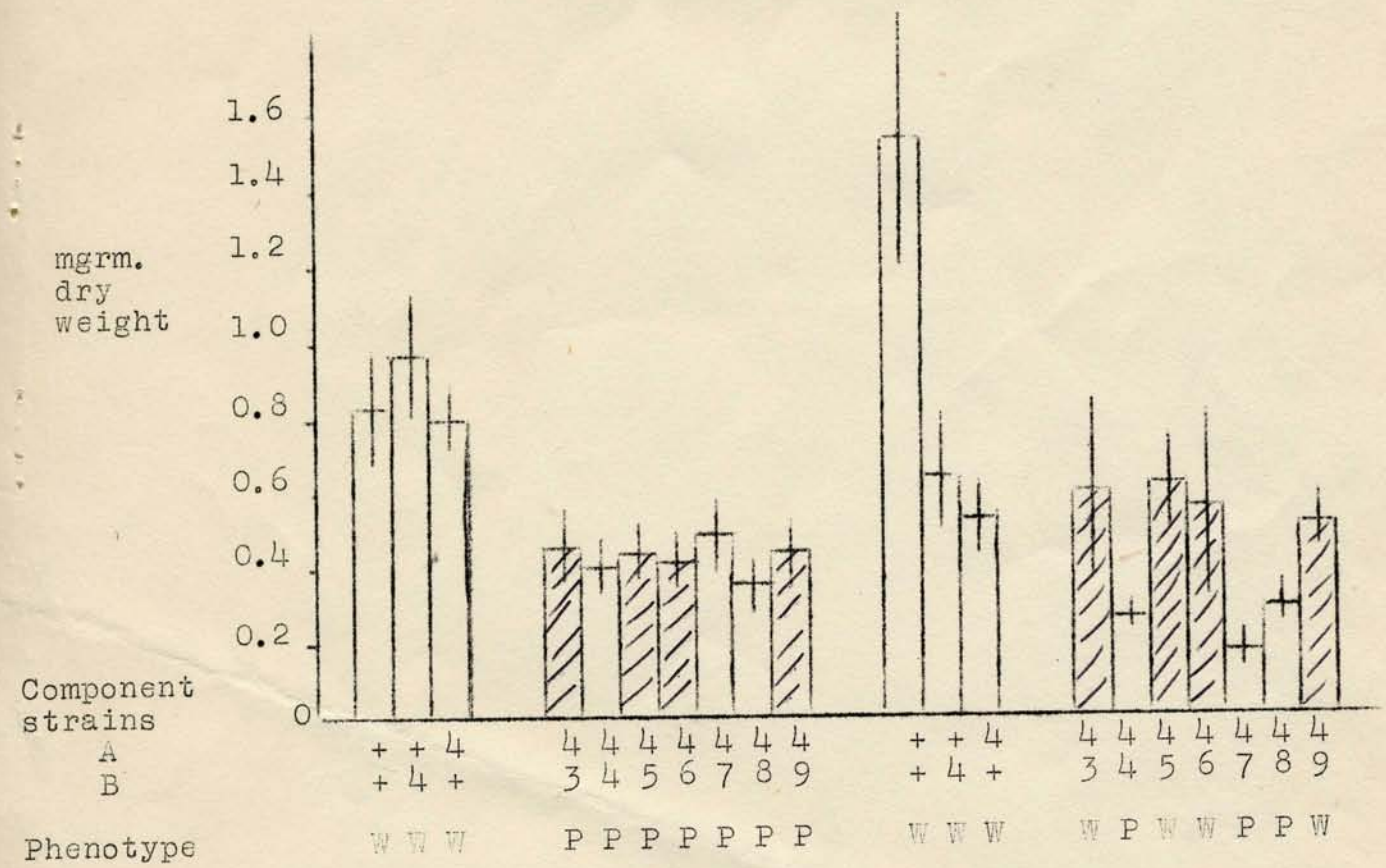


FIGURE I

The heterokaryons were grown on unsupplemented sorbitol for 7 days at 37°C and the diploids for 5 days. The histograms represent the mean dry weights of discs of mycelium cut from three different colonies of each organism and the verticle lines the standard deviations. The phenotypes of the different combinations are indicated, W = wild type, T = total mutant and P = partial mutant. Combinations which are complementary in diploids are cross-hatched.

Component strain A = ribolprolbil;w3;sb
B = adl4pabaly;sb