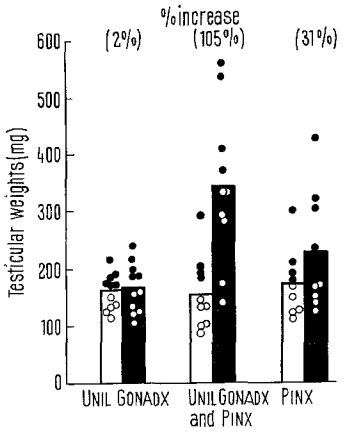

Influence of pinealectomy on compensatory testicular enlargement in blinded adult hamsters. Height of hollow bars represents mean weight of the original testis (taken at the beginning of the experiment). Height of solid bar represents mean weight of remaining testis (taken at the conclusion of the experiment). Each point represents the weight of a single testis. Mean per cent increase in testis weight is in parentheses at top. UNIL GONADX, unilateral gonadectomy; PINX, pinealectomy.

substances also curtails compensatory growth of the gonads[4-6]. The fact that pinealectomy alone did not stimulate a significant regrowth of the testes within 3 weeks confirms earlier findings; usually about 4 weeks is required before the testes of blinded hamsters will show evidence of regeneration after pinealectomy[9]. Presumably, the pineal gland of blinded hamsters secretes a substance or substances which act on the neuroendocrine axis to restrict gonadotropin synthesis or secretion[10].

Zusammenfassung. Halbseitige Kastration führt bei geblendeten Goldhamstermännchen mit Atrophie der Geschlechtsorgane nur nach Pinealektomie zu kompensatorischer Hodenhypertrophie.

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A Nutritional Method for the Isolation of Morphological Mutants of *Neurospora crassa*

In spite of the acknowledged importance of morphological mutants in understanding developmental processes, no method exists for their systematic isolation. They have been observed from time to time in *Neurospora*, an organism in which they should prove particularly useful due to its extensive use for genetic and biochemical studies. We have found a method, based largely upon the filtration enrichment technique of CATCHESIDE[1] (as later modified[2]) for the isolation of large numbers of morphological mutants. This came from the earlier observation that many known morphological mutants of *Neurospora* fail to grow, or exhibit a lag when sodium acetate in combination with glutamate or aspartate replace the usual sucrose and potassium nitrate of minimal medium. Wild type strains, on the other hand, grow on this combination, thus allowing a method for selection of morphological mutants.

The enrichment technique is based on the elimination of germinated conidia by means of successive filtrations through gauze filters which will pass conidia. Thus conidia

Growth response of several known morphological mutants to the selective media used for concentrating unknown mutants as described in the text

Locus	Allele isolate No.	Linkage group	FGSC[a] No.	Growth response Acetate + glutamate	Acetate + aspartate
Amycelial	K422	I	305	—	+
Biscuit	B6	V	277	—	—
Plug	B118	V	96	—	+
Colonial-4	70007	IV	67	—	—
Fluffy	L	II	45	—	+
Spray	B132	V	68	—	—
Frost	B110	I	103	—	—
Dapple	R2375	II	1077	+	—
Doily	LD-55-51	VII	177	+	—
Carpet	P564	II	292	—	—
Medusa	R2401	IV	1403	—	—
Crisp	B122	I	804	+	+
Colonial-2	Y5331	VII	172	+	+
STA_4	(wild type)	–	262	+	+
LIND +	(wild type)	–	853	+	+

[a] FGSC-Fungal Genetics Stock Center, Dartmouth College, Hanover (New Hampshire, USA).

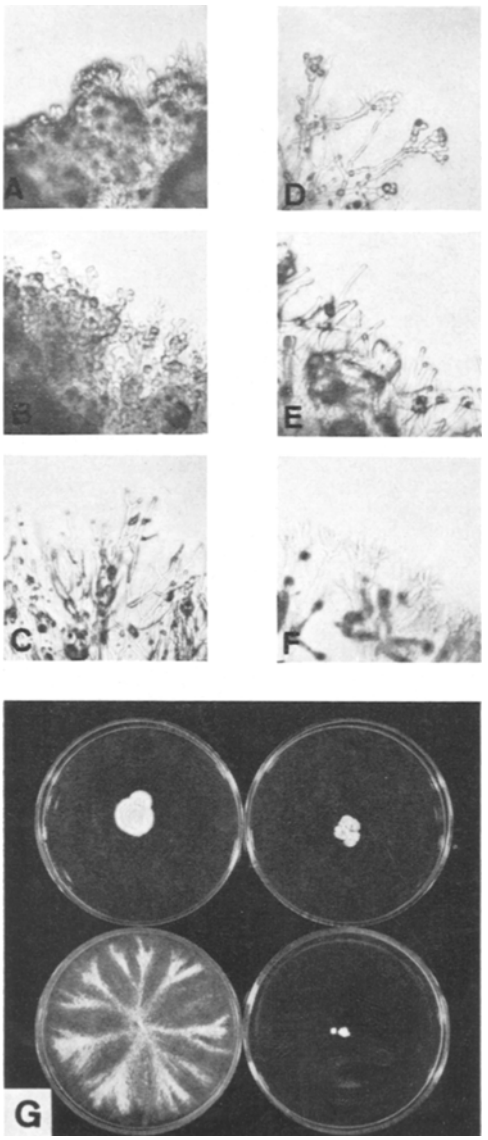
which grow on a particular selective medium are filtered out and mutants unable to grow are enriched. In our procedure a suspension of wild type conidia (STA4 is used here) containing approximately 10^7 cells per ml is irradiated with UV-light to produce 85–90% kill. After a 35 min period in the dark[3], 5 ml of the suspension are inoculated into an aeration tube containing 40 ml WESTERGAARD and MITCHELL minimal medium[4] with sodium acetate (2%) and either glutamic or aspartic acid ($10^{-2}M$) as sole sources of carbon and nitrogen respectively. The pH is adjusted to 6.0. Large test tubes 27×200 mm are used for incubation as well as for subsequent filtrations. The incubation mixture is aerated in these tubes with wet, sterile air through a disposable glass transfer pipette held in place with cotton. The filters packed with sufficient gauze to filter mycelia are stored in cotton plugs in the large test tubes. The used filter is removed from the tube and the pipette with its cotton plug is inserted to complete the new aeration tube. Incubation is carried out at 25 °C with filtrations being made at approximately 12, 18, and 24 h, and then a 12–24 h interval (depending on growth) to a total incubation time of 72 h.

After 72 h the ungerminated conidia are concentrated either by filtration or centrifugation and resuspended in the 2–3 ml sterile, distilled water. All of the cells are then spread on 10–15 petri plates containing solid WESTERGAARD and MITCHELL minimal medium.

The identification and isolation of the morphological mutants is carried out with a dissecting microscope as soon as possible, ordinarily 24–36 h after plating. This is necessary to avoid overgrowth by wild type cells surviving the filtrations. Alternatively it is possible to plate the cells on minimal sorbose agar plates but recognition of the mutant colonies under these conditions is more difficult. An average run yields approximately 20 mutants.

The Figure shows representative isolates that were obtained with this method. Interestingly, no single class or type of morphological mutants seems to be favoured. This was somewhat expected, however, since a wide variety of known morphological mutants also fail to grow in the selective conditions as shown in the Table.

Of the underlying biochemical basis for these growth responses very little is known. Preliminary experiments using the morphological strains 'amycelial' and 'biscuit' have shown, however, that malate or α-ketoglutarate ($10^{-2}M$) will alleviate the inhibition seen on acetate-glutamate plates. Accompanying this growth response, moreover, there is an induction of conidiation and other morphological alterations not typically seen in either of these strains but reminiscent of similar morphological modifications reported earlier using amino acids or certain combinations of Krebs cycle intermediates[5]. These results again stress the importance of oxidative metabolism for the maintenance of normal morphology in *Neurospora* and suggest a new method of grouping morphological mutants that may be helpful for future work.



A–C, unmapped morphological mutants obtained as described in the text when $10^{-2}M$ aspartate was present as the sole nitrogen source. D–F, same but with glutamate ($10^{-2}M$) as nitrogen source. G, selected isolates after 4 days growth on WESTERGAARD and MITCHELL[4] minimal medium; lower left, a 'snow-flake' colony.

Résumé. Une méthode sélective d'isolement de mutants morphologiques de *Neurospora crassa*, basée sur leur incapacité d'utiliser l'acétate et glutamate (ou l'aspartate) comme seules sources de C et d'N, est proposée.

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