7/5/45. — [commonplace notes. See refs to sex, crossing. 2/98]

X a. Asmedical Monilias, now in Fungi imperfecti, test for sex by crossing different "species". What is a sp. in an organism that cannot be hybridized? Consider range of genic variation in Dm, Nc or Hs.!!

[Drosophila, Neurospora, Homo]

✓ b. For variability in N. crassa adaptants, plate out microconidia in "Fluffy" strains.

[lysine being used] ✓ c. For limiting growth to produce "resting" N.c., use a deficiency mutant, e.g. pantothenic acid in a medium containing limiting amounts.

d. For stats. in adaptation, adapt leucineless by keeping varying lengths at lighting, then being to see when adaptation does occur.

Pab inhibitor ?? e. Feature of solid media accelerating in pab! adaptation.

X f. Quick means of sexing Neurospora?

anthranilic acid. g. Yellow pigment produced by 10575 (tryptophaneless) when adapted.

Kohn et al. – Induction of a methionine requirement. Is this selection of a mutant? Does selection require both sulfanilamide + methionine? Aminal culture of methionineless and parent E. coli of Kohn's strain should be possible by such selection. Speeded by single cell isolation. Are both factors necessary both for mutation + selection. Alternate transfers in normal + methionine-sulfanilamide medium.

In N. tetrasperma, a deficiency mutant can be introduced by crossing ♀ crassa + backcrossing successively. Then regular bicarya are produced. Purification by selection would be indicated by a failure to produce perithecia spontaneously.

Sex det'd by 2 genes? in basidiomycetes?

Relationships between color and pot-less?

Methionineless in Neurospora.

Valine-Isoleucine, separate deficiencies?

deficiencies not linked

If adaptation can be prevented, diplophase in bacteria can be selected for by using two different mutant strains of E coli and growing in continuously renewed minimal medium. Zygmeal, a sexual process could be demonstrated by plating out mixed cultures + finding a wild. Do all strains adapt? Transformation?

(✓ heat lysing)

Are genes determining type specificity in pneumococcus alleles? Meaning of allelism in bacteria.

Production of recognized mutations – use E. coli strains, absorbing lysed cells over deficiency anti-sera, and immunizing against purified antigen. These antisera should then induce mutation.

(Kabat thinks impracticable)

Does adaptation occur w/ selection? To a 1633-5531 mutant make pant. limiting. When growth is complete to an intermediate level, wash mycelium by removal of soln c̄ sterile pipette + replace c̄ min. Fries. After various periods, add soln pant sterilely. If adaptation has taken place, it should grow immediately. Or, if a pab level that does not cause de-adaptation can be found, washing is unnecessary.

(See Ec-lys expt.)

(Intracaryal selection.)

One can also transfer + wash 1633 mycelium in buffer c̄ sucrose + determ adapt. by adding nutrient.

In bacteria, a selection hypothesis for dissociation could always be tested by recovery of the dissociant form a mixed culture under the conditions of dissociation, in a sign. shorter time than normal dissociation. In Neurospora one can mark the adapted component, combine c̄ another marked, and attempt recovery.

Emerson '45 describes an adaptation which looks much like mine — heterocaryotic. It is genetic, he seems to disregard pab production as a factor! Also selection. Why is this heterocaryon not selected for??

Assay for pab: anti sulfathiazole activity in presence of excess methionine, etc. (v. Snell)

Ask Beadle for his recurrence of 1633

Allelism in N. crassa: irradiate 1633 to non-adaptability. Possible alleles: non-adapting; partially adapted stable + unstable. (Compare a, a^s, a^{bs}, a^p ... A series in Maize interacting c̄ Dt.) Here, quantitative. Here a 1633^p would gradually increase

Compare mutability of 8829, 3375 7. at 25°, 30°. The former is probably lighter.

PAB-synthesis is a limiting reaction in 1633 d strains. Temperature studies? Compare 1633^2 ± 1633 + enough pab to give same growth rate at 25°. Penetration – temperature? Nondissociation + temperature compare p 111?
Growth tubes + liquid: 30° – 25° ?!!

Review types of pabless adaptations!

By bacterial reversion, estimate micropopulation size by sectioning + staining agar.

Examine Beadles genetic data on 16117. Note:
21 asci = 168 spores from 16117 × 15300a.
all asci were 1:1 but a total of 517 spores, including these and random isolates were 273 (–) : 244 (+).
Comparing random isolate data ± expectation of 1:1 $\chi^2 \doteq 2$. that they are the same; comparing the 42 chromatid recombinations ± an expectation of 5% computed from random isolate data, $\chi^2 = 2$. They certainly therefore have not conclusively shown that 16117 is a single gene.

The best way to refute them is to obtain monogenic strains by mutation!

✓ Compare 1633^{+} and 1633° ε^{+} as limiting sulfanilamide.

in rate as plasmagens accumulated!!!

v. Rhoads

Growth factor requirements of tissue cells, cancer?

Maintenance of aneuploid cells in man. Symplasm + Heterocaryosis. (Bacterial association?)

Lederberg Tatum v.s.

"Adaptation" to biotin-requiring in Neurospora. Mass isolation. (Enzymes?)

Braddock missing point.

Transformation in heterocaryon. a+b x a-b. find ++. Either mutation or transformation or sexual process. (Presence of a-b in a+b? Demonstrate easily. Would certainly be selected against.) Or a + "killed" b.

Lys-arg.

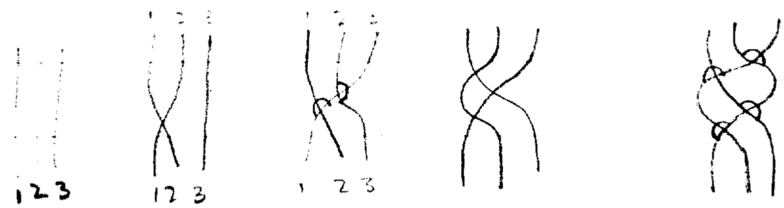
Cytoplasmic "inheritance": a+b, monoconidial isolation - if still grows, ? how genetically (a) Would prove plasmagene hypothesis [a lethal??].

See Tatum

Multiple mutants in bacteria by sulfa + methionine selection and plating out of various heterotrophic strains. Simultaneously, SA-resistance is obtained

Fabrics (braids) + permutation groups.

e.g. $n = 3$. $(32)(12) = (123)^n$

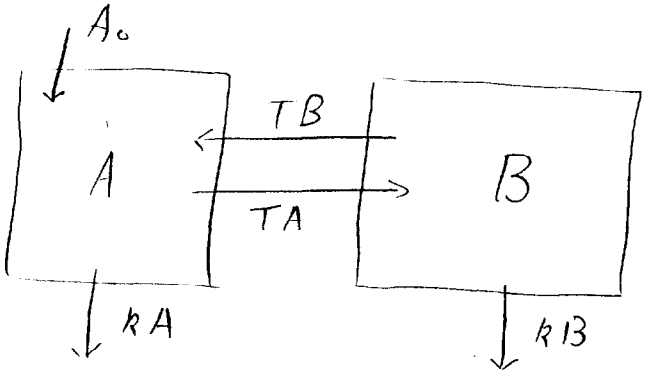


12 32 12 32 etc.

Mathematical formulation of gene distribution [illegible] required. Mathematical analogy for n genes [illegible] of ~~a~~ $f_1(a_n) \times f_2(a_n)$. (Vector analytic?)

9/15/45.

Plasma dye disappearance curves in pediatrics.



T = transfer coefficient

k = disappearance "

$u = -(k+T)$

$$\dot{A} = -(k+T)A + TB = uA + TB$$

$$\dot{B} = uB + TA.$$

$$\ddot{B} - 2u\dot{B} + (u^2 - T^2)B = 0.$$

~~$B_{max} \cdot uB = -TA.$~~

~~$\frac{B_{max}}{A} = \frac{T}{k+T}$~~ $\quad \frac{B_{max}(+)}{A(+)} = \frac{T}{k+T}$

$$A = \frac{A_0 e^{-kt}}{2}\left(3 - e^{-2Tt}\right) \qquad B = \frac{3A_0 e^{-kt}}{2}\left(1 - e^{-2Tt}\right)$$

$$A/B = \frac{3 - e^{-2Tt}}{3 - 3e^{-2Tt}} \qquad B/A = 3 \cdot \frac{1 - e^{-2Tt}}{3 - e^{-2Tt}}$$

Using the Ryan-Lederberg method of determining mutation rate one can do studies on influence of such factors as temperature, hot-cold shock, radiation, antibodies, etc. [incl. genetic background] on a large scale. One could also, if controlling genes were discovered, examine their effect in heterocaryon. This is a powerful tool! [for studying gene structure. E.G. influence of adding extracts of Neurospora [transformation experiment

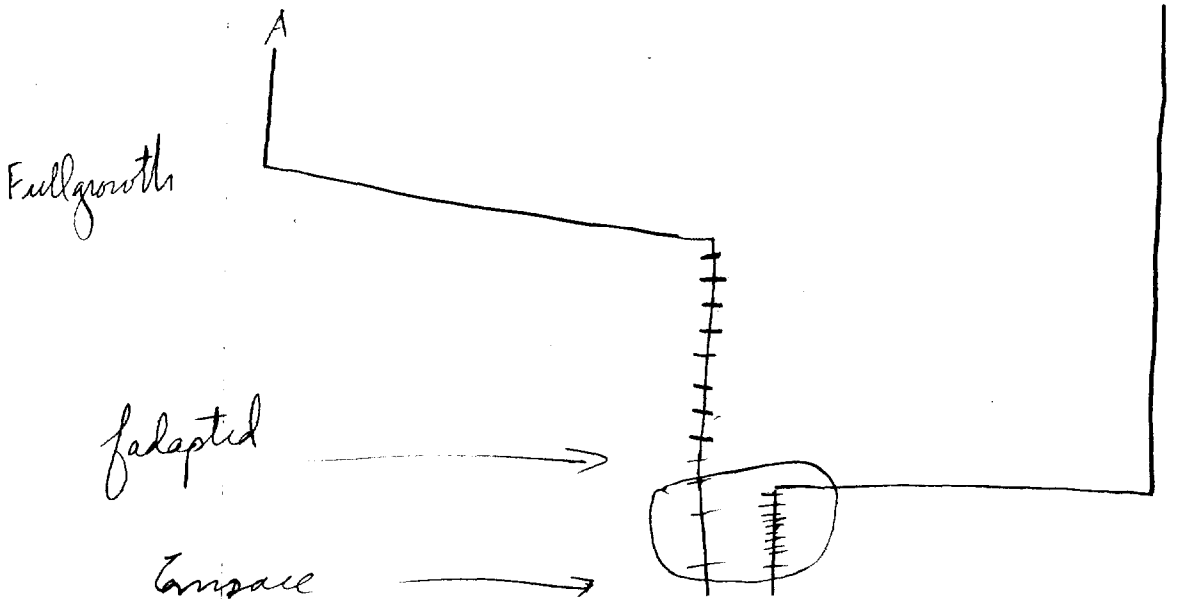
One can also calculate the composition of heterocaryotic mycelium using color marked lc strains, like + different test genes. E.g. ~~pant~~ pant-lc -+ and lys-lc 15300 The proportion of + adapted to 15300 adapted cultures is a measure of their proportions in the mycelium.

Expt.

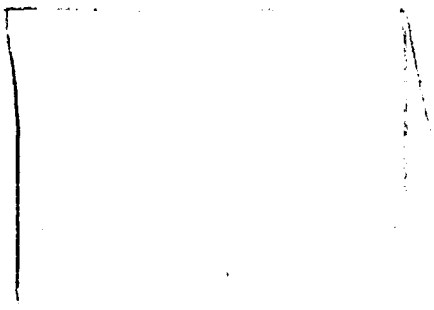
1. ~~Leu~~ Iron Fuci ~ .25, .50 mg leucine. Stationary at 3-4 days. Watch for adaptations on stationary mycelium by noting increased growth [Show that leucine was limiting.]

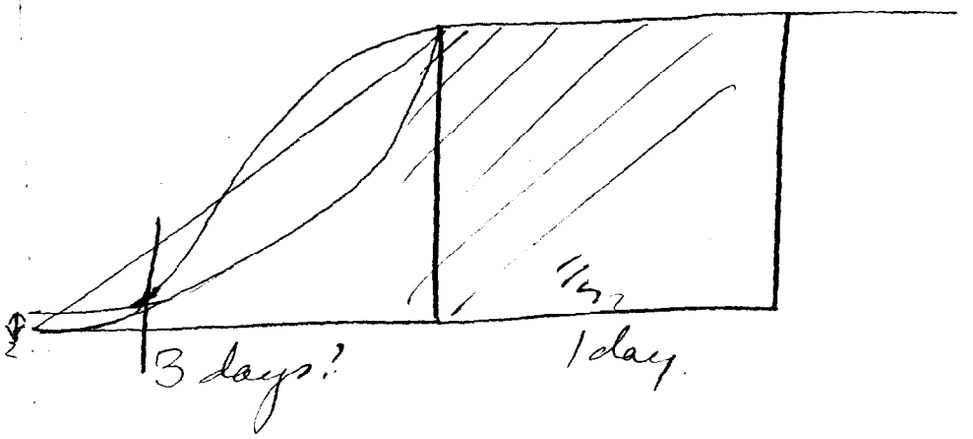
2. Lys-Leu Stock. Use limiting ~~Leu~~ lysine, supraopt. leucine. After 3-4 days, take K and wash mycelium, transfer to excess lysine, limiting leucine, and watch for adaptations. When the half-life is reached [should be calculable from (1) data, transfer the other 1/2 of series in same way. [Show that lysine was limiting]

Lysine limiting Excess leucine.	Leucine — Lysine +	Lys + Leu —



This will determine whether, in a stationary culture, leucineless mutations occur whether leucine is limiting, or leucine is in excess and lysine is limiting. Therefore the mutations that occur in a stationary culture in which leucine is limiting do not occur in response to the absence of leucine, but due to an unrelated cause, with a uniform probability per unit mass of mycelium. In order to calculate the adaptations expected during growth, plot area under curve. Use an empirical approximation.





$A_m = A_0 e^{kt}$

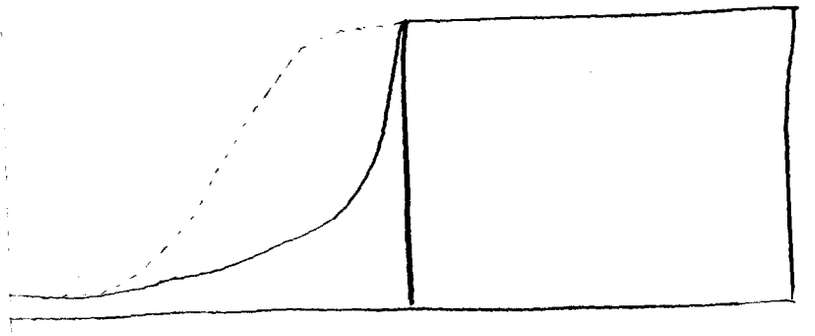
$$\int_0^m A_0 e^{kt} = A_0 \left[k e^{kt} \right]_0^m$$

$$= \cancel{A_0} \underline{kA_m} - kA_0$$

negligible.

$\text{area} = k \cancel{A_0} A_0$

$\text{area} = ca\, kA_m.$



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