

How to determine recessive-lethal mutation rates.

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Background

Recessive lethal mutations provide an objective measure of mutation rate. Development of a method for measuring the frequency of recessive lethals in the X chromosome of *Drosophila* made it possible for H. J. Muller (1927) to demonstrate that X rays are mutagenic, a finding which led to the Nobel Prize. Muller's method using the *CIB* chromosome depended on the suppression of crossing over by a heterozygous inversion, which kept the irradiated X chromosome intact through a cross, and on the fact that only one copy of the X chromosome is present in male *Drosophila*. Presence of a lethal mutation anywhere in the X chromosome was revealed by absence of males in the progeny of females that were heterozygous for the irradiated chromosome.

Because *Neurospora* is haploid, and because recessive genes can be sheltered in a heterokaryon, presence of a recessive lethal mutation is revealed by the absence of colonies representing one of the component nuclear types when conidia from a heterokaryotic mycelium are plated. This enables lethals anywhere in the entire genome be detected, in contrast to *Drosophila*, where lethals are readily detected only in one chromosome of the complement.

Neurospora heterokaryons were first employed in this way by Atwood (1950) and Atwood and Mukai (1953), using complementing colonial and nutritional markers to force the heterokaryon. More recently, David Stadler developed a refined system that reveals the presence of lethal mutations by using replication to transfer conidiating colonies from a heterokaryon onto diagnostic medium (Stadler and Crane 1979). See Figure 22 in Perkins et al. 2001. Stadler's powerful method enables spontaneous and induced mutation frequencies over the entire genome to be measured with accuracy -- a tour de force that has never been properly recognized. Dose-rate effects were determined (Stadler and Macleod 1984). Very low doses of radiation were shown to induce repair mechanisms (Stadler and Moyer 1981). Different types of induced mutations were identified (Stadler *et al.* 1987). The method has also been used extensively to determine the mutator effects of mutagen sensitive mutants (Kafer 1981, 1984).

Stadler *et al.* employed multiply marked, complementing strains as components of forced heterokaryons in experiments that were designed to reveal the inability of one component to grow as a homokaryon when it had acquired a recessive lethal mutation. Colonies originating from single conidia from a marked, forced heterokaryon were replicated to diagnostic media (Stadler and Crane 1979, Stadler and Macleod 1984, Stadler *et al.* 1987). Alternatively, conidia from a heterokaryon were streaked on diagnostic sorbose media (Stadler and Moyer 1981, Stadler 1983).

Procedure

As an example, Stadler and Macleod (1984) used *sn cr-1 trp-2 lys-5 mtr A* as one component and *sn cr-1 trp-2 ad-2 cyh-1 A* as the second. Both components were of the same mating type, as is necessary for a heterokaryon to form, and both were *sn cr-1*, to allow for replication. *lys-5* and

ad-2 serve as forcing markers. The *lys-5 mtr* component can grow as a homokaryon on lysine + fluorophenylalanine (+ anthranilic acid). The *ad-2 cyh-1* component can grow on adenine + cycloheximide (+ anthranilic acid). (All media were supplemented with anthranilic acid to satisfy the requirement of *trp-2*, which was irrelevant to these experiments.)

The following protocol for this experiment is from Stadler and Macleod (1984):

"Conidia were harvested from a 5-10 day tube culture of a heterokaryon into water or buffer or liquid culture medium, sonicated to break up clumps, filtered through glass wool and counted in a haemocytometer. The suspension was diluted to a concentration of 10^3 per ml. 10 ml of this suspension in a glass petri dish was treated with UV and samples were taken before and after treatment for plating. 200 cells (0.2 ml) were spread on each of a series of plates of minimal medium (+ anthranilic acid) to permit growth of the surviving heterokaryotic conidia into colonies; typically each plate produced about 50 colonies. The plates were incubated 3 days in the dark at 33°C plus one day in the light at room temperature (22°C) to produce ideal conidiation for replica plating. Each of these master plates was replicated (via velveteen) to one plate supplemented with lysine and FPA and a second with adenine and CYH (both with ANTH). The replicas were incubated about 30 h at 33° before being examined for nongrowers which signalled recessive lethal mutations. Putative mutant-bearing heterokaryons were marked on the master plates and confirmed by a second growth test: drops of conidial suspensions were incubated on the same media used on the replica plates. Those heterokaryons which failed to grow on one or the other of the test media in this second test were scored as recessive lethal mutations. Fig. 1 illustrates the main principles of the method." [The figure is reproduced as Fig. 22 in Perkins *et al.* 2001.]

The following strains are available: FGSC: 7880-7883 (*sn cr-1 trp-2 lys-5 mtr A* (FGSC 7881), and *sn cr-1 trp-2 ad-2 cyh-1 A* (7883), *sn cr-1 trp-2 lys-5 mtr uvs-2 A* (7880), and *sn cr-1 trp-2 ad-2 cyh-1 uvs-2 A* (7882). The stock center catalog also lists similar *sn cr-1* strains with different marker combinations (FGSC 5211-5218, from E. Kafer).

References

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