A highly fertile fluffy allele, fl^Y, which produces macroconidia.

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Abstract
In 1964, the FGSC obtained a pale-yellow stock which was listed as ylo-3 (Y234M474) [FGSC no. 902].
In 1964, the FGSC obtained a pale-yellow stock which was listed as ylo-3 (Y234M474) [FGSC no. 902]. The strain was deposited by Alan M. Kapular, with information that the mutant originated from wild-type 74A following UV treatment in experiments of T. Ishikawa at Yale University, that the locus did not show linkage to ylo-1 or ylo-2, and that cultures are slow to conidiate. Subden and Threlkeld (Can. J. Genet. Cytol. 10:351, 1966) included Y234M474 in a table with carotenoid mutants, where ylo-3 is listed as being in IIR.

In our hands, Y234M474 maps at the locus of fl:fluffy in IIR. No wild-type recombinants were obtained among 207 progeny from Y2334M474 x fl^P. We propose to designate the Y234M474 allele fl^Y ("fluffy-yellow").

The previously known fl alleles do not normally produce macroconidia (although some may be induced to conidiate sparingly with special media). The new fl^Y allele regularly produces macroconidia on minimal synthetic cross medium (SC) or Vogel's minimal, with sucrose as carbon source, but conidia are seen only several days after fl^+ controls have conidiated, and in lower numbers. Conidiation of fl^Y was not enhanced on the conidio-genic medium of Turian (1964 Nature 202:1240). No difficulty was experienced in classifying progeny of a fl^Y x fl^P cross in 12 x 175 mm minimal slants (34°) after the cultures had been held long enough for conidiation to occur.

The color of fl^Y depends on temperature of incubation during growth and conidiation. Conidia and mycelia were both yellow when growth was at 34°, but they were orange when growth was at 25° and 38°, on minimal and on complete medium in 150 mm slants. Cultures may either be grown in a lighted incubator or grown in the dark and then brought into a lighted room for induction of carotenoids. It is not unusual for morphological mutants at other loci to appear yellowish rather than orange or to have carotenoid synthesis attenuated so that the mutant appears paler than wild type.

It should be noted that the hue and intensity of carotenoids in mutants and in wild type can appear quite different when they are viewed in natural daylight compared to artificial light. Carotenoid mutants remain distinguishable under both conditions, however, although they may be more distinct in one than the other. We routinely examine cultures in a laboratory illuminated with white fluorescent lights.

fl^Y behaves like other fluffy alleles in producing microconidia profusely when combined as a double mutant with dn (dingy). Slant cultures of fl^Y; dn appear grey-brown after microconidia are produced. No orange or yellow macroconidia are visible to the naked eye. fl^Y; dn has not been examined microscopically for macroconidia. Presumably pe fl^Y would resemble fl^Y; dn.

Vegetative growth of fl^Y is vigorous. Linear growth on race tubes is similar to that of Oak Ridge wild type (3.8 mm/h on minimal medium at 25° C). Morphology of young cultures resembles other fluffy alleles such as fl^L and fl^P.

Strains of fl^Y are extremely fertile. Perithecia may be visible four days after opposite mating types are inoculated together onto slants of synthetic cross medium (SC) in 150 mm tubes at 25°C. As with fl^P, 4-day old fl^Y slants can be stored at 5° for at least two weeks while retaining full fertility.

Our laboratory has long used fl^P A and fl^P a as standard testers on 12 x 75 mm SC slants to determine the mating type and chromosome sequence of progeny from crosses, to detect new chromosome rearrangements, to score Spore killer genes, and to determine the species of wild-collected Neurospora isolates. We had hoped that fl^Y might prove superior to fl^P and other fluffy alleles as a tester, because it would let us inoculate large numbers of tubes with a conidial suspension. (With the nonconidiating fluffy alleles, we inoculate them with a suspension of small particles of macerated mycelia, which is more work to prepare.) However, we found that the production of conidia by fl^Y, even though delayed, sometimes impedes observation and interferes with scoring. A more serious disadvantage is that large protoperithecia ("false perithecia") are sometimes produced in unfertilized single-mating-type cultures of fl^Y. These could be mistaken for young perithecia or for barren perithecia and could lead to false readings for mating type and for fertility. False perithecia can be a major problem when duplication-generating rearrangements are being analyzed, because Duplication strains characteristically produced barren perithecia in test crosses (Perkins and Barry 1977, Adv. Genet. 19:133-285). (False perithecia also occur occasionally in some fl^P strains, but not in those we have selected as testers.)
Thus fl^Y is less useful than fl^P for our purposes. It might, however, be useful to others in studies of regulation and development.

Strains fl^Y A (FGSC 4240) and fl^Y a (FGSC 4241) have been deposited in FGSC. These are progeny from backcrosses of the original strain Y234M474 to OR wild type. ---

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Colonies of pabaA6 yA2 adE20 were grown on complete medium (CM) with 40 mM hydroxyurea (HU), a concentration which reduced the linear growth rate to about 15% of the CM control. After 10 days incubation, 30 stunted, poorly-conidiated colonies had produced 45 vigorous, HU-resistant sectors. Of these, 28 gave stable cultures which were wild type in growth rate and morphology. The remaining 17 were all unstable on vegetative growth; in this, in their colony morphology, analysis, they showed the characteristics of known duplication strains, such as those selected on the basis of a leaky, rate-limiting mutation (Sexton and Roper 1984 J. Gen. Microbiol. 130:583-595). Four resistant strains, HU-R1, 2, 3, and 4 were analyzed. Each was combined in diploid with a translocation-free master strain (MS); the known markers in the resistant haploids were on chromosome I, and the MS carried markers on the remaining 7 chromosomes (wa3; galA1; pyrA4; facA303; sB3; nicB8 riboB2). Two strains, HU-R1 and HU-R2, were also crossed to the MS. Mitotic and meiotic segregants were tested for resistance on CM with 40 mM HU, a concentration suitable for distinguishing high and intermediate resistance from the substantially lower, but variable, resistance of stock strains.

Meiotic and mitotic analysis of HU-R1, a stable isolate, showed that its resistance arose from mutation in a single gene. The meiotic segregation was 80 resistant : 74 sensitive, with free recombination between the resistance gene and all other segregating markers. The diploid, HU-R1//MS, had a standard, low degree of spontaneous mitotic instability on CM. On average, each 7-day colony gave only one mitotic segregant with a conidial color (dark green, yellow or white) different from the paler green parent, over which these segregant sectors showed no growth advantage. Haploidization analysis located the gene for resistance to chromosome V; there were 10 resistant fac^- and 20 sensitive fac^+ haploids, with markers on all other chromosomes segregating independently.

Colonies of HU-R2, 3 and 4 had a linear growth rate on CM less than that of wild type, and a modified surface morphology seen in various, known duplication strains. Their vegetative behavior suggested also that the proposed duplications determined HU resistance. Colonies of these strains gave clear-cut, faster-growing sectors. The majority of these sectors had wild type growth and were HU sensitive; they could be interpreted as arising by spontaneous, mitotic deletion of a duplicate chromosome segment which determined resistance and modified growth. A minority of sectors were still resistant, but they yielded second-order, sensitive sectors; this was consistent with the known stepwise loss of duplicate chromosomal material (Bainbridge and Roper 1966 J. Gen. Microbiol. 42:417-424). Diploids formed between HU-R2, 3, 4 and MS were of intermediate resistance. They were all extremely unstable on CM; each 7-day colony yielded between 5 and 14 large, faster-growing sectors, all of them HU-sensitive. In each case about 90% of the sectors were diploids with the standard, low degree of mitotic instability; the remainder were stable haploids with wild type morphology and growth rate. Such mitotic instability has been shown previously in diploids known to be heterozygous for a duplication (Case and Roper 1981 J. Gen. Microbiol. 124:9-16). Balanced haploids and diploids, which have lost the duplication by one of another process, overwhelm the diploid parent and any other unbalanced mitotic segregants. The haploids arise by the normal haploidization process, but with total selection against those slower growing haploids which have the duplication-bearing homologue; this allows location of the duplication to its chromosome. The diploids arise by mitotic crossing over between the chromosome arm bearing the duplication and its normal homologue. With appropriate segregation of chromatids, this gives a balanced, faster growing diploid. Suitable markers in the master strain allow location of the duplication to its chromosome arm. Figure 1. illustrates the processes, with HU-R2 as an example.