

Hsu, K. S. Acriflavin resistance controlled by chromosomal genes in *Neurospora*.

Prototrophic strains of 74A and 73a background differed in sensitivity when 2 µg acriflavin (Nutritional Biochem. Co.) per ml was present in the minimal slants. A cross between resistant (STA 4) and sensitive (Pa) strains indicated a 2:2 segregation for sensitivity in the tetrad.

The gene in strain KHI, responsible for this low level of resistance, was located in the left arm of linkage group I and designated acr-1. A second gene, in strain KH2, was isolated when the sub-culture of a single strain continued to grow in the presence of 10 µg/ml of the dye while others did not. Again, the difference was due to a single gene, designated ocr-2, which was located to the left of the group III centromere marker sc (scumbo) in a 3-point cross, acr-2 x sc tryp-1 (Table 2). Tetrad data (unordered) from a cross using the same markers is consistent with this order. Assuming the order

ocr-2 sc tryp-1,

I | tetrads of the 141 analyzed were singles in I, 69 were singles in II, 3 were 4-strand doubler in II, 1 was single in I and double in II, and 5 were I, II doubles. (Map distance acr-2 - sc, 6.0 units, C = 0.6). Since sc is

This note brings up to date results obtained with acriflavin-resistant mutants in *N. crassa*. Preliminary results have been reported in NN#1:5 (1962) and Genetics 47:961 (1962).

Table 1. Loci and strains of origin of acriflavin-resistant mutants.

Isolation No.	Locus	Strain of origin*
KH1	<u>acr-1</u>	STA4
KH2	<u>acr-2</u>	<u>cr^L</u> , <u>nit-1</u> (34547), <u>aur</u> (34508) a
KH4**	<u>acr-2</u>	<u>cr^L</u> ; <u>cot</u> (C102); <u>ylo</u> (Y30539y) A
KH5	<u>acr-2</u>	<u>cr</u> (B123); <u>cot</u> (C102) A
KH6	<u>acr-2</u>	<u>cr</u> (B123); <u>bal</u> (B56) A
KH8	<u>acr-3</u>	<u>cr</u> (B123); <u>cot</u> (C102) A
KH9**	<u>acr-2</u>	<u>cr</u> (B123); <u>bal</u> (B56) a
KH10**	<u>acr-2</u>	<u>cr</u> (B123); <u>ylo</u> (Y30539y) a
KH14	<u>acr-3</u>	<u>pe</u> (Y8743m), <u>f1^L</u> A
KH15	<u>acr-3</u>	<u>pe</u> (Y8743m), <u>f1^L</u> a

* All in the background of St. Lawrence stocks 74A and 73a except the pe f1 strains which are in Lindgren background.

** Isolated from the plates where conidia had been exposed to UV.

about 2 units right of the centromere (Hungate, F. P. 1946 Ph. D. Thesis. Stanford University), it seems possible that *acr-2* is just left of the centromere.

For the isolation of mutants resistant to higher acriflavin concentration, conidia harvested from both macroconidial and microconidial strains were plated on the surface of minimal agar containing 50 µg acriflavin/ml. The plated conidia were exposed to ultraviolet irradiation giving about 75% killing for some of the macroconidial strains. Resistant colonies were isolated after 5 or 6 days at WC, not more than one for each culture. Eight strains so isolated were single-gene mutants. Five of them, KH 4, KH 5, KH 6, KH 9, and KH 10, were assigned to the *acr-2* locus because no wild-type progeny were observed from crosses with KH 2. (40, 41, 54, 32, and 54 progeny were tested, respectively.) KH 8, KH 14, and KH 15, were assigned to a third locus, *ocr-3*, in the left arm of group I, proximal to mating-type and probably proximal to *acr-1*. That these three alleles presumably represent mutations at a single locus distinct from *acr-1* was based on observations that recombinants were recovered in crosses involving KH 1 and each of the three alleles, and that no wild type was obtained among 145 progeny tested from KH 8 × KH 14, nor among 130 from KH 14 × KH 15. Table 1 gives the loci and the strains of origin of these mutants. Linkage data of *acr-1*, *acr-2*, and *ocr-3* based on random segregants from 3-point crosses are summarized in Table 2.

Table 2. Linkage data on random segregants from crosses involving *acr-1*, *acr-2* and *acr-3*. (The top number in each pair represents the class that has the + (or A, or S) allele of the leftmost marker.)

Zygote genotype and recombination percent	Parental combinations	Recombination			Total and percent germination	Marker isolation numbers
		Singles region 1	Singles region 2	Doubles regions 1 & 2		
A <i>acr-1</i> ^R <i>cr</i> a <i>acr-1</i> ^S + 7.9 26.5	69 80	7 2	25 24	5 3	215 (60%)	sex KH1 <i>cr</i> ^L
<i>acr-1</i> ^S a <i>cr</i> <i>acr-1</i> ^R A + 12.3 24.5	36 36	7 1	10 11	3 2	106 (53%)	KH1 sex <i>cr</i> ^L
+ SC <i>tryp-1</i> <i>acr-2</i> ^R + + 3.2 27.3	76 74	1 6	31 28		216 (48%)	KH2 5801 10575
+ SC <i>tryp-1</i> <i>acr-2</i> ^R + + 3.1 28.1	30 36	0 3	14 13	0 0	96 (18%)	KH5 5801 10575
A <i>acr-3</i> ^R <i>cr</i> a + + 5.3 7.7	95 87	1 8	8 6		207 (49%)	sex KH8 B123
+ <i>ad-3B</i> + <i>acr-3</i> ^R + <i>cr</i> 13.8 6.3	30 35	6 4	1 3	1 0	80 (52%)	KH8 35203 B123
+ <i>ad-5</i> + <i>acr-3</i> ^R + <i>cr</i> 8.3 11.1	18 12	2 0	2 1	1 0	36 (50%)	KH8 Y152M40 B123
+ A <i>acr-3</i> ^R <i>leu-3</i> a + 9.3 4.0	35 31	4 2	1 1	1 0	75 (63%)	R156 sex KH14
A <i>acr-3</i> ^R a + <i>cr</i> 5.4 18.9	34 51	4 1	11 9	0 1	111 (50%)	sex KH14 B123
A + <i>cr</i> a <i>acr-3</i> ^R + 6.1 25.0	19 22	3 1	10 5	0 0	60 (60%)	sex KH15 B123

The acriflavin concentrations mentioned above are not the maximum concentrations at which these alleles can survive, but rather the convenient concentrations for differentiating resistant from sensitive isolates. In fact, in acriflavin slants, KH 1 still grew at 4 µg/ml, and other mutants continued giving slight growth at as high as 0.5 mg/ml. Correspondingly lower concentrations were required to differentiate resistance and sensitivity when the isolates were tested in liquid culture. The tolerance of

both resistant and sensitive strains was enhanced when hydrolyzed yeast nucleic acid was added to the acriflavin minimal medium. The double mutant acr-2^r; acr-3^r was more resistant than either single mutant. In scoring acriflavin resistance, false negatives may result if inocula are too small.

acr-1^r appeared to be recessive, while both acr-2^r and acr-3^r were dominant. This was inferred from the observation that at the same concentrations, the amount of growth of acr-1^r + ocr-1^s heterocaryons was close to that of the wild type, while growth of acr-2^r + acr-2^s, or acr-3^r + acr-3^s heterocaryons was close to the mutant type. The dominance of ocr-2^r and acr-3^r was in agreement with the fact that at least five of the seven mutants isolated from the macroconidial strains were heterocaryotic for the mutant alleles. Such heterocaryons would probably not be able to survive the inhibitory effect of ocriflavin if the mutant alleles were recessives.

All acr-2 mutants were cross-resistant to 3-amino-1,2,4-triazole, and all ocr-3 mutants, to malachite green. The double mutant acr-2^r; ocr-3^r could grow in the presence of either chemical. The concentrations at which resistance and sensitivity could be differentiated were 0.25% in agar slants and 0.1% in liquid culture for aminotriazole, and 2 µg/ml in slants and 0.5 µg/ml in liquid for malachite green. These mutants did not differ appreciably from wild type in their sensitivity to acridine orange, proflavine, thionine, and crystal violet.

Mutations from ocriflavin sensitivity to resistance seem to be frequent. Mutants at either ocr-2 or ocr-3 have been recovered from most of the strains upon first testing when between 10^6 and 10^7 conidia from each strain were plated on the ocriflavin plates. Mutants resistant to the dye have also been obtained by Howe and Terry (1962 *Canad. J. Genet. Cytol.* 4: 447) and by M. E. Case (personal communication). ■ ■ ■ Department of Biological Sciences, Stanford University, Stanford, California.