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Strains for identifying and studying individual vegetative (heterokaryon) incompatibility loci in Neurospora crassa

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Abstract

Genetic and molecular studies of vegetative incompatibility are proceeding in several Neurospora labs. The purpose of this note is to present an expanded list of strains in the Fungal Genetics Stock Center that are potentially useful when partial diploids are employed to identify different alleles at any of the 11 known *het* loci of *N. crassa*. Some of the strains are newly deposited in FGSC. Others have previously been listed under other categories in the stock list.

Strains for identifying and studying individual vegetative (heterokaryon) incompatibility loci in *Neurospora crassa*.

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Genetic and molecular studies of vegetative incompatibility are proceeding in several Neurospora labs. The purpose of this note is to present an expanded list of strains in the Fungal Genetics Stock Center that are potentially useful when partial diploids are employed to identify different alleles at any of the 11 known *het* loci of *N. crassa*. Some of the strains are newly deposited in FGSC. Others have previously been listed under other categories in the stock list.

Wild populations of *N. crassa* are polymorphic for *het* genes (Mylyk 1976 Genetics **83**:275-284). Laboratory strains, which have come from varied lineages, frequently differ from one another in *het* genotype. This polymorphism and the multiplicity of *het* loci often make it difficult to use heterokaryon tests for genetic analysis, because failure to complement may result from allelic differences at any one of numerous *het* loci. Extraneous *het* genes other than at the locus of interest will usually not be a problem when duplications (partial diploids) are used.

Duplications of known content can be obtained for defined chromosomal segments in progeny of crosses heterozygous for insertional or terminal translocations (see Perkins and Barry 1977 Advan. Genet. **19**:133-295). Because the duplications exist in an otherwise haploid genome, they make it possible to identify individual vegetative incompatibility (*het*) genes and to study them one by one without the necessity of making strains isogenic or homozygous for other *het* genes located outside the duplicated segment. If the translocation and normal-sequence parents differ with respect to alleles at a *het* locus within the duplication, then duplication progeny heterozygous for the included incompatible allelic combination display a characteristic inhibited growth with abnormal morphology and pigment (Newmeyer and Taylor 1967 Genetics **56**:771-791; Perkins 1975 Genetics **80**:87-105; Mylyk 1975 Genetics **80**:107-124, **83**:275-284). These heterozygous (*het*x/*het*^{y}) duplications are clearly distinguishable from homozygous (*het*^{x}/*het*^{x}) or (*het*^{y}/*het*^{y}) duplication strains, which are usually phenotypically normal or nearly so.

Heterokaryon incompatibility has been shown to correspond with phenotypic abnormality of heterozygous duplications for *het* genes at seven loci - (*mating type* [Newmeyer 1970 Can. J. Genet. Cytol. **12**:914-926], *het-c, d, -e, -5,* and *-8* [see Mylyk 1976 Genetics **83**:275-284], and *het-6* [D. J. Jacobson unpublished]). Three loci, *het-7, -9,* and *-10,* have been defined solely on the basis of their behavior in duplications. Presumably unlike alleles at these three loci are also heterokaryon incompatible, although this has not been tested because strains are not available that are known to differ only at the *het* locus in question but not at other loci. *het-i* has been defined only by behavior in heterokaryons; it differs from other *het* genes in such a way that incompatibility of different *het-i* alleles may not be detectable in duplications (Pittenger and Brawner 1961 Genetics **46**:1645-1663). Stocks with forcing markers are available for heterokaryon tests of *het-c, -d,* and *-e* in eight genotype combinations (prepared by L. Garnjobst and J. Wilson). These are listed in part VII.D.1 of the FGSC Stock List. Strains in this set are

probably identical to the Oak Ridge (OR) wild type and its derivatives at *het* loci other than *het-c, -d,* and *-e.* OR strains are *het-C het-d het-e het-i het-5^{OR} het-6^{OR} het-7^{OR} het-8^{OR} het-9^{OR} het-10^{OR}*. A few wild strains carry *tol,* a recessive suppressor of the *het* incompatibility associated with mating type, but OR and most other *N. crassa* strains are *tol^{+}*.

Genetic evidence suggests the existence of multiple alleles at two loci-*het-c* and *het-8* (Howlett, Leslie, and Perkins, 1993 Fungal Genet. Newsl. 40). However, multiple allelism could be simulated by two alleles at each of two closely linked *het* loci, and this alternative has not been ruled out.

The listing that follows (Table 1) is comprised of reference strains and strains with relevant linked markers, both in normal sequence and in the sequence of rearrangements capable of generating duplications that include the locus in question. Different *het* alleles are denoted by superscripts based on the wild strains of origin or on a laboratory reference strain, for example ^{AD} - Adiopodoumé, ^{CR} - Costa Rica, ^{HO} - Houma, ^{LI} - Liberia, ^{OR} - Oak Ridge, ^{PA} - Panama. Symbols for *het-c, -d, -e,* and *-i* are exceptions, with unraised capital or small letters used to specify the first two alleles, e.g. *het-D, het-d.* These, together with mating type, were the first *het* loci to be identified. Map relations of the markers and loci are shown in Figure 1. Updated versions of the list will appear in the FGSC Stock List (Part VII.D, Special-Purpose Stocks). (Contribution No. 93-355-A from the Kansas Agricultural Experimental Station, Manhattan.)

Table 1. Strains for studying individual *het*-loci of *N. crassa*

Genotype	FGSC No. A	a
het-c (IIL) (all are het-6OR)		
het-C (OR wild types)	2489	4200
het-c	7335	7336
het-C pyr-4	4030	4031
het-c pyr-4	7145	7146
cot-5 het-C	3560	3561
cot-5 het-c	7447	
cot-5 het-C pyr-4 thr-2	7355	7356
T(IIL VR)NM149 het-C	3879	3880
T(IIL VR)NM149 het-c	1483	1482
T(IIL VR)NM149 het-C pyr-4		3136
T(IIL VR)NM149 het-C ro-3	2011	2012
het-cAD	430	2614
het-cAD pyr-4 thr-2	7313	
T(IIL VR)NM149 het-cAD	2191	2192
T(IIL VR)NM149 het-cAD pyr-4	7314	7315
het-d (IIR) (all are het-C)		
het-D (RL wild types)	2218	2219
het-d (OR wild types)	2489	4200
T(IIR VL)ALS176 het-D	2414	3014
T(IIR VL)ALS176 het-d	3013	2415
T(IIR IVR)OY337 het-D	7472	7473
T(IIR IVR)OY337 het-d	3666	3667

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Genotype	FGSC No. A	a
het-e (VIIL)		
het-E (RL wild types)	2218	2219
het-e (OR wild types)	2489	4200
T(VIIL IVR)T54M50 het-E	2603	2604
T(VIIL IVR)T54M50 het-e	2466	2467
T(VIIL IVR)T54M50 het-e nic-3	3132	3133
het-i (I or II by linkage to translocation 4637 al-1)		
het-I al-2 nic-1	7343	
het-i al-2 nic-1		7344
het-I T(I;II)4637 al-1; pan-1	7342	
het-i (ST74A, 8-1a)	262	988
het-5 (IR)		
het-5PA (Panama CZ30.6)	1131	
arg-13 het-5PA (b11 OR)	7345	
thi-1 ad-9 nit-1 het-5PA (b10 OR)	7348	7349
T(IR VIR)NM103 het-5PA (b4 OR)	7346	7347
het-5OR (OR wild types)	2489	4200
T(IR II)MD2 het-5OR	3826	3827
T(IR VIR)NM103 cyh-1 al-1 arg-13 R het-5OR		3135
het-6 (IIL)		
Where not specified, the strain is het-C. Duplications from translocation NM149 include		
both the het-c locus and the het-6 locus. Whether het-6 heterozygosity contributes to an		
incompatible phenotype detected using NM149 can be determined by progeny-testing with		
AR18 or P2869.		
het-6PA (Panama CZ30.6, CZ30.4 (het-C?))	1131	1130
het-6PA (Probably het-C)	2189	2190
het-6PA arg-12 (b9 from Spurger P836)	7350	7351
T(IIL VR)NM149 het-6PA (b7 from P836)	7352	7353
T(IIL VR)NM149 het-6PA (Probably het-C)	2647	2188
het-6OR (OR wild types)	2489	4200
un-24 het-6OR	7354	
T(IIL IIIR)AR18 het-6OR	1561	1562
T(IIL VI)P2869 het-6OR	1828	1829
T(IIL VR)NM149 het-6OR	3879	3880
T(IIL VR)NM149 het-6OR (het-c)	1483	1482
T(IIL VR)NM149 het-6OR pyr-4		3136
T(IIL VR)NM149 het-6OR ro-3	2011	2012
het-7 (IIIR)		
het-7LI (Liberia UA-1)	961	
het-7OR (OR wild types)	2489	4200
T(IIIR X;IIIR;VIIL)D305 het-7OR	2139	2140
T(IIIR X;IIIR;VIIL)D305 het-7OR dow	3150	3151
het-8 (VIL)		
het-8PA (Panama CZ30.6, Marrero-1d)	1131	2224
T(VIL IR)T39M777 het-8PA	7413	7412
het-8OR (OR wild types)	2489	4200
chol-2 nit-6 het-8OR	7212	
ser-6 het-8OR ad-8		7213

Genotype	FGSC No. A	a
T(VIL IR)T39M777 het-8OR	2133	2134
T(VIL IR)T39M777 nit-6 het-8OR	7409	7408
T(VIL IR)T39M777 ser-6 het-8OR	7406	7407
T(VIL IR)T39M777 ad-8 het-8OR	3187	3188
het-8HO (Houma-1n, 1)	2220	3943
chol-2 nit-6 ser-6 het-8HO	7485	7486
T(VIL IR)T39M777 het-8HO	7411	

het-9 (VIR)

het-9PA (Panama CZ30.6)	1131	
het-9OR (OR wild types)	2489	4200
T(VIR IVR)AR209 het-9OR	1931	1932

het-10 (VIIR)

het-10CR (Costa Rica UFC205a)	851	
het-10OR (OR wild types)	2489	4200
T(VIIR IL)5936 het-10OR	2104	2105

mating type (IL)

(In a^{m1}, the mating and het-incompatibility functions of a are both inactive;
in a^{m33}, the hetfunction is inactive but the a mating function remains intact.
(Griffiths and DeLange 1978 Genetics 88:239-254).
tol is an unlinked recessive suppressor of A/a het-incompatibility.)

a^{m1} ad-3B cyh-1		4564
a^{m33}		5382
a^{m33} arg-3		5383
a^{m33} ad-3B		4568
tol (N83)	2338	1946
tol trp-4	2336	2337
leu-3 suc; tol pan-1		7322
leu-3 cyt-1 arg-3; tol	7337	
T(IL=> IIR)39311	1245	1246
T(IL=> IIR)39311 am33		6705
T(IL=> IIR)39311; tol trp-4	2985	2976
T(IL=> IIR)39311 ser-3 arg-1; tol		3220
In(IL=> IR)H4250	1563	1564
In(IL=> IR)H4250; tol	1947	2975
In(IL=> IR)H4250 leu-3; tol	3253	3254

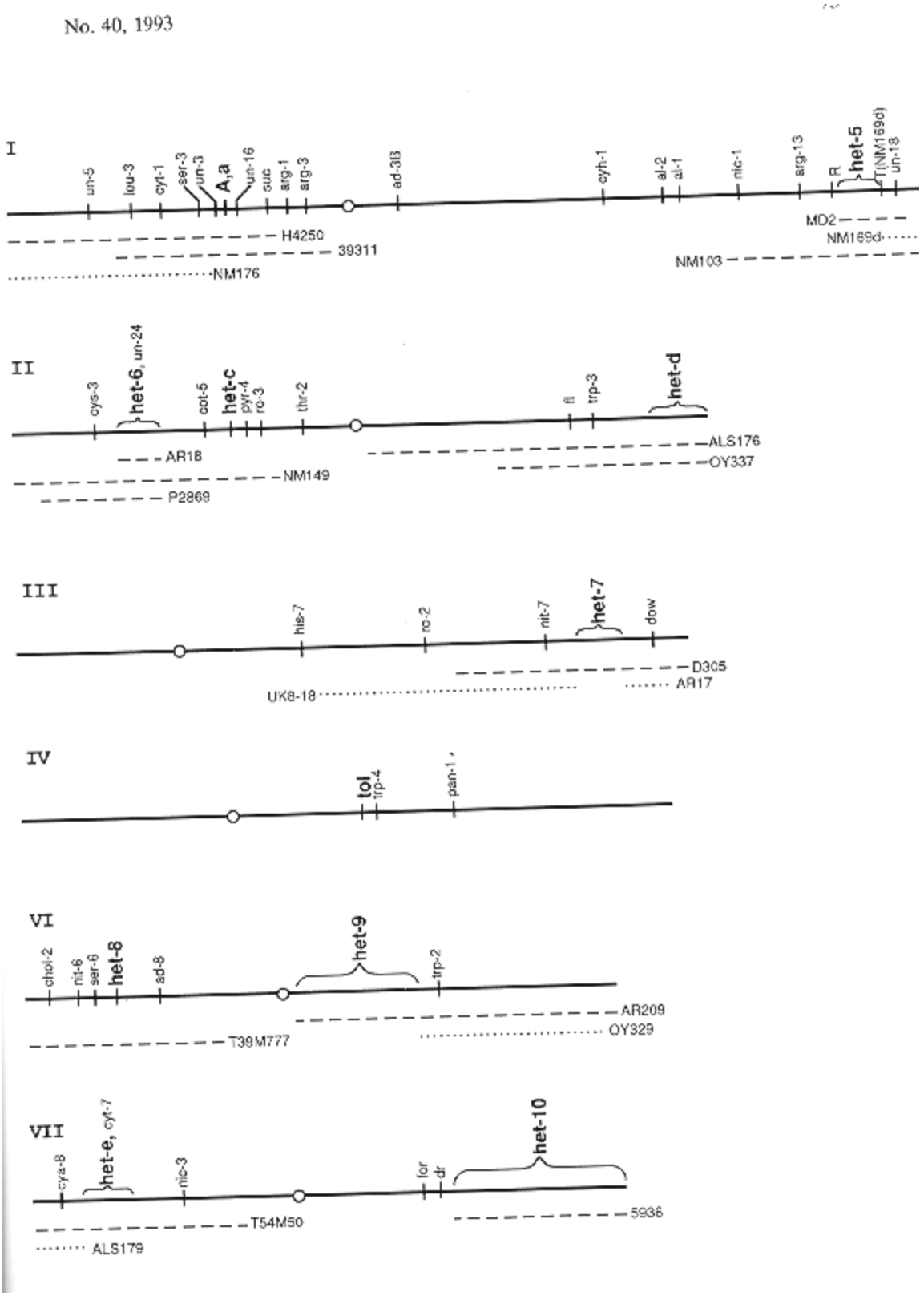

Figure 1. *N. crassa* linkage groups showing the sequence of markers and rearrangements relevant to known *het* loci. Dashed lines below the linkage groups show the extent of duplications

produced from the crosses between normal sequence and the respective chromosome rearrangements that produce duplications containing a *het* locus. Dotted lines below the maps show the extent of duplications that do not include a known *het* locus. For example, in a cross of insertional translocation AR18 *het-6*^{OR} × normal sequence *het-6*^{PA}, one third of the viable progeny are duplicated for the segment marked AR18. These duplications are heterozygous *het-6*^{OR}/*het-6*^{PA} but haploid for genes outside the duplication. For more complete maps, see Fungal Genet. Newsl. **39**:61-70, 1992 or *Genetic Maps*, 6th edition.

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