Multiple origins of the *i h*eterokaryon gene in Rockefeller-Lindegren strains

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The Fungal Genetics Stock Center has been able to revive four old lyophilized cultures of the mutants used by Garnjobst in her pioneering 1953 study of vegetative incompatibility in *Neurospora crassa*. One of them, inositolless (37401), tests as *het-c,* het-d, het-E,* as described by Garnjobst. This strain cannot be tested completely for *het-I/i,* but it is not *het-I/i* compatible with the FGSC riboflavinless *het-cdE I* tester strain. The other early cultures were the standard Rockefeller-Lindegren *het-CDEI.* A lineage chart of the *het-e* strains in the Wilson-Garnjobst heterokaryon testers shows that many of the *inl* testers are *het-i*, which probably accounts for the erratic performance observed in growth tubes with these testers. A list of *het-I/i* genotypes of 31 FGSC strains is included.

The recently reported *Hi/hi* genes in Rockefeller-Lindegren strains are similar, if not identical, to the *I/i* genes in Oak Ridge-St. Lawrence strains
(J.F. Wilson, et al.,1999. Fungal Genet. Newsl. **46**:25-30). Assuming identity, (*I/i*) will be used in the rest of the article in place of (*HI/hi*).

Het-I/i is unlike the other heterokaryon incompatibility genes that prevent the initial formation of a heterokaryon by killing the fused hyphal segments. In *I/i* combinations, forced heterokaryons form vigorous heterokaryons initially, but these heterokaryons are unstable, and become homokaryotic on transfer, due to elimination of the *het-i* component. Most, but not all OR-SL nutritional mutants examined are *het-i* genotype, and most RL mutants tested are *het-I* genotype, suggesting that the OR-SL wild types are *het-i;* the RL wild types; *het-I* , although they cannot be tested directly. However, in a cross between a RL *het-I* nutritional mutant and a St. Lawrence wild type carrying a presumed *het-i* gene, only one incompatibility gene segregated (Wilson, et al., 1999).

Since the *het-I* genotype seemed to be the norm in RL strains, it was surprising to find that five inositolless f_3 *het-CDE* strains sent to us by Dr. Garnjobst in 1960 were *het-i* instead of *het-I.* These isolates were only three generations removed from the original inositolless mutant produced from a Lindegren wild type by Beadle and Tatum (G.W. Beadle and E.L. Tatum, 1945. Am. J. Bot. **32**:678-686). We know that the present RL wild types were developed directly from the Lindegren wild types, and the two wild types are compatible in all other *het* genes. (Beadle and Tatum, 1945; D. Newmeyer, et al., 1987, Fungal Genet. Newsl. **34**: 46-51).

We asked FGSC to send us the earliest representatives they had of the five mutants Dr. Garnjobst used in her initial experiments (Laura Garnjobst, 1953. Am. J. Bot. **40**:607-614; Beadle and Tatum, 1945). Dr. McCluskey was able to revive four of the mutants from early lyopilized cultures (Kevin McCluskey, 2000. Fungal Genet. Newsl. **47**:110)). We used our own silica gel stock as a substitute for the missing fifth mutant, *al-2* a(15300).

These cultures, with isolation numbers and lyopil dates were:
inl A (37401)1958
pan-1 A (5531)1946
nic-3 A (Y31881) 1956
rib-2 a (Y30539) 1962
al-2 a(15300) 1961 (silica gel)

We first checked nutritional genotype and mating type,then determined the *het-CDE* genotype with known *het-CDE* testers (which are not necessarily the same as FGSC testers), and finally, tested for het-*I/i* genotype, using the protocol outlined in J.F.Wilson, et al., 1999.

The five mutants were tested against all combinations of *het-C/c* and *D/d,* but only in *het-E* at first, on the assumption that *het-E* was probably unchanged. If *het-E* had changed we would know soon enough, because all the combinations would be incompatible.The results of all tests except those of *inl* (37401) were positive with *het-CDE* testers only (FGSC 2000. Catalog of Strains, Part VII.D.1). The five mutants were then tested against all *het-e* combinations, and all tests were negative.

The mutant, *inl* (37401), gave a positive heterokaryon test solely with *het-cdE*. As an additional test, hyphal fusions between *inl* and all *het-E* combinations were examined. Interstrain incompatibility reactions were observed with *het-CDE, cDE,* and *CdE*, but fusion and continuing flow were seen through interstrain fusions with *het-cdE.*

The results of the *I/i* tests for all mutants except *inl* were also unam-biguous. All were *I*, suggesting strongly again that the original Lindegren wild types were *het-C,D,E, I.* The *inl* culture could not be tested thoroughly because we had no testers of genotype *het-cdE* with known *het-I/i* genotypes. However, we tested the *inl* culture against *rib-2* (y30539) *het-cdE A*, FGSC 475, and it was *het-I/i* incompatible. Limited heterokaryon tests across a single gene *het-E/e* difference, while not definitive, suggest that FGSC 475 is *het-I.* If so, the 1958 *inl* mutant is *het-i.* More crosses will be needed to provide additional proof.

If the 1958 *inl* is indeed *het-i*, it could account for the presence of the allele in Rockefeller-Lindegren mutant stocks prior to the *het E/e* work (Wilson, J.F.and L.Garnjobst 1966. Genetics **53:**(No. 3) 621-631). The *het-E/e* research and consequent production of the *het*-testers introduced still more *het-i* genes, since the OR-SL wild-type strain we used in the initial cross was *het-i*. What we did not know at the time was that the *inl* (37401) f_3 *het-CDE* mutant we picked as the other parent was also *het-i!* The progeny of this cross was the source of all *inl het-e* combinations placed in FGSC. Chart 1 shows the lineage of the *inl het-e* FGSC tester strains. The lineage of the *pan-1; al-2* (5531;15300) FGSC testers is not available for publication yet. Our records show that *het-I/i* genotypes of the critical early *pan-1;al-2* parents were never checked, but we have them in silica gel. However, since all the *inl het-e* FGSC testers are probably *het-i*, two or three serial transfers of heterokaryons of *inl* and the companion *pan-1;al-2* strains should establish their *het-I/i*. genotype. As noted below, some combinations may give ambivalent responses.

Tables 1 and 2 list all the FGSC strains we have tested for *het-I/i.* Table 2 illustrates the problems encountered in determining the *het-I/i* genotypes of some *het-Cde* strains. In *het-Cd*e and other combinations we have found a number of strains of uncertain *het-I/i* genotype. The apparent ambivalence in OR strains may be due to the operation of the APittenger effect@, in which *het-i* nuclei are not eliminated when they reach a threshold of 70% of the nuclei in the heterokaryon. The results were unambiguous in all the *het-CDE* cultures (over a hundred) we have tested. The APittenger effect@does not seem to be operable in a *het-CDE* environment (Wilson, et al.1999).

It should be emphasized again that differences in *het-I/i* in the Wilson-Garnjobst *het* testers do not interfere with *het-CDE* determinations as long as growth tube tests are avoided. Jacobson, et al. (1995 Fungal Genet. Newsl. **42**: 34-40) encountered just such a problem with their growth tube tests of the Wilson-Garnjobst testers. We ran the same tests on minimal slants and found that all except one of the pairs they found to be erratic were *het I/i* incompatible. The one exception was a combination including a *mat* mutant. This combination was perfectly compatible in our serial transfer tests (Wilson, et al.1999).

If growth tubes are used, *het-I/i* differences will become apparent in 36-48 hr as the supply of *het-i* nuclei runs out. In contrast, if one grows the components at 30EC for 24 hr, and inoculates minimal slants with superimposed aerial hyphae of the two strains, the resulting heterokaryon, if fully compatible, will cover the slant in 24 hr or less at 30EC. Mixed aerial hyphal-conidial inocula should be avoided, because *het-C/c and het-E/e* differences can be blurred. Heterozygosity in *het-I/i* shows up with 1-3 serial transfers of the heterokaryon (Wilson, et al.1999). In *het-I/i* tests one can use mixed mycelial-conidial inocula with no problem.

There seems to be no way of proving that this lyophilized sample of *inl*(37401) is indeed a subculture of the original Beadle-Tatum *inl*(37401). However, it has the same *het-cd* genotype that Garnjobst attributed to the original mutant. In addition, all the cultures Dr. Garnjobst ever sent to our laboratory were clearly and extensively labelled with mutant name, isolation number, cross number, ascus and ascospore number, and filial or backcross number. This culture was simply marked *inos* 37401 *A*, and it was deposited by Dr. Garnjobst about the time she left Stanford to go to Rockefeller University.

We do not know whether the information is available, but it would be of interest to know whether subsequent early *inl* mutants were tested against this one for complementation. If they were, we may have an explanation for the curious lack of new *inl* mutations. A two-gene difference, involving *het-C/c,D/d*
would certainly have been enough to prevent the formation of the heterokaryon.

* We are using the older terminology for het-C^{OR}/*het-*c^{PA} because combining these superscripts with *het* genes D/d and E/e makes the text difficult to read.

Chart 1. Lineage of *het-e* Tester Strains in FGSC

1) Incompatible b_9 St. L *het-CDe i a* X compatible b_9 St. L *het-Cde i A*
|
ascus 23

1, 2 *het-Cde i A*
5, 6*het-Cde i a*
3, 4*het-CDe i A*
7, 8*het-CDe i a*

2) 23-5 *het-Cde i a* X *inl* 37401-f^3-1 (4-5) *CDE i A* *
|
inl 37401 *het-CDe i*
inl 37401 *het-Cde i*

3)*inl* 37401 *het-Cde i* X *inl* 37401 *het-cdE i*
|
inl 37401 *het-cde i*

4)*inl* 37401 *het-Cde i* X *inl* 37401 *het-cDE i*
|
inl 37401 *het-cDe i*

*I/i genotype not known at the time of the cross

Analysis of the heterokaryon genotype of the Oak Ridge-St. Lawrence wild types started with twenty isolates sent to us by Dr. St. Lawrence (Wilson, J.F.and L. Garnjobst 1966. Genetics **53:**(No. 3) 621-631). These isolates were from a sib cross of b_9 isolates arising from a series of back crosses to ST74A. Only half the *mat-A* isolates were compatible with ST74A, as determined by the presence or absence of incompatibility reactions at hyphal fusions. Similar results were obtained in tests of the *mat-a* isolates against 74-OR21a. Above is a reconstruction of the lineage of what is now known about the presence of *het-I/i* in the Wilson-Garnjobst heterokaryon testers in FGSC. Although all the *inl het e* testers cannot be checked, the inadvertent selection of *inl het-i A* for the first cross suggests that they are *het-i* .

Table 1. ***Het I/i* Genotypes of Some FGSC Cultures**

het type	mating type	*het-I/i*	marker	FGSC #
CDE	*A*	*I*	*rib-2*	478
CDE	*A*	*i*	*inl*	479*
CDE	*A*	*I*	*pan-1;al-2*	1423
CDE	*a*	*I*	*pan-1;al-2*	1427
Cde	*A*	*I*	*inl*	1453
Cde	*a*	*i*	*arg-12*	1527
Cde	*a*	i	*inl*	1438
Cde	*A*	*i*	*pan-1;al-2*	2658
Cde	*a*	*i*	*ad-2 ;al-2;*	956
Cde	*a*	I	*pan-1;al-2*	2657
Cde	*A*	*i*	*alcoy*	997
Cde	*a*	*i*	*alcoy*	998
Cde	*a*	*i*	*ad-3B*	4564
Cde	*a*	*i*	*cot-1*	*4066*
Cde	*a*	*i*	*arg-12*	1527
Cde	*A*	*I*	*arg-5;ylo*	6828
CDe	*A*	*I*	*pan-1;al-2*	2656
CDe	*a*	*i*	*inl*	1439
CDe	*a*	*I*	*pan-1;al-2*	2661
cDE	*A*	*i*	*pan-1;al-2*	*1425*
cDE	*A*	*I*	*inl;al-2*	*476*
cDE	*a*	*I*	*pan-1;al-2*	142

***** No longer listed in FGSC collection, but JFW strain is healthy.

Table 2. Ambivalent Strains

het type	mating type	*het-I/i*	marker	FGSC #
Cde	a	*I/i* <	*his-1*	680
Cde	A	*I/i* <	*his-1*	681
Cde	A	*I/i* <	*his-3*	455
Cde	A	*I/i* <	*his-5*	456
Cde	A	*I/i* <	*his-6*	457
Cde	A	*I/i* <	*ad-6*	664
Cde	A	*I/i* <	*ad-1*	672
Cde	A	*I/i* <	*ad-8*	451
Cde	a	*I/i* <	*arg-5;ylo*	6829

< Inconsistent results with Cde testers of known *I/i* genotype.