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Abstract

Recent interest and accelerated research into the genetics of heterokaryon incompatibility (HI) in *Neurospora crassa* has led to increased use of the original Wilson-Garnjobst HI tester strains available from FGSC (1994 Catalog of Strains, Part VII.D.1.). We have found inconsistencies and abnormalities in both growth of heterokaryons and segregation of markers in crosses using these strains. First noticed was a lack of vigor and incomplete complementation of markers in forced heterokaryons when compared to compatible heterokaryons with known Oak Ridge (OR) background. Secondly, skewed allele ratios were recorded in crosses between the Wilson-Garnjobst strains and strains with OR background. Perkins and Bjorkman raised a cautionary note about these strains (1978 *Neurospora Newsl.* **25**:24-25), however, they concentrated primarily on the scot mutant present in these and other strains originating from the Rockefeller-Lindegren (RL) background. We have attempted to further characterize the erratic behavior of Wilson-Garnjobst strains and determine if the scot mutant or other modifiers of HI are responsible.

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The Wilson-Garnjobst heterokaryon incompatibility tester strains of *Neurospora crassa* contain modifiers which influence growth rate of heterokaryons and distort segregation ratios.

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Recent interest and accelerated research into the genetics of heterokaryon incompatibility (HI) in *Neurospora crassa* has led to increased use of the original Wilson-Garnjobst HI tester strains available from FGSC (1994 Catalog of Strains, Part VII.D.1.). We have found inconsistencies and abnormalities in both growth of heterokaryons and segregation of markers in crosses using these strains. First noticed was a lack of vigor and incomplete complementation of markers in forced heterokaryons when compared to compatible heterokaryons with known Oak Ridge (OR) background. Secondly, skewed allele ratios were recorded in crosses between the Wilson-Garnjobst strains and strains with OR background. Perkins and Bjorkman raised a cautionary note about these strains (1978 *Neurospora Newsl.* **25**:24-25), however, they concentrated primarily on the *scot* mutant present in these and other strains originating from the Rockefeller-Lindegren (RL) background. We have attempted to further characterize the erratic behavior of Wilson-Garnjobst strains and determine if the *scot* mutant or other modifiers of HI are responsible.

To date, all Wilson-Garnjobst strains tested by us have show the *scot* phenotype at 39°C, confirming results of Perkins and Bjorkman (Figure 1). FGSC 1527 is the only strain listed in Part VII.D.1. that was not expected to contain *scot* because it is of OR rather than RL background; this proved to be true (data not shown).

Linear growth rate in race tubes was used to quantify and compare heterokaryon formation among the strains tested. Loopfuls of conidia from two strains were co- inoculated at one end of a 25 ml disposable pipet containing 15 ml Vogel s minimal medium N. (A similar method is described by White and Woodward 1995 *Fungal Genetics Newl.* **42**) The experiment was limited to the Wilson-Garnjobst strains that differ at *het-c* (Table 1); the *am1 ad-3B cyh-1R* helper strain (FGSC 4564) was included as an OR background standard. Strains of like mating type with complementary markers were co-inoculated in all possible combinations. No attempt was made to control nuclear ratio. Single strains inoculated on race tubes were used as controls. All tubes were incubated at 34°C and each combination of strains was tested three times. The starting point was marked as the leading edge of the colony after overnight growth; measurements were recorded at 24 hr intervals (Figure 2). Although *scot* is reported to have a slightly abnormal phenotype at 34°C, it is erratic and difficult to see on Vogel s minimal medium N (Perkins and Bjorkman 1978 *Neurospora Newsl.* **25**:24- 25). We saw no evidence of the *scot* phenotype in these experiments. Heterokaryon formation was confirmed by testing hyphal tips for prototrophic growth. The leading margin of the colony from the race tube was transferred to a fresh plate of minimal medium. These cultures were incubated for 1 day at 34°C and 10 single hyphal tips from the colony were subcultured on to separate 10 x 75 mm tubes of minimal medium. Prototrophic growth in any of the 10 tubes, scored at 2 days, was considered a positive

heterokaryon. The results of hyphal tip subculturing is indicated on Figure 2 with either a (+) or (-) associated with the line of each combination tested.

The combination of strains with known OR background, FGSC 1527 and FGSC 4564, consistently gave the fastest growth; we consider this characteristic of a strong positive heterokaryon. All other combinations, whether alike or different at *het-c*, showed both slower or stalled growth and inconsistency among replications (Figure 2). Only FGSC 1527 + FGSC 1438, *het-C* + *het-C*, grew the entire length of the race tube (25 cm). However, this happened only twice in the three replications, with one replication growing less than 1 cm in 9 days. Moreover, FGSC 1438 + FGSC 4564, the other OR strain, never grew more than 5 cm. This was generally true of the other *het-Cde* strains with FGSC 4564 (data not shown).

Few combinations other than FGSC 1527 + FGSC 4564 yielded heterokaryotic hyphal tips, including those with like *het-c* alleles. Correlated with this was apparent incomplete complementation of the *al-2* mutant seen as either white or mixed white and orange conidia along the length of the race tube. We interpret these two results as either failure to establish a true heterokaryon or maintain a stable heterokaryon during continued growth in the race tube. Of interest is the one *het-c* + *het-C* pairing that appeared to form a heterokaryon. This was probably due to escape from incompatibility.

Additional growth rate tests were performed with other Wilson-Garnjobst strains at 30°C. One set was inoculated with equal number of conidia in an attempt to control for nuclear ratios. All these additional tests gave similar results as described above (data not shown).

The growth curves in our Figure 2 resemble the seven types of heterokaryotic growth reported by Holloway (1955 Genetics **40**:117-129, see his Figure 1). In fact, Holloway used some strains from the Garnjobst collection in his study. In addition, personal communication from James F. Wilson to David D. Perkins (an unpublished letter) states, "The *inos* testers do show a single gene difference in the ability to form a heterocaryon which will grow the length of a growth tube, but it is not C, D, or E, does not involve cytoplasmic incompatibility and does not affect heterocaryon test on slants." Crosses were performed to determine if *het*-like genes (such as Holloway's *W*, *X*, *Y*, and *Z*) were responsible for the growth curves observed, especially the reduced vigor of OR and Wilson-Garnjobst *het-Cde* combinations.

Four Wilson-Garnjobst strains were crossed with multiply marked centromere (multicent) linkage testers (Table 1), which are OR background and fully heterokaryon compatible with FGSC 4564. Progeny were scored for all markers and compatibility with the OR parental background (Table 2). Heterokaryon formation was tested by pairing all progeny containing a forcing marker with FGSC 4564 in 10 x 75 mm tubes of minimal medium. The difference between strong positive compatibility and weak or no prototrophic growth was clear at 2 days. This trait segregated in a 1:1 ratio in the three crosses homozygous for *het-C*. Alleles of at least one other marker in each cross showed deviation from the expected 1:1 ratio, affecting linkage calculations from these data. Skewed allele ratios were also seen when strains derived from the Wilson-Garnjobst strains were crossed with other strains of OR background (compatible with FGSC 4564) and in one cross between Wilson-Garnjobst *inl* and *pan-1* strains (data not shown). No marker was consistently skewed in any of these crosses when repeated. However, *het-c* and

mt (the only scorable markers) were not skewed in crosses between *pan-1* strains (data not shown).

Of particular interest is linkage between compatibility with OR background and other markers, as this should indicate approximate location of gene(s) affecting compatibility. The clearest instance of linkage to OR compatibility is *inl* in cross 1027. However, this is complicated by the skewed allele ratio of *acr-2*. Both *inl* and *at* (linkage group V) show linkage to *acr-2* (III) and as such OR compatibility also appears linked to *acr-2*. In contrast, cross 1028 shows an apparent linkage between OR compatibility and each *ylo-1* (VI) and *wc-1* (VII). Again, a skewed allele ratio of *wc-1* confounds this analysis. Unlike cross 1027, there is no apparent linkage between OR compatibility and linkage group III or V markers.

An unexplainable excess of recombinant progeny is seen between OR compatibility and each *pan-1*, *psi*, and *wc-1* in crosses 1030 and 1034. If the locus modifying compatibility with OR is independent of *het-c*, as indicated by the lack of linkage to *arg-5*, the expectation for the cross heterozygous at *het-c* would be a 1:3 ratio of OR compatible to OR incompatible. The data reveal an opposite ratio of 3:1 compatible:incompatible. This does not correlate with the observed skewed ratio of mating type. At present, we do not have a good explanation for the distorted segregation patterns seen in these crosses.

The spreading colonial phenotype of *scot* is probably not responsible for the inconsistent growth or incomplete complementation seen, even though OR compatibility is apparently linked to linkage group V in cross 1027. First, there is no such linkage in the other three crosses. Second, although *scot* is erratic at 34°C, similar results were obtained from a subsample of pairings repeated at 30°C and 25°C, both permissible temperatures (data not shown). It is unclear if *scot* may influence growth rate when heterozygous in heterokaryons, for example the Wilson-Garnjobst + OR combinations, or if the gene has a more general pleiotropic effect in modifying heterokaryotic growth. The linkage data leave this possibility open, however, other loci on linkage group V or other linkage groups may also be responsible for modifying compatibility. Since *scot* expression may affect growth rate directly, it is also possible that modifiers of the *scot* gene cause growth variability in heterokaryons. In any case, inconsistencies among the *pan-1*; *al-2* strains (crosses 1028, 1030, 1034) and in other repeated crosses does not allow the interpretation of these data as indicating the location of any loci responsible for skewing or incompatibility with OR background.

The skewed ratios and apparent linkage of markers on separate chromosomes may suggest a chromosomal difference between the Wilson-Garnjobst and multicent OR parents. Although a relatively high number of white inviable ascospores were noted in the crosses listed in Table 2, there was no regular patterns of black to white ascospores among shot asci, which would indicate chromosome aberrations (data not shown). Although we could not completely reject chromosomal differences, presence of other inviability factors or segregation distorters seems a more likely explanation.

To further disseminate this information and warn workers of possible complications using the Wilson-Garnjobst strains, we have requested that the following statement be added to Part VII.D.1. of the FGSC Catalog: These strains contain *scot* and probably other genes in their

background that affect both growth of heterokaryons and segregation of markers in crosses. Heterokaryon tests should be performed at permissible temperature (25°C) and caution should be exercised when using these strains for genetic studies.

These results also raise the issue of standardizing definitions of compatibility and incompatibility. This is becoming more important as workers proceed with the molecular dissection of HI. There is a need to further identify these factors which may modify the reactions of *het* genes at the molecular, cellular, and colony level. In an effort to facilitate standardization in future studies of HI in *N. crassa* we suggest that 1) OR background (*het-Cde* and OR alleles at *het-6* through *het-10*) be considered the standard against which HI is measured (FGSC 4564 is especially useful in this context, see Perkins 1984 Neurospora Newsl. **31**:41-42); 2) genes of interest (*het* genes, modifiers, suppressors, etc.) be crossed into OR background to measure their effect; and 3) characterizations of compatibility or incompatibility be quantified, when necessary, by growth rates in race tubes.

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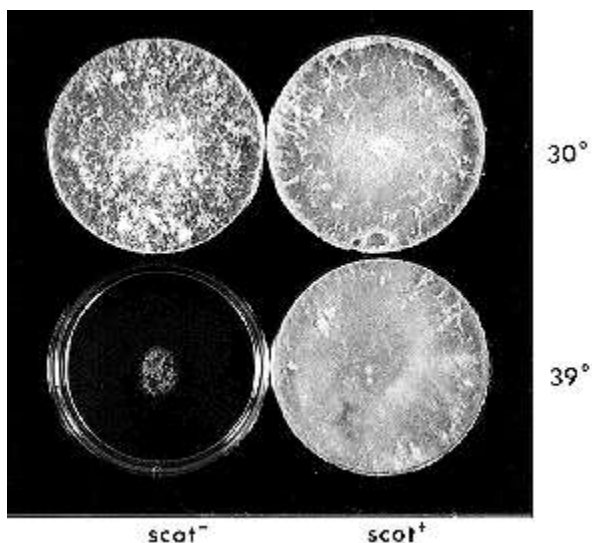


Figure 1. Phenotype of *scot* at permissive (30°C) and restrictive (39°C) temperatures. Plates show 3 days growth on Vogel's minimal medium N supplemented with pantothenic acid where required. *Scot-* strain is FGSC 2659 -- Wilson-Garnjobst *het-cDe; pan-1; al-2 a*. *Scot+* is FGSC 6714 -- Oak Ridge background, T(VL)MB67.

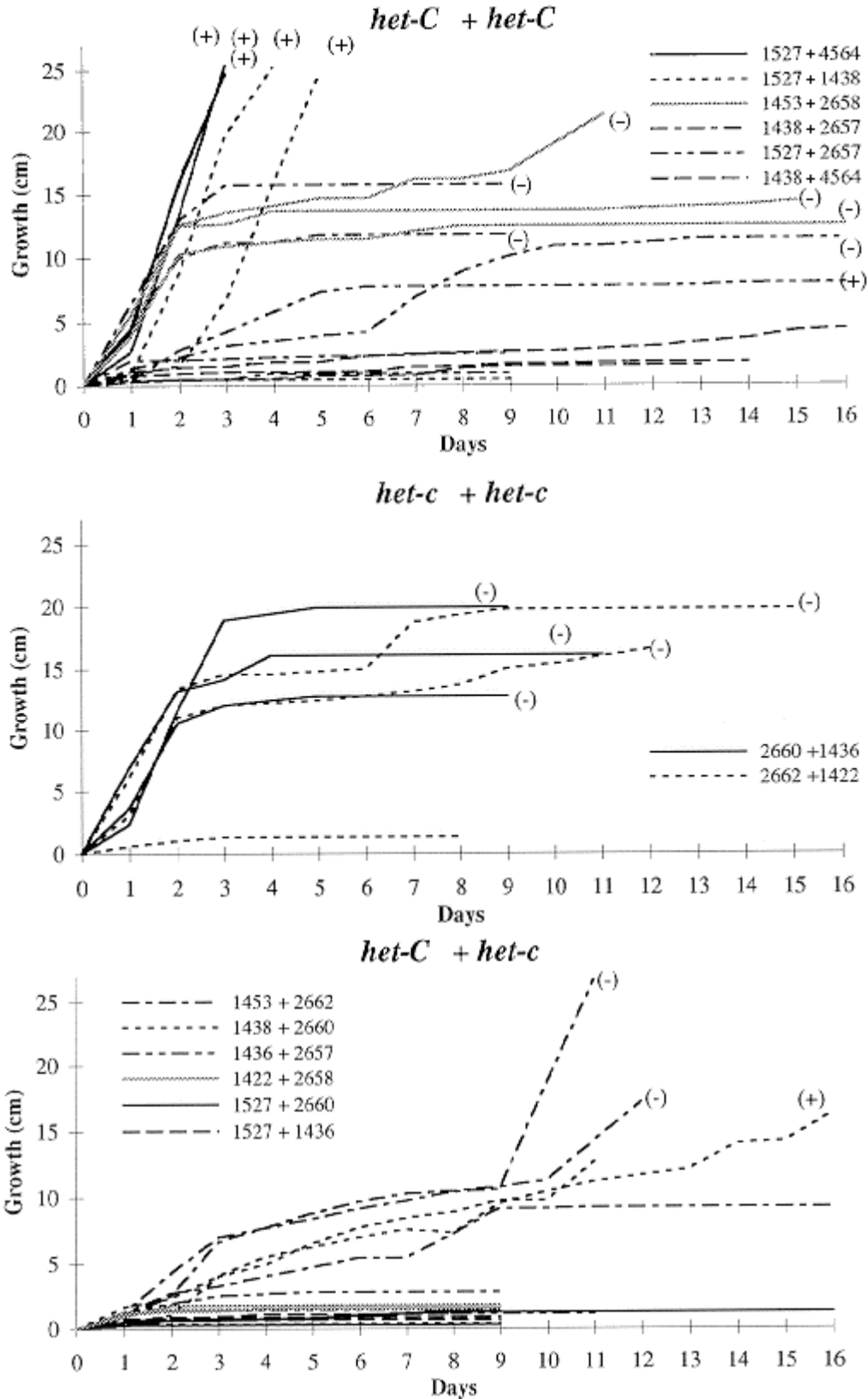


Figure 2. Growth rates of forced heterokaryons in race tubes at 34 C. The *het-c* alleles of each pair is indicated at the top of each graph. Each line represents a pair of strains; each pair was

tested three times. Pairings tested for heterokaryotic hyphal tips have a symbol in parentheses at the end of the line, with (+) indicating prototrophic hyphal tips recovered and (-) indicating only auxotrophic hyphal tips recovered (see text for details).

Table 1. Strains used for heterokaryon growth rate tests illustrated in Fig. 1 and crosses listed in Table 2.

het genotype	Mating type	Markers	FGSC Number
het-Cde	A	inl	1453
het-Cde	A	pan-1; al-2	2658
het-Cde	a	inl	1438
het-Cde	a	pan-1; al-2	2657
het-Cde	a	arg-12	1527
het-Cde	am1	ad-3B cyh-1R	4564
het-Cde	a	multicent-4	
		(arg-5;acr-2;psi;at;ylo-1;wc-1)	6829
het-Cde	A	multicent-4	
		(arg-5;acr-2;psi;at;ylo-1;wc-1)	6828
het-cde	A	inl	1422
het-cde	A	pan-1; al-2	2662
het-cde	a	inl	1436
het-cde	a	pan-1; al-2	2660

Note: All strains are Wilson-Garnjobst heterokaryon incompatibility testers except FGSC 1527, 4564, 6828, and 6829. They are, respectively, an *arg-12* mutant, a mating- type mutant with inactive heterokaryon incompatibility function of the mating type gene, and the two multiply marked centromere testers; these strains are all Oak Ridge background.

Table 2. Allele ratios of markers and apparent linkage among markers in crosses of Wilson-Garnjobst testers and multiply marked centromere (multicent) testers.

Cross 1027: *het-Cde; inl A 1453 X multicent-4 a 6829* Allele Ratios of Markers

mt	arg-5	acr-2	psi	at	ylo-1	wc-1	inl	OR(a)
A:a	+:-	+:-	+:-	+:-	+:-	+:-	+:-	+:-
54:50	48:56	74:30*	48:56	51:53	58:46	60:44	51:53	46:45

Linkage Among Markers

	inl & at	inl & acr-2	at & acr-2	inl & OR	acr-2 & OR
Parentals	65*	75*	65*	80*	62*
Recombinants	38	29	39	11	29

Cross 1028: *het-Cde; pan-1; al-2 A 2658 multicent-4 a 6829* Allele Ratios of Markers

mt	arg-5	acr-2	psi	at	ylo-1	wc-1	pan-1	al-2	OR
A:a	+:-	+:-	+:-	+:-	+:-	+:-	+:-	+:-	+:-
26:36	26:36	32:30	38:24	28:34	18:16	27:7*	33:29	34:28	28:30

Linkage Among Markers

	mt & al-2	psi & pan-1	ylo-1 & OR	wc-1 & OR
Parentals	40*	51*	22*	22*
Recombinants	22	11	10	10

Cross 1030: *het-Cde; pan-1; al-2 a 2657 multicent-4 A 6828* Allele Ratios of Markers

mt	arg-5	acr-2	psi	at	ylo-1	wc-1	pan-1	al-2	OR
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A:a +:- +:- +:- +:- +:- +:- +:- +:- +:-
 66:55 65:56 52:69 63:58 67:54 44:22* 33:31 64:57 64:57 53:68

Linkage Among Markers

	mt & al-2	psi & pan-1	pan-1 & OR	psi & OR	wc-1 & OR
Parentals	77*	101*	46*	37*	23*
Recombinants	44	20	76 (b)	84 (b)	42 (b)

Cross 1034: *het-cde; pan-1; al-2* a 2660 multicent-4 A 6828 Allele Ratios of Markers

mt	arg-5	acr-2	psi	at	ylo-1	wc-1	pan-1	al-2	OR
A:a	+:-	+:-	+:-	+:-	+:-	+:-	+:-	+:-	+:-
63:38*	54:47	50:51	49:52	57:44	25:27	28:24	55:46	52:49	71:30*

Linkage Among Markers

	mt&al-2	psi&pan-1	pan-1&OR	psi&OR	mt&OR	al-2&OR	arg-5&OR
Parentals	60*	82*	39*	36*	61	54	49
Recombinants	41	19	62 (b)	55 (b)	40	47	52

Notes: Strain numbers are FGSC numbers. Number of progeny tested are listed for each ratio; number of progeny tested for *ylo-1* and *wc-1* were limited to albino+ progeny where *al-2* was present in one parent. An (*) for any ratio indicates deviation from a 1:1 ratio at a 5% significance level. Other unlisted linkage tests between markers are all not significantly different from 1:1. Marker locations are: mt, linkage group I; *arg-5*, II; *acr-2*, III; *psi*, IV; *at*, V; *ylo-1*, VI; *wc-1*, VII; *inl*, VR; *pan-1*, IVR; *al-2*, IR.

(a)OR signifies Oak Ridge het background (multicent parent), with (+) indicating heterokaryon compatibility and (-) indicating incompatibility with FGSC 4564 (*am1 ad-3B cyh-1R*). Number of progeny tested were limited to those containing an appropriate forcing marker.

(b) Ratios with unexplained excess of Recombinant progeny.