Investigation of the *het* genes that control heterokaryon incompatibility between members of heterokaryon-compatibility (h-c) groups A and G1 of *Aspergillus nidulans*

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A chromosome assay method was used to determine the heterokaryon compatibility relationships between strains belonging to heterokaryon-compatibility (h-c) groups A and G1 of *Aspergillus nidulans*. A hybrid strain (RD15) was isolated following protoplast fusion of strains 65-5 (h-cA) and 7-141 (h-cG1). The morphology of RD15 was severely abnormal compared to diploid strains of *A. nidulans* produced from heterokaryon-compatible haploid parents. Inocula of RD15 were induced to haploidize on medium containing Benlate and a parasexual progeny sample of 291 haploid segregants was obtained. The progeny strains were genotyped for standard markers. Allelic ratios and pairwise marker segregations were determined. Pairs of progeny strains that carried different alleles for the standard markers on each linkage group in turn were tested for compatibility. Strain pairs that possessed different alleles for the markers on linkage groups II, III, V, VI and VII were incompatible indicating the presence of heterokaryon-incompatible (*het*) genes on these linkage groups. Backcrosses to an h-cG1 strain showed that two *het* genes were located on linkage group III and confirmed a total of six *het* gene differences between the h-cA and h-cG1 strains.

Introduction

Wild isolates of *Aspergillus nidulans* have been classified into heterokaryon-compatibility (h-c) groups based on whether mixed cultures form heterokaryotic mycelium (Grindle, 1963*a, b*). Compatibility tests can be done under selective or non-selective conditions. Selective methods usually require the prior introduction of selectable markers into isolates and conditions in which neither parental homokaryon can grow. Complementing nutritional requirements have been commonly used and selection can be imposed by inoculating the parental strains together onto minimal medium. Heterokaryotic mycelium grows out in a sectoring pattern, though mass hyphal subculture to fresh minimal medium may be necessary before such a growth habit is observed (Pontecorvo *et al.*, 1953; Roper, 1966). Heterokaryon-incompatible pairings, on the other hand, do not grow or show only limited growth from the initial centres of inoculation, even after subculture (Dales *et al.*, 1983).

Detection of heterokaryotic mycelium is helped by using parental strain combinations that differ for conidiospore colour. Heterokaryosis can then be detected by the sporadic occurrence within the mixed culture of individual conidiospore heads in which both parental spore colours are displayed in adjacent chains of spores. These mixed sporeheads have a vertically striped appearance (Pontecorvo *et al.*, 1953) and indicate the heterokaryotic nature of the conidiophore vesicle from which the phialides and ultimately the conidial chains have been derived by mitosis. Observation of striped heterokaryotic sporeheads has been used as a definitive test for heterokaryon compatibility in *A. nidulans* and is the basis for screening under non-selective conditions (Grindle, 1963*a, b*; Caten & Jinks, 1966; Croft & Jinks, 1977).

Using these methods 100 wild isolates of *A. nidulans* in the University of Birmingham strain collection have been assigned to 19 h-c groups (h-cA, h-cB etc.) (Grindle, 1963*a, b*; Croft & Jinks, 1977). None of these strains are compatible with the commonly held laboratory stocks of *A. nidulans* derived from NRRL 194 (Thom & Raper, 1945) which have been assigned to a twentieth group (h-cG1) (Croft & Jinks, 1977). These heterokaryon compatibility differences do not disrupt the sexual cycle and pairs

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of heterokaryon-incompatible strains will readily form hybrid cleistothecia in the laboratory (Jinks *et al.*, 1966; Butcher, 1968). Analysis of sexual cross progenies has indicated that heterokaryon incompatibility in *A. nidulans* is heterogenically controlled by a series of nuclear *het* genes. An allelic difference between parental strains at one or more *het* loci causes incompatibility (Croft & Jinks, 1977).

Protoplast fusion has been used to produce somatic hybrids from heterokaryon-incompatible strains (Dales & Croft, 1977, 1983; Dales *et al.*, 1983). The colonies produced are often diffuse mycelial mats with sparse conidiation. Individual sporeheads are usually small, enclosed in an aqueous exudate and only rarely develop pigmentation. The hyphae, though, are often pigmented and sometimes this pigmentation is secreted into the surrounding medium. Analysis of colony morphology, stability and, more importantly, the segregation of genetical markers among breakdown sectors collected from hybrids has indicated that both heterokaryotic and diploid colonies can be isolated following protoplast fusion of heterokaryon-incompatible parental strains (Dales & Croft, 1977, 1983; Dales *et al.*, 1983). This has allowed the parasexual analysis of *het* genes. Two *het* loci, *hetA* and *hetB*, were identified which segregated among haploid segregants obtained from a diploid formed between a strain belonging to h-cB and an h-cG1 master strain (Dales *et al.*, 1983). With two *het* genes segregating there were four progeny compatibility classes of which two were parental and two recombinant. The recombinant classes were composed of strains that differed from both parental h-c group strains at a single *het* locus. It was straightforward to observe how the parental markers segregated with each recombinant compatibility class and this indicated *het* gene locations for *hetA* and *hetB* on linkage groups V and VI, respectively (Dales *et al.*, 1983).

Situations which involve more than two segregating *het* genes are much more difficult to analyse in this way, as it is not easy to generate the necessary compatibility tester strains. To avoid this problem a chromosome assay method has been used to investigate the haploid segregants obtained from a hybrid strain produced by protoplast fusion of an h-cQ strain with an h-cG1 master strain (Dales & Croft, 1983). The use of this method presumes complete linkage through the parasexual cycle of a *het* gene, or genes, and a marker gene located on the same chromosome. Pairs of parasexually derived progeny strains were selected that had alternative parental alleles for the marker on one linkage group, the linkage group under test, and identical alleles for the markers on the remaining linkage groups, with the exception of linkage group I. Heterozygosity for linkage group I was required to provide a spore colour difference. These

strain pairs were compatibility tested. No *het* genes were detected on linkage group I. Consequently, a compatible result indicated there were no *het* gene differences, between the parental h-c group strains, on the linkage group under test. Incompatibility indicated that *het* gene differences were present on that linkage group. Each linkage group was tested in turn using a small number of different progeny strain pairings. Thus rare parasexual recombinants which had arisen by the disruption of the linkage between a *het* gene, or genes, and the marker gene on the same chromosome, could be detected and not confound the assay.

Although the h-cQ/h-cG1 hybrid was not heterozygous for the marker on linkage group VI it was still possible to complete the chromosome assay. Subsequent sexual crosses of selected parasexually derived progeny strains with h-cG1 strains established the number of *het* gene locations on each linkage group (Dales & Croft, 1983). It was shown that the h-cQ strain differed from h-cG1 strains at five *het* loci. Two genes were detected on linkage group III and one gene on each of linkage groups V, VI and VII.

This chromosome assay method followed by selected sexual crossing has now been used to investigate the number and location of *het* genes acting between a strain of h-cA and an h-cG1 master strain.

Methods

Strains. The strains of *A. nidulans* used are described in Table 1.

Media. Czapek (Cz) medium was derived from the formula of Thom & Church (1926). The ingredients per litre were: NaNO₃, 2.0 g; KCl, 0.5 g; MgSO₄·7H₂O, 0.5 g; ZnSO₄·7H₂O, 0.01 g; K₂HPO₄, 1.0 g; FeSO₄·7H₂O, 0.01 g; sucrose, 30.0 g; agar (Oxoid no. 3), 15.0 g. This medium was used routinely as a minimal medium. Auxotrophic strains were cultured on Cz supplemented with the particular nutrient(s) required. The ability to utilize lactose as the sole carbon source was tested by using Cz medium in which lactose (10.0 g l⁻¹) was used in place of sucrose.

Strain culture. General culture methods and methods for the production and analysis of sexual crosses were derived from those of Pontecorvo *et al.* (1953) and Clutterbuck (1974). Methods for heterokaryon compatibility testing, the isolation, fusion and regeneration of protoplasts, the haploidization of diploid strains, and the detection of *het* genes by chromosome assay have been described previously (Dales & Croft, 1977, 1983; Dales *et al.*, 1983).

Terminology. Throughout this paper the terms compatible and incompatible will mean heterokaryon compatible and heterokaryon incompatible respectively.

Results and Discussion

Protoplast fusion of strains 7-141 (h-cG1) and 65-5 (h-cA)

Dense protoplast suspensions ($>5 \times 10^6$ protoplasts ml⁻¹) were prepared from strains 7-141 and 65-5. These

Table 1. *Strains of A. nidulans*

Strains 7-37 and 7-141 are members of h-cG1 and are derivatives of NRRL 194. Strain 7-141 is FGSC 375. Gene symbols and locus letters are as defined by Clutterbuck (1974). Strain 65-5 is a UV-induced mutant derivative of wild isolate 65 in the Birmingham strain collection and is a member of h-cA. A sexual cross with wild isolate 65 (data not shown) generated only a low frequency of aneuploid types among the progeny. This suggests that strain 65-5 is translocation-free (Pollard *et al.*, 1968; Upshall & Käfer, 1974) with respect to wild isolate 65. Gene symbols are as defined by Clutterbuck (1974). Locus letters are arbitrary, e.g. *paba-65.1* represents the first *para*-aminobenzoic acid (PABA) requiring mutant to be isolated from wild isolate 65. Roman numerals indicate linkage group.

Birmingham collection strain no.	Genotype
7-37	<i>pabaA6</i> , <i>yA2</i> I I
7-141	<i>suA1adE20</i> , <i>adE20</i> , <i>biA1</i> ; I I I <i>acrA1</i> ; <i>sA4</i> ; <i>pyroA4</i> ; <i>pA2</i> ; II III IV V <i>lacA1</i> ; <i>nicB8</i> ; <i>riboB2</i> VI VII VIII
65-5	<i>paba-65.1</i> , <i>y-65.1</i> I I

were mixed in approximately equal concentrations as estimated by haemocytometer counts and induced to fuse by treatment with polyethylene glycol (30%, w/v, PEG 4000 in 0.1 M-CaCl₂) as described by Dales & Croft (1977). The protoplast fusion mix was plated out on osmotically stabilized agar (Cz + 0.6 M-KCl) in soft agar overlays and incubated at 35 °C. Small colony centres composed of just a few hyphae were visible on these plates after 3 d incubation. Several hundred such colony centres regenerated but on prolonged incubation the majority showed no further mycelial growth and excreted a dark-red pigment into the surrounding agar. Only 12 colony centres showed continued growth and after 7 d incubation these colonies showed the fan-shaped sectors that are characteristic of nutritionally complementing, balanced heterokaryons (Dales & Croft, 1977, 1983; Dales *et al.*, 1983). The mycelium of these colonies, though, was severely affected when compared with balanced heterokaryons from compatible pairings and was composed of a loose network of hyphae with most of the growth being below the agar surface. A dark-red pigmentation developed within these hyphae and diffused into the surrounding agar. The sparse hyphae on the agar surface produced very few conidiophores and the sporeheads produced were small, watery and unpigmented.

A total of 350 small agar block subcultures were made both from the 12 sectoring-growth colonies and by

complete transfer of non-sectoring colony centres to fresh plates of Cz agar. Of these 350 inoculations only 17 showed a sectoring growth pattern and these were all subcultures from the 12 original sectoring growth colonies.

The 17 colonies on Cz agar were morphologically similar to the 12 colonies on Cz + 0.6 M-KCl agar. The mycelial growth was again mainly below the agar surface, occasionally surfacing to form small regions of aerial growth. Consequently, the colonies appeared to be composed of discrete clumps of surface mycelium separated by bare agar surface. This effect was accentuated as the subsurface hyphae were deeply pigmented due to the accumulation of red pigment, while the aerial hyphae were hyaline. The rare conidial heads were again small, watery and generally unpigmented, though faint pale-green or yellow sporeheads were occasionally observed. Striped heterokaryotic conidial heads were not detected.

Subcultures to plates of Cz agar fully supplemented for all the auxotrophic markers carried by both of the parental strains, i.e. the removal of nutritional selection, led to the growth of vigorous sectors of both parental phenotypes. Analysis of these sectors confirmed that they were parental genotypes and indicated the probable heterokaryotic nature of the colonies from which these sectors arose, though it was possible that the 17 colonies could have been mixed cultures of the original parental strains that were complementing on minimal medium by cross-feeding.

A dense suspension of conidia and hyphal fragments was prepared from the aerial clumps of the sectoring growth colonies on Cz agar. This suspension was plated in soft agar overlays on fresh plates of Cz agar. After 4 d incubation at 35 °C a confluent lawn of pink-pigmented mycelium was produced on all of the plates. Single-spore purifications gave rise to symmetrically round colonies of thin, pink-pigmented mycelium that was stable on transfer to fully supplemented agar. Sporehead formation was rare, though all were pale green. The mean conidial diameter was larger ($\sim 1.3\times$) than that of haploid conidia. These pink colonies were assumed to be diploid and a single culture (RD15) was retained for further analysis.

Haploidization and chromosome assay

Small agar blocks were cut from a colony of strain RD15, transferred to plates of fully supplemented Cz agar containing 1.0, 1.1, 1.2, 1.3, 1.4 or 1.5 µg Benlate (E. I. du Pont de Nemours) ml⁻¹, and incubated at 35 °C for 5 d to induce haploidization. These transfers produced normal (wild-type) green, normal yellow as well as pale-green and pale-yellow spored sectors from restricted colony

centres at all the Benlate concentrations used. This segregation of conidial colour was good evidence for the hybrid nature of the RD15 strain and, by extrapolation, of the heterokaryotic nature of the 17 colonies isolated following protoplast fusion.

A parasexual progeny sample of 300 segregants (RD15-1 to RD15-300) was collected by subculturing from the sectors produced on the Benlate-containing plates. Only one segregant sector per colony centre was sampled to avoid re-isolating the same, or the reciprocal product, of a single haploidization event. The segregants were purified by spreading to give single colonies. An inoculum from each purification spread was collected onto 4 × 4 master plates and replicated onto a range of differential media to analyse the segregation of standard markers (Tables 2, 3 and 4). The master plates were also

inoculated onto fully supplemented Cz agar containing 1.5 µg Benlate ml⁻¹ to test whether the segregants were haploid (Upshall *et al.*, 1977). This test, in combination with conidial diameter measurements (data not shown), indicated that nine of the progeny strains were still diploid and these have been excluded from further analysis.

Tables 2 and 3 show that all of the remaining 291 progeny strains were wild-type for the *lacA* marker on linkage group VI. This indicates that RD15 is a recombinant diploid in which structural homozygosity has been achieved for part, or all, of chromosome VI. Similar observations have been reported for h-cB/h-cGl and h-cQ/h-cGl diploids (Dales *et al.*, 1983; Dales & Croft, 1983). It is suspected that *het* gene heterozygosity on this linkage group can generate a strong selective

Table 2. Segregation of standard markers among the 291 parasexual progeny derived from the recombinant diploid RD15

Strain RD15 was obtained by protoplast fusion of strains 65-5 and 7-141. The genotype classes recovered are numbered in parentheses and the number of progeny strains obtained for each genotype class are presented.

III <i>sa4</i>	IV <i>pyroA4</i>	V <i>pA2</i>	VI <i>lacA1</i>	VII <i>nicB8</i>	VIII <i>riboB2</i>	I			
						paba-65.1 y-65.1 + bi + paba + ⁺ <i>biA1</i>			
						II		II	
						+ <i>acr</i>	<i>acrA1</i>	+ <i>acr</i>	<i>acrA1</i>
+	+	+	+	+	+	(48) 3	(47) 2		
+	+	+	+	+	<i>ribo</i>	(10) 4		(75) 6	(87) 2
+	+	+	+	<i>nic</i>	+				
+	+	+	+	<i>nic</i>	<i>ribo</i>	(26) 2	(20) 2	(61) 2	(71) 2
+	+	<i>p</i>	+	+	+	(49) 1			
+	+	<i>p</i>	+	+	<i>ribo</i>	(6) 5	(18) 1	(81) 2	(83) 1
+	+	<i>p</i>	+	<i>nic</i>	+	(33) 1			
+	+	<i>p</i>	+	<i>nic</i>	<i>ribo</i>	(25) 3		(74) 1	(69) 1
+	<i>pyro</i>	+	+	+	+	(45) 12	(4) 3	(55) 3	(57) 2
+	<i>pyro</i>	+	+	+	<i>ribo</i>	(9) 2	(8) 3	(84) 3	(86) 2
+	<i>pyro</i>	+	+	<i>nic</i>	+	(30) 2	(29) 3	(92) 5	(97) 2
+	<i>pyro</i>	+	+	<i>nic</i>	<i>ribo</i>	(2) 6	(40) 2	(67) 2	(73) 2
+	<i>pyro</i>	<i>p</i>	+	+	+	(46) 4	(1) 9	(56) 3	(51) 2
+	<i>pyro</i>	<i>p</i>	+	+	<i>ribo</i>	(5) 3	(13) 2	(89) 2	(85) 3
+	<i>pyro</i>	<i>p</i>	+	<i>nic</i>	+	(34) 4	(31) 1	(90) 3	(93) 2
+	<i>pyro</i>	<i>p</i>	+	<i>nic</i>	<i>ribo</i>	(42) 4	(23) 6	(72) 2	(59) 5
<i>s</i>	+	+	+	+	+	(50) 1			
<i>s</i>	+	+	+	+	<i>ribo</i>	(16) 3		(88) 1	
<i>s</i>	+	+	+	<i>nic</i>	+				
<i>s</i>	+	+	+	<i>nic</i>	<i>ribo</i>	(3) 2		(60) 1	(62) 1
<i>s</i>	+	<i>p</i>	+	+	+				
<i>s</i>	+	<i>p</i>	+	+	<i>ribo</i>	(11) 2	(7) 7	(78) 2	(80) 2
<i>s</i>	+	<i>p</i>	+	<i>nic</i>	+	(35) 2			
<i>s</i>	+	<i>p</i>	+	<i>nic</i>	<i>ribo</i>	(43) 1	(39) 2	(68) 2	(66) 2
<i>s</i>	<i>pyro</i>	+	+	+	+	(44) 2	(24) 2	(52) 1	(58) 2
<i>s</i>	<i>pyro</i>	+	+	+	<i>ribo</i>	(12) 5	(15) 2	(77) 6	(79) 6
<i>s</i>	<i>pyro</i>	+	+	<i>nic</i>	+	(32) 5	(37) 1	(91) 4	(95) 4
<i>s</i>	<i>pyro</i>	+	+	<i>nic</i>	<i>ribo</i>	(27) 3	(41) 2	(65) 8	(63) 3
<i>s</i>	<i>pyro</i>	<i>p</i>	+	+	+	(22) 6	(19) 2	(53) 2	(54) 1
<i>s</i>	<i>pyro</i>	<i>p</i>	+	+	<i>ribo</i>	(14) 2	(17) 3	(76) 4	(82) 4
<i>s</i>	<i>pyro</i>	<i>p</i>	+	<i>nic</i>	+	(36) 1	(28) 2	(96) 1	(94) 2
<i>s</i>	<i>pyro</i>	<i>p</i>	+	<i>nic</i>	<i>ribo</i>	(21) 7	(38) 6	(64) 6	(70) 5

Table 3. Segregation of alleles among the 291 parasexual progeny derived from the recombinant diploid RD15

Chi-square (χ^2) tests of significance were done using Yates' correction. The probability levels are abbreviated as follows: * probability (P) between 0.05 and 0.01; ** P between 0.01 and 0.001; *** P less than 0.001; NS represents non-significance (P greater than 0.05).

Alleles	Nos of progeny strains	χ^2	Significance
+ γ : γ -65.1	130 : 161	3.09	NS
+ <i>paba</i> : <i>paba</i> -65.1	130 : 161	3.09	NS
<i>biA1</i> : + <i>bi</i>	130 : 161	3.09	NS
<i>acrA1</i> : + <i>acr</i>	121 : 170	7.92	**
<i>sA4</i> : + <i>s</i>	141 : 150	0.22	NS
<i>pyroA4</i> : + <i>pyro</i>	219 : 72	73.25	***
<i>pA2</i> : + <i>p</i>	147 : 144	0.01	NS
<i>lacA1</i> : + <i>lac</i>	0 : 291	289.00	***
<i>nicB8</i> : + <i>nic</i>	138 : 153	0.67	NS
<i>riboB2</i> : + <i>ribo</i>	183 : 108	18.82	***

disadvantage (Dales & Croft, 1983). Apart from *lacA*, biased allelic ratios were observed for *acrA*, *pyroA* and *riboB* (Table 3). These probably reflect differential allele viability. The allelic segregation of *adE* was masked by the presence of the suppressor of adenine requirement (*suA1adE20*). All the progeny were prototrophic for adenine and so segregations for *adE* and *suA1adE20* could not be included in Tables 2, 3 and 4.

Linkage was detected between the γ -65, *paba*-65 and *biA* loci on linkage group I as was expected (Table 4). Linkage was also indicated for *acrA* with *pA*, *acrA* with *lacA*, and *sA* with *riboB*. It is unlikely that linkage is involved, though, as haploidization should lead to the assortment of complete linkage groups (McCully & Forbes, 1965). Mitotic recombination should only disrupt such linkages in approximately 1% of haploidization events (Käfer, 1961, 1977). Values as high as 43.3% (*acrA* : *pA*), 41.6% (*acrA* : *lacA*) and 42.6% (*sA* : *riboB*)

Table 4. Pairwise segregation of markers among the 291 parasexual progeny derived from the recombinant diploid RD15

χ^2 tests were done and probability levels indicated as described for Table 3.

Marker pair	Progeny nos		Recombination frequency	χ^2	Significance
	Parental	Recombinant			
γ -65 : <i>paba</i> -65	291	0	0.000	289.00	***
γ -65 : <i>biA</i>	291	0	0.000	289.00	***
γ -65 : <i>acrA</i>	156	135	0.464	1.37	NS
γ -65 : <i>sA</i>	160	131	0.450	2.69	NS
γ -65 : <i>pyroA</i>	146	145	0.498	0.00	NS
γ -65 : <i>pA</i>	134	157	0.540	1.66	NS
γ -65 : <i>lacA</i>	161	130	0.447	3.09	NS
γ -65 : <i>nicB</i>	159	132	0.454	2.32	NS
γ -65 : <i>riboB</i>	160	131	0.450	2.69	NS
<i>acrA</i> : <i>sA</i>	151	140	0.481	0.34	NS
<i>acrA</i> : <i>pyroA</i>	143	148	0.509	0.06	NS
<i>acrA</i> : <i>pA</i>	165	126	0.433	4.96	*
<i>acrA</i> : <i>lacA</i>	170	121	0.416	7.92	**
<i>acrA</i> : <i>nicB</i>	148	143	0.491	0.06	NS
<i>acrA</i> : <i>riboB</i>	145	146	0.502	0.00	NS
<i>sA</i> : <i>pyroA</i>	151	140	0.481	0.34	NS
<i>sA</i> : <i>pA</i>	155	136	0.467	1.11	NS
<i>sA</i> : <i>lacA</i>	150	141	0.485	0.22	NS
<i>sA</i> : <i>nicB</i>	158	133	0.457	1.98	NS
<i>sA</i> : <i>riboB</i>	167	124	0.426	6.06	*
<i>pyroA</i> : <i>pA</i>	143	148	0.509	0.06	NS
<i>pyroA</i> : <i>lacA</i>	72	219	0.753	73.25	***
<i>pyroA</i> : <i>nicB</i>	156	135	0.464	1.37	NS
<i>pyroA</i> : <i>riboB</i>	131	160	0.550	2.69	NS
<i>pA</i> : <i>lacA</i>	144	147	0.505	0.14	NS
<i>pA</i> : <i>nicB</i>	150	141	0.485	0.22	NS
<i>pA</i> : <i>riboB</i>	157	134	0.460	1.66	NS
<i>lacA</i> : <i>nicB</i>	153	138	0.474	0.67	NS
<i>lacA</i> : <i>riboB</i>	108	183	0.629	18.82	***
<i>nicB</i> : <i>riboB</i>	156	135	0.464	1.37	NS

Table 5. *Heterokaryon compatibility tests between selected pairs of strains to test for het gene interaction*

Linkage group under test	Strain pair	Genotype class*	Compatibility test†	Linkage group under test	Strain pair	Genotype class*	Compatibility test†			
I	{	RD15-29	10	+	V	{	RD15-45	38	—	
		RD15-170	75				RD15-190	63		
		RD15-35	26				RD15-59	41		—
		RD15-181	61				RD15-237	70		
		RD15-26	25				RD15-29	10		—
		RD15-271	74				RD15-189	81		
		RD15-57	45				RD15-63	46		—
		RD15-210	55				RD15-210	55		
II	{	RD15-45	38	—	VI	{	RD15-151	36	—	
		RD15-193	64				RD15-192	91		
		RD15-29	10				RD15-237	70	—	
		RD15-231	87				7-37			—
		RD15-1	1				RD15-245	70	—	
		RD15-214	56				7-37			—
		RD15-3	3				RD15-284	70	—	
		RD15-182	62				7-37			—
RD15-53	44	RD15-45	38	—						
RD15-272	58	7-141			—					
III	{	RD15-45	38	—		VII	{	RD15-84	38	—
		RD15-174	59		7-141				—	
		RD15-30	11		RD15-45			38		—
		RD15-189	81		7-141				—	
		RD15-29	10		RD15-196			82		—
		RD15-234	88		RD15-29			10	—	
		RD15-3	3		RD15-181			61		—
		RD15-181	61		RD15-57			45	—	
RD15-7	19	RD15-191	92	—						
RD15-171	51	RD15-50	7		—					
IV	{	RD15-45	38	+		VIII	{	RD15-240	66	—
		RD15-240	66		RD15-32			30	—	
		RD15-88	48		RD15-210			55		—
		RD15-210	55		RD15-13			28	+	
		RD15-127	49		RD15-237			70		+
		RD15-214	56		RD15-88			48	+	
		RD15-59	41		RD15-172			75		+
		RD15-182	62		RD15-57			45	+	
RD15-46	39	RD15-217	84	+						
RD15-237	70	RD15-168	50		+					
		RD15-234	88	+						
		RD15-59	41		+					
		RD15-212	95	+						

* Genotype classes are derived from Table 2.

† +, Compatibility; -, incompatibility.

must, therefore, reflect distortions from random segregation. Distorted pairwise segregations were also obtained for *pyroA* and *riboB* with *lacA*. In both cases excess recombinant types were recovered. These unexpected segregations may reflect chance variation or sampling error compounded with differential allele viability. Despite deviations from 50:50 ratios the observed segregations suggest a translocation-free relationship between the h-cA and h-cGl parental strains with the possible exception of linkage group VI.

Pairs of progeny strains were selected (Table 5) that differed for chromosome I markers. This provided a spore colour difference for compatibility testing. Five such pairs of strains were used that carried identical combinations of alleles for the markers on linkage groups II to VIII. These were tested for compatibility to screen for *het* gene interaction on linkage group I. No *het* genes were detected on this linkage group (Table 5). Other sets of five pairs of strains carried allelic differences for each of the markers on linkage groups II, III, IV, V, VII and

Table 6. Sexual crosses done to establish the number of *het* gene differences operating on linkage groups II, III, V, VI, and VII between the parental strains 65-5 and 7-141, of h-cA and h-cG1 compatibility respectively

Cross number	Strains crossed*	Linkage groups under test	Total progeny sample	Compatibility classes		No. of <i>het</i> genes	Linkage group location
				Parental	Recombinant		
				A	G1		
RD24	RD15-237 × 7-37 (70)	VI	100	47, 53		1	VI
RD25	RD15-193 × 7-37 (64)	II and VI	64	16, 19	15, 14	2	VI, II
RD26	RD15-174 × 7-37 (59)	III and VI	102	16, 17	15, 14, 6, 17, 9, 8 28, 29	3	VI, III, III
RD27	RD15-190 × 7-37 (63)	V and VI	100	19, 24	21, 29	2	VI, V
RD28	RD15-196 × 7-37 (82)	VI and VII	97	26, 21	21, 29	2	VI, VII

* The numbers in parentheses denote genotype class (derived from Table 2).

VIII in turn. These strains were compatibility tested to screen for *het* gene activity on each of these linkage groups. The lack of segregation for linkage group VI prevented the selection of strain pairs from within the RD15 progeny that carried alternative alleles at the *lacA* locus. Two progeny genotype classes were detected that carried marker alleles derived from the h-cG1 parent (7-141) on all linkage groups except VI (genotype class 70) or I and VI (genotype class 38). Representatives of these two genotype classes were compatibility tested with the h-cG1 strains 7-37 or 7-141, respectively, to test for *het* gene interaction on linkage group VI. The results presented in Table 5 show that *het* genes were segregating among the RD15 progeny on linkage groups II, III, V, VI and VII.

Determination of the total number of het gene differences between the parental strains 65-5 and 7-141

Selected RD15 progeny strains were backcrossed to the h-cG1 strain 7-37. Progeny samples were purified, collected onto 4 × 4 master plates and genotyped for standard markers and compatibility. The crosses and the numbers of progeny compatibility classes generated are presented in Table 6. These indicate a total of six *het* gene differences between the original parental strains 65-5 and 7-141. Two of these differences are located on linkage group III and one on each of linkage groups II, V, VI and VII.

These results are similar to those obtained previously from the analysis of a hybrid strain produced by protoplast fusion of an h-cQ strain (106-2) and an h-cG1

master strain (7-21), where five *het* gene differences were detected (Dales & Croft, 1983). No *het* gene location was detected on linkage group II between these h-c groups. Whether any of the *het* genes on linkage groups III, V, VI and VII in h-cA strains are identical to, or allelic with, the *het* genes on the corresponding linkage groups of h-cQ strains has not yet been determined.

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