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Two locally acting genetic controls of gene conversion, *ccf-5* and *ccf-6*, in *Ascobolus immersus*

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SUMMARY

Two new conversion control factors (*ccf*s), *ccf-5* and *ccf-6*, have been characterized in the Pasadena strains of *Ascobolus immersus*. Both are monogenic, with two known allelic forms (called *A* and *B*) of each factor, and affect the frequency of meiotic gene conversion at a *white* (*w*) ascospore locus closely linked to it, *ccf-5* affecting *w-9* and *ccf-6* affecting *w-BHj*. The *ccf*s appear to be specific to their own target site, with no effect on at least nine unlinked *w* mutations. Conversion of the *w* locus affected was studied in $+ \times w$ crosses with all four possible *ccf* arrangements: for example, for $+ \times w\text{-}9$, with *ccf-5*(*A*) in both parents, with *ccf-5*(*B*) in both parents, with *ccf-5*(*A*) in +, *B* in *w-9*, and with *ccf-5*(*B*) in +, *A* in *w-9*. For both *ccf*s, there were slight differences between crosses homozygous for *A* and those homozygous for *B*, and also slight differences between the two forms of heterozygous cross, *A*/*B* and *B*/*A*, but the major effect was for heterozygosity for the control factor to depress conversion frequency of the *w* locus, compared with either homozygous state. These two *ccf*s are compared with other sites affecting recombination in fungi and higher eukaryotes. Two possible modes of action of *ccf*s *5* and *6* are (i) on pairing closeness before hybrid DNA initiation, and (ii) on later stages such as the spread of hybrid DNA from an initiation point.

1. INTRODUCTION

In the Pasadena strains of *Ascobolus immersus* several genetic factors are known which control the parameters of gene conversion at various sites in the genome. The system now known as conversion control factor 1 (*ccf-1*) affects the conversion behaviour of closely linked white ascospore pigmentation mutation *w-62* such that crosses homozygous for either known form of *ccf-1* give much higher *w-62* conversion frequencies than heterozygous crosses (Emerson & Yu-Sun, 1967). Conversion at ascospore pigmentation locus *wI*, which is unlinked to *w-62*, is controlled by at least three separate factors (*ccf*s, *-2*, *-3* and *-4*), the different genotypes of which give conversion frequencies averaging from 0·66% to about 27% for mutation *wI-78* (Helmi & Lamb, 1983). *ccf-2* (which is closely linked to

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wI) consists of three forms showing incomplete dominance to each other and cis/trans position effects on *wI* conversion (Lamb & Helmi, 1978). The 'Super' factor (Helmi & Lamb, 1979) has been shown to consist of two unlinked, interacting components, *ccf-3* and *ccf-4*, and approximately doubles whatever gene conversion frequency at *wI* is determined by the *ccf-2* factors. The interactions between *ccf-3* and *ccf-4* are dependent on the genotypes of the *ccf-2* factors present (Helmi & Lamb, 1983).

Lamb & Ghikas (1979) studied the gene conversion properties of 29 induced and 9 spontaneous white ascospore pigmentation mutations. Six mutations (other than those at *wI*) showed different conversion behaviour according to which derived wild-type strains they were crossed to. This paper describes an investigation of the genetic determination of the conversion behaviour of two of those mutations, *w-9* and *w-BHj*, which are unlinked to each other and to *w-62* and *wI* (Ghikas, 1978; Howell, 1982). In both cases hitherto unknown conversion control factors, *ccf*s-*5* and -*6*, were responsible, such that crosses homozygous for either form of *ccf-5* gave much higher conversion frequencies of the closely linked *w-9* mutation than heterozygous crosses. The two forms of *ccf-6* acted in a similar but less pronounced way on linked mutation *w-BHj*. *ccf*s-*5* and -*6* appear very similar to *ccf-1* in the Pasadena strains of *Ascobolus immersus*, the *cv* modifiers in the European strains of the same species (Girard & Rossignol, 1974), the *ss* recombination modulator in *Neurospora crassa* (Catcheside, 1981) and probably to the controls of intragenic recombination in the *adh-1* locus of *Zea mays* (Freeling, 1978) and in the *rosy* gene of *Drosophila melanogaster* (Chovnick, Ballantyne & Holm, 1971).

2. MATERIALS AND METHODS

Strains were descended from the two red-spored wild-type strains P5- and K5+ isolated in Pasadena by Dr Yu-Sun. These original strains were not used in this work, and so all wild-type strains used will be referred to as derived wild-type to denote that they are laboratory strains derived from the original isolates from the wild. White ascospore pigmentation mutations *w-9* and *w-BHj* were ultraviolet light-induced by Lamb & Ghikas (1979).

Methods were basically as described by Ghikas & Lamb (1977) and Wickramaratne & Lamb (1978), with all crosses incubated at 17·5 °C. Those crosses involving individual spore isolates were performed in duplicate, while crosses with the various combinations of the *ccf* factors comprised many individual crosses with replicates for each combination of *ccf* factors. Wild-type red spores were germinated using horse-dung extract agar (Lamb & Helmi, 1978) with 2 h heat shock at 50 °C. *w-9* white ascospores germinated optimally on horse-dung extract agar with 4 h heat shock at 50 °C, while the optimum germination conditions for *w-BHj* ascospores were 2 % water agar and 30 min heat shock at 50 °C (Howell, 1982).

3. RESULTS

The genetic controls of w-9 *conversion behaviour*

(i) *Preliminary experiments*

w-9 mutant strains were crossed to seven derived wild-type strains and the results from these crosses are shown in Table 1. For simplicity only data for *w-9*

narrower ratio conversion classes (4:4, 6:2, 2:6, 5:3 and 3:5) are given because the wider ratio classes (8:0, 0:8, 7:1 and 1:7) were much rarer and their omission has little effect. The results show that the narrower ratio conversion frequencies fall into two clearly defined categories. The first two crosses showed much lower conversion frequencies (3·14 and 3·47%) than the remaining five crosses (10·14–15·44%). No intermediate conversion frequency was obtained. The two

Table 1. *Conversion data from* + × w-9 *crosses*

Mutant strain	Crossed to*	Octad classes (+ : *w*), % 4:4	6:2	2:6	5:3	3:5	%NRCF†	Total octads
w-9+	92−	96·82	1·68	0·55	0·68	0·23	3·14	4711
w-9−	93+	96·53	1·42	1·54	0·51	—	3·47	777
w-9+	7R7−	87·91	6·49	0·89	3·17	0·48	11·03	4162
w-9−	25-5R+	89·76	5·21	1·23	3·60	0·09	10·14	1055
w-9+	RS8-1−	84·56	7·87	1·11	5·55	0·91	15·44	991
w-9+	RS17-1−	88·61	5·18	0·62	4·55	1·04	11·39	966
w-9+	RS17-3−	88·27	6·54	0·59	3·81	0·64	11·59	4203

* Strains are all derived wild types. Final + and − indicate mating type.

† NRCF = narrower ratio conversion frequency $= \frac{(6:2+2:6+5:3+3:5)\times 100}{\text{total octads}}$.

low-conversion-frequency (LCF) crosses were homogeneous ($\Sigma\chi^2 = 0{\cdot}10$, $P = 0{\cdot}70$–$0{\cdot}80$, 1 D.F.) and if the data from cross *w-9*+ × RS8-1− are excluded, the high-conversion-frequency (HCF) crosses also show good homogeneity for conversion frequency ($\Sigma\chi^2 = 1{\cdot}86$, $P = 0{\cdot}50$–$0{\cdot}70$, 3 D.F.). The difference in conversion frequency for the two sets of crosses was in good agreement with the earlier results of Lamb & Ghikas (1979) and strongly suggested that the conversion behaviour of *w-9* is under genetic control; the system involved cannot be *ccf-2* since different derived wild-type strains (92− and 7R7−) carrying the same, *P*, form of *ccf-2* can give LCF and HCF when crossed to the same *w-9* mutant strain. A simple, easily testable model of conversion control was assumed (a closely linked, monogenic system) as the basis for the following experimental programme of crosses.

When strain *w-9*+ was crossed to derived wild-type strains 92− and 7R7−, very different conversion frequencies resulted (Table 1). Since the *w-9*+ strain was the same in each case, if the conversion control of this mutation is monogenic, strains 92− and 7R7− must carry different forms of the conversion control factor (*ccf*) so that either LCF cross *w-9*+ × 92− or HCF cross *w-9*+ × 7R7− must be heterozygous for different forms of the *ccf*. In a cross doubly heterozygous for a white mutation and a closely linked conversion control factor, analysis of the conversion behaviour of the 6 wild-type spore isolates from 6+ : 2*w* convertant octads (whether they arise by symmetrical or asymmetrical hybrid DNA formation) should reveal that four spores carry the same form of the control factor as the derived wild-type parent while two spores will have inherited the control factor originally present in the white mutant parent. This will be so if the white mutation and its *ccf* do not usually co-convert and will be apparent unless certain forms of dominance are involved.

(ii) *Conversion behaviour of wild-type isolates from* 6+:2w *octads from cross* w-9+ × 92−

Wild-type spores were isolated from 35 6+:2*w* convertant octads from LCF cross *w-9*+ × 92−. Since ascospore germination was poor and it was necessary to cross all wild-type isolates to the same *w-9*+ strain (since our only *w-9*− strain may not have carried the same form of the *ccf* as *w-9*+), complete analysis of the conversion behaviour of all six wild-type spore isolates in any octad was not possible. Thus the data must be dealt with as originating from wild-type spores randomly isolated from a population of 6+:2*w* octads. If little or no co-conversion of the *w-9* mutation and its *ccf* occurs and the latter is indeed monogenic and closely linked, a ratio of 2 LCF:1 altered CF among the crosses described would be expected, with the provisos already mentioned.

Twenty-five wild-type spore isolates were crossed to strain *w-9*+ and generally 1000–2000 octads were scored per cross, although some sample sizes fell outside this range. Twenty-one crosses showed LCF (1·50 % [data from one replicate only] to 5·72 %, S.E. ±0·20) while 4 crosses showed altered, high conversion frequencies of 11·91 % ±0·31 to 13·51 % ±0·11 (Howell, 1982). These values of 21:4 fit the expected 2:1 ratio of 2 LCF:1 altered CF crosses (χ^2, applying Yates' correction, = 2·64, P = 0·10–0·20, 1 D.F.), but fewer segregants than expected with altered conversion properties were detected. Perhaps this was due to sampling effects, or some co-conversion of *w-9* and its *ccf* could be occurring.

The results from this analysis suggested that LCF cross *w-9*+ × 92− is heterozygous for at least one *ccf* affecting the conversion of *w-9*. This *ccf* may be closely linked to *w-9* since some co-conversion of the two sites is consistent with the data. On this basis, HCF cross *w-9*+ × 7R7− should be homozygous for the *ccf* in question and a similar analysis of the conversion properties of wild-type isolates from 6+:2*w* octads should reveal that none exhibits altered conversion behaviour in backcrosses to *w-9* strains.

(iii) *Conversion behaviour of wild-type isolates from* 6+:2w *octads from cross* w-9+ × 7R7−

Wild-type spores were isolated from 26 6+:2*w* octads from HCF cross *w-9*+ × 7R7−. Thirty-four spores germinated, from which 18 fertile crosses resulted. All of the crosses were of the HCF type, with conversion frequencies ranging from 9·03 % ±0·38 to 15·59 % ±0·27 (Howell, 1982). The *w-9*+ (13 crosses) and *w-9*− (5 crosses) tester strains behaved in the same way, indicating that both strains probably carry the same form of the *ccf* in question. The variation in conversion frequency could be due to the segregation of minor modifiers of gene conversion in the cross, or imperfectly controlled environmental effects.

Taken together, the above two experiments point to the existence of a *ccf* primarily responsible for determining the degree of gene conversion of mutation *w-9*, giving high conversion frequencies (*c.* 9–15 %) in crosses homozygous for one form of the factor (let this be designated *A*) with reduced conversion frequencies when the factor is heterozygous, at least in *w-9*, *A*, × +, *B* crosses (where *B* is the other form of the factor).

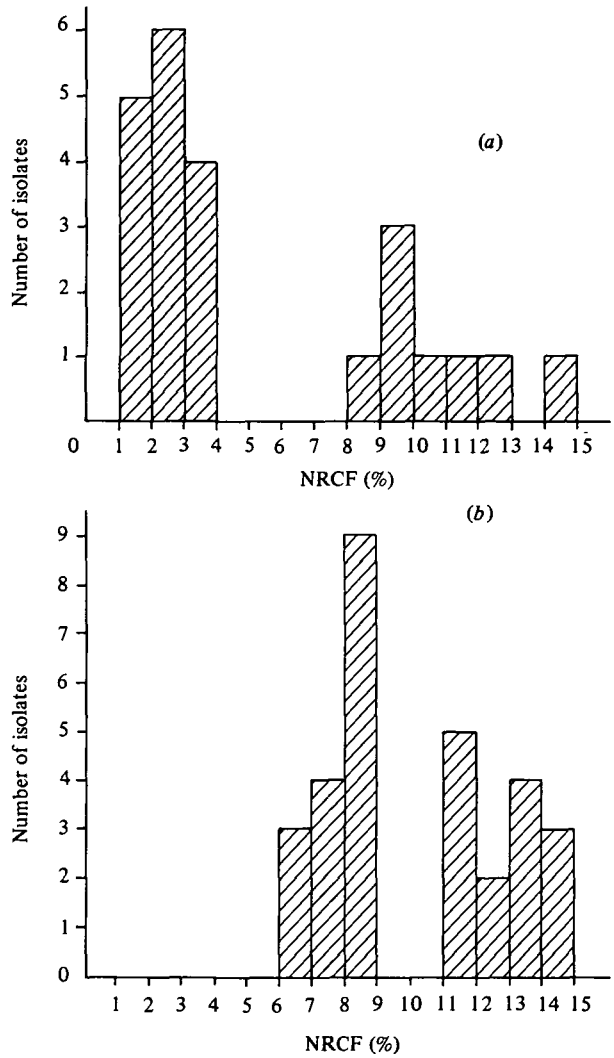

Fig. 1 (*a*). Narrower ratio conversion frequencies of random wild-type isolates from cross 25-5R+ × 92− (i.e. +, *ccf-5*(*A*) × +, *ccf-5*(*B*)) when crossed to *w-9*+.

Fig. 1 (*b*). Narrower ratio conversion frequencies of random wild-type isolates from cross 25-5R+ × BH19-1− (i.e. +, *ccf-6*(*B*) × +, *ccf-6*(*A*)) when crossed to *w-BHj*+ or *w-BHj*−.

(iv) *Monogenic nature of* w-9 *conversion control*

From other data (Howell, 1982) it was deduced that a cross between derived wild-type strains 25-5R+ and 92− would be heterozygous for the *ccf* or *ccfs* responsible for *w-9* conversion control. To distinguish between these alternatives, 200 wild-type spores were randomly isolated from the progeny of cross 25-5R+ × 92− (all octads were 8+ :0*w*).

Fifty-six spores germinated and 23 of these isolates gave fertile crosses with the

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w-9+ tester strain. The data obtained are shown in Fig. 1 (*a*), and it can be seen that 15 isolates gave LCF crosses (with conversion frequencies of 1·27 % ±0·23 to 3·08 % ±0·36) behaving like the 92-parent, while 8 gave HCF crosses (with conversion frequencies of 8·92 % ±0·17 to 14·14 % [data from one replicate only]) behaving like the 25-5R+ parent. If the conversion control of *w-9* is monogenic, a 1:1 ratio of LCF and HCF crosses should be expected from this type of experiment, and the observed values of 15:8 are an acceptable fit to such a 1:1 ratio ($\chi^2 = 2{\cdot}13$, $P = 0{\cdot}10$–$0{\cdot}20$, 1 D.F.). However, the data are also consistent with two pairs of factors interacting, producing two phenotypes either in a 2:1 or, more likely, a 3:1 ratio (χ^2 for 2:1 = 0·02, $P = 0{\cdot}80$–$0{\cdot}90$, 1 D.F.; χ^2 for 3:1 = 1·17, $P = 0{\cdot}20$–$0{\cdot}30$, 1 D.F.). Nevertheless, the results do rule out polygenic control of *w-9* conversion. The possible existence of a second factor affecting *w-9* conversion requires investigation.

The results point to the existence of at least a single conversion control factor acting on *w-9*. This factor is designated as conversion control factor 5 or *ccf-5* (since it was the fifth such factor to be discovered in the Pasadena strains of *Ascobolus*), remembering that it may act on *w-9* in conjunction with other factors. Crosses of the type *w-9*, *ccf-5*(*A*) × +, *ccf-5*(*B*) are LCF and *w-9*, *ccf-5*(*A*) × +, *ccf-5*(*A*) are HCF.

(v) *Conversion behaviour of* w-9 *in* ccf-5(B) *homozygous crosses*

w-9 strains carrying *ccf-5*(*B*) were obtained by isolating white spores from 2+ :6*w* octads from a *w-9*, *ccf-5*(*A*) × +, *ccf-5*(*B*) LCF cross (cross *w-9*+ × 92− : Table 1). If *ccf-5* is closely linked to *w-9*, four white spore isolates from such octads should carry *ccf-5*(*A*) and give LCF crosses with *ccf-5*(*B*) derived wild-type strains, while two white spore isolates should carry *ccf-5*(*B*) and hence may show altered conversion behaviour in crosses to the same tester strains. This will only be seen in all such octads if no co-conversion of *w-9* and *ccf-5* occurs. Due to poor ascospore germination, complete analysis of the conversion behaviour of the six white spore isolates from any octad was impossible. Thus the data were treated as originating from white spores randomly isolated from a population of 2+ :6*w* octads. Accordingly, a ratio of 2:1 for LCF:altered CF crosses might be expected if *w-9*, *ccf-5*(*A*) × +, *ccf-5*(*B*) and *w-9*, *ccf-5*(*B*) × + *ccf-5*(*B*) crosses show different conversion frequencies.

Forty-seven white spore isolates gave fertile crosses with *ccf-5*(*B*) derived wild-type strains. Forty-three crosses were LCF (with conversion frequencies of 1·13 % ±0·21 to 4·57 % ±0·29) while four crosses showed altered, elevated conversion frequencies of 7·86 % ±0·19 to 11·54 % ±0·22 (Howell, 1982). These data do not fit the expected 2:1 ratio (χ^2, applying Yates' correction = 11·94, $P < 0{\cdot}01$, 1 D.F.). Nevertheless, it would still seem that the four isolates showing altered, enhanced conversion frequencies arose by conversion at the *w-9* site and therefore carry *ccf-5*(*B*). So *w-9* appears to show a high conversion frequency in *ccf-5*(*B*) homozygous crosses, very similar to that seen in *ccf-5*(*A*) homozygous crosses.

Thus far fewer segregants with altered conversion frequencies were detected than was expected. Appreciable co-conversion of *w-9* and *ccf-5* along with chance random sampling would explain this. Alternatively, a second *ccf* may be acting

on *w-9* and interacting with *ccf-5* to mask some conversion enhancing combinations of *ccf-5* in a cross (in any experiments which showed fewer than expected segregants of a particular conversion type, it was always the HCF class which was deficient).

(vi) *Conversion behaviour of* w-9 *in* w-9, ccf-5(B) × +, ccf-5(A) *crosses*

From the previous experiment four *w-9* isolates known to carry *ccf-5(B)* were available. Two were of (+) mating type and two of (−). These mutant isolates were crossed to derived wild-type strains 7R7− and 25-5R+ respectively, both shown to carry *ccf-5(A)*. Three of the four crosses were fertile and all showed low *w-9* conversion frequencies of 1·58 % ±0·20 to 2·21 % ±0·14, with a similar conversion spectrum to that seen in *w-9*, *ccf-5(A)* × +, *ccf-5(B)* crosses.

(vii) *Linkage of* w-9 *and* ccf-5

Wild-type and white mutant spores were randomly isolated from 4+ :4*w* octads from a *w-9*, *ccf-5(A)* × +, *ccf-5(B)* LCF cross (*w-9*+ × 92− : Table 1). If *w-9* and *ccf-5* are closely linked, most, if not all, wild-type spores so isolated should carry *ccf-5(B)* and show conversion behaviour like the wild-type parent, while the white spore isolates should mostly carry *ccf-5(A)* and behave like the white-spored parent. Recombinants for the two sites would show altered conversion behaviour in suitable test crosses.

Forty-five random wild-type isolates gave LCFs (1·27 % ±0·28 to 5·35 % ±0·38) when crossed to *w-9*+, i.e. all wild-type isolates showed conversion properties like their derived wild-type parent. Eight random white spore isolates also gave LCFs (1·06 % ±0·18 to 2·51 % ±0·27) when crossed to 92−, i.e. all white spore isolates exhibited conversion properties like the white mutant parent. So no progeny among 53 tested showed recombination between *w-9* and *ccf-5*, showing that the two sites are closely linked.

(viii) *Separate identity and specificity of* ccf-5

ccf-5 did not produce any effect on the conversion behaviour of mutations known to be affected by *ccf*s *-1*, *-2*, *-3*, *-4* and *-6*. Thus *ccf-5* appeared to be in no way associated with any known *ccf* and has a separate identity. Furthermore, *ccf-5* had no effect on the conversion behaviour of 10 unlinked ascospore colour mutations of loci *wII*, *wIII* and *wIV* plus solitary mutations *w-BHj* and *NGw14* (Howell, 1982) and thus its effect appears to be strictly local and possibly specific to *w-9*.

A summary of the action of *ccf-5* on *w-9* is given in Table 2. From this it can be seen that *w-9* conversion frequency is high in *ccf-5(A)* homozygous and *ccf-5(B)* homozygous crosses (11·08 % ±0·11 and 9·24 % ±0·28 respectively) and reduced about fivefold in *ccf-5(A)* × *ccf-5(B)* heterozygous crosses to values of 1·96 % ±0·12 with *ccf-5(A)* in cis to *w-9* and 2·37 % ±0·04 with *ccf-5(A)* in trans. The conversion frequency differences between *ccf-5(A)* and *ccf-5(B)* homozygous crosses are highly significant ($\Sigma\chi^2 = 33{\cdot}42$, $P < 0{\cdot}01$, 1 D.F.) as are the slight differences between the two types of *ccf-5(A)* × *ccf-5(B)* heterozygous cross ($\Sigma\chi^2 = 8{\cdot}55$, $P < 0{\cdot}01$, 1 D.F.). However, the main effect of *ccf-5* is to reduce *w-9* conversion frequency when heterozygous in a cross and possible cis/trans position effects of *ccf-5* on *w-9*

conversion frequency, although significant, are only slight. The relative frequency of post-meiotic segregation (PMS) octads is highest in *ccf-5(A)* homozygous crosses (37·31 %) and about the same (19·52–21·65 %) in the other three types of cross, although a wider spread of values can be seen in the original data (Howell, 1982). Conversion is predominantly to wild-type, ranging from 70·91 % conversion to + in *w-9*, *ccf-5(A)* × +, *ccf-5(B)* crosses to 89·76 % in *ccf-5(A)* homozygous crosses.

Table 2. *Conversion data from* + × w-9 *crosses with different combinations of* ccf-5 *factors**

ccf-5 factor in		Octad classes (+ : *w*), %					Total	%NRCF	Relative frequency	% conversion
+	*w-9*	4:4	6:2	2:6	5:3	3:5	octads	±S.E.	of PMS†, %	to +‡
A	A	88·65	6·25	0·70	3·69	0·44	81517	11·08±0·11	37·31	89·76
A	B	97·61	1·50	0·40	0·43	0·04	142685	2·37±0·04	19·79	81·42
B	A	98·03	1·07	0·51	0·32	0·06	12789	1·96±0·12	19·52	70·91
B	B	90·72	5·67	1·57	1·70	0·30	10500	9·24±0·28	21·65	79·69

* Each cross category consists of pooled data from a number of different individual crosses.

† PMS = post-meiotic segregation $= \frac{(5:3+3:5)\times 100}{6:2+2:6+5:3+3:5}$.

‡ % Conversion to + $= \frac{(6:2+5:3)\times 100}{6:2+2:6+5:3+3:5}$.

The genetic controls of w-BHj *conversion behaviour*

(i) *Preliminary experiments*

w-BHj mutant strains were crossed to 17 derived wild-type strains and the results from these crosses are shown in Table 3. The narrower-ratio conversion frequencies fall into two broad categories. The first eight crosses in the table show lower conversion frequencies (5·74–8·86 %) than the remaining nine crosses (11·70–18·42 %). In the latter HCF category, apart from one cross (*w-BHj* + × 7P −) giving 11·70 %, no other cross gave a conversion frequency of less than 13·97 %. Both the LCF ($\Sigma\chi^2 = 80{\cdot}20$, $P < 0{\cdot}01$, 7 D.F.) and the HCF ($\Sigma\chi^2 = 93{\cdot}11$, $P < 0{\cdot}01$, 8 D.F.) groups of crosses were heterogeneous with respect to conversion frequency as replicates of the same cross often were. For example, seven replicates of LCF cross *w-BHj* + × 25-5R + were performed, all giving sample sizes of over 1000 octads. The range of conversion frequencies was 5·41–8·85 % ($\Sigma\chi^2 = 24{\cdot}58$, $P < 0{\cdot}01$, 6 D.F.). Conversion frequency heterogeneity among crosses of the same group (LCF or HCF) may largely be due to imperfectly controlled environmental conditions, but the role of minor modifiers of conversion cannot be ruled out. No cross gave both LCF and HCF replicates. The difference in conversion frequency between the two sets of crosses strongly suggested that the conversion behaviour of *w-BHj* is under genetic control, and the system involved cannot be *ccf-2* since all the derived wild-type strains used (with the exception of KIII −, KIV + and 42 −) carried the same, *P*, form of *ccf-2*. As in the case of *w-9* conversion control, a simple, easily testable model (a closely linked, monogenic system) was assumed at the outset and the experimental programme outlined for *w-9* was repeated, initially selecting one LCF cross for further study.

(ii) *Conversion behaviour of wild-type isolates from* 6+:2w *octads from cross* w-BHj− × 25-5R+

Wild-type spores were isolated from 40 6+:2*w* convertant octads from the LCF cross *w-BHj*− × 25-5R+. Ascospore germination was very poor, but in four octads all six wild-type spores germinated. These wild-type isolates were crossed to

Table 3. *Conversion data from* + × w-BHj *crosses*

Mutant strain	Crossed to	Octad classes (+:*w*), % 4:4	6:2	2:6	5:3	3:5	%NRCF	Total octads
w-BHj−	9r+	92·73	4·31	1·17	0·80	0·86	7·14	8867*
w-BHj−	25-5R+	92·47	3·49	1·39	1·25	1·00	7·12	13560
w-BHj+	6P−	91·76	4·12	2·25	1·00	0·75	8·11	801
w-BHj+	9P−	91·08	4·92	1·85	1·23	0·86	8·86	1625
w-BHj+	16P−	90·65	5·69	1·45	0·96	0·58	8·68	1037
w-BHj+	18P−	91·04	5·09	1·17	1·57	0·91	8·75	2298
w-BHj+	KIII−	94·26	3·82	0·57	0·00	1·34	5·74	1046*
w-BHj−	KIV+	93·05	3·67	0·79	1·29	0·99	6·75	1007
w-BHj+	42−	85·61	8·81	1·87	2·13	1·42	14·23	7805*
w-BHj+	7R7−	80·41	9·48	3·59	2·90	2·45	18·42	4685
w-BHj+	4P−	82·81	9·46	2·48	2·44	1·53	16·90	1047
w-BHj+	5P−	83·33	8·97	3·65	2·17	1·78	16·57	1014
w-BHj+	7P−	88·12	6·84	2·11	1·67	1·08	11·70	3889
w-BHj+	11P−	84·72	8·54	3·06	1·58	1·79	14·96	949
w-BHj+	17P−	85·79	8·57	2·06	2·11	1·23	13·97	4132
w-BHj+	24P−	83·58	8·82	2·45	1·47	2·94	15·68	408
w-BHj−	1D2+	84·35	8·69	2·65	2·40	1·42	15·16	4458

* Data of Lamb & Ghikas (1979).

w-BHj+ and *w-BHj*− tester strains. Considering the conversion data from each octad in turn, in octad 8, four wild-type isolates gave fertile crosses, of which three were LCF (6·61 % ± 0·34 to 8·67 % ± 0·16) while one isolate gave an altered, enhanced conversion frequency of 17·11 % ± 0·42. In octad 9, four of the six wild-type isolates again gave fertile crosses. Three of these crosses are classifiable as LCF, although the conversion frequencies (9·97 % ± 0·21 to 10·51 % ± 0·23) were rather higher than those for other LCF crosses in this experiment or others. One isolate gave an altered, enhanced conversion frequency of 14·63 % ± 0·22. In octad 11, five of six possible crosses were fertile. Three were clearly LCF, with conversion frequencies of 7·84 % ± 0·14 to 8·57 % ± 0·11. Two isolates showed enhanced conversion frequencies of 12·10 % ± 0·12 and 12·92 % ± 0·13. These isolates were of the same mating type and are almost certainly sister spores. Finally, in octad 19, all six wild-type isolates gave fertile crosses, with four isolates giving LCF crosses (6·40 % ± 0·26 to 8·18 % ± 0·42), while two isolates – of the same mating type – gave altered, enhanced conversion frequencies of 13·10 % ± 0·29 and 15·25 % ± 0·33. These latter two isolates are presumably sister spores, so the variation in conversion frequency between them can be attributed to environmental effects.

The results from this analysis, although based on few octads, do seem to indicate

that the LCF cross *w-BHj* − × 25-5R+ is heterozygous for at least one *ccf* affecting the conversion of *w-BHj*. In none of the four octads analysed did the *ccf*(s) co-convert with *w-BHj*, since isolates with altered conversion characteristics were recoverable in all four octads. If *w-BHj* and its *ccf*(s) were loosely linked or unlinked, then crossing-over between the sites, unassociated with conversion, could produce four wild-type isolates with altered conversion properties per octad. No such octad was observed, indicating that if a single *ccf* is involved it is likely to be linked to *w-BHj*, giving 'low' conversion frequencies (*c.* 6–9 %) in crosses heterozygous for two forms of this factor (let these forms be designated *A* and *B*, where the *w-BHj* strains carry the *A* form), with 'high' conversion frequencies (*c.* 12–15 %) in crosses homozygous for the *A* form of the *ccf*.

(iii) *Monogenic nature of* w-BHj *conversion control*

From other data (Howell, 1982) it was deduced that a cross between derived wild-type strains 25-5R+ and BH19-1− would be heterozygous for the *ccf* or *ccfs* responsible for *w-BHj* conversion control. To distinguish between these alternatives, 350 wild-type spores were randomly isolated from the progeny of cross 25-5R+ × BH19-1− (all octads were 8+ :0*w*).

Thirty isolates gave fertile crosses with either *w-BHj*+ or *w-BHj*− tester strains, and the data obtained are shown in Fig. 1(*b*). From this it can be seen that 16 isolates gave LCF crosses (with conversion frequencies of 6·05 % ±0·17 to 8·93 % ±0·31) behaving like the 25-5R+ parent, while 14 gave HCF crosses (with conversion frequencies of 11·15 % ±0·24 to 14·80 % ±0·29), behaving like the BH19-1− parent. Both the *w-BHj*+ and *w-BHj*− tester strains exhibited similar behaviour. If the conversion control of *w-BHj* is monogenic, a 1:1 ratio of LCF and HCF crosses should be expected from this type of experiment, and the observed values of 16:14 are a very close fit to such a ratio ($\chi^2 = 0{\cdot}13$, $P = 0{\cdot}70$–$0{\cdot}80$, 1 D.F.). Although the difference in conversion frequency between the LCF and HCF crosses is not great, in no case did replicates of the same cross yield conversion frequencies which straddled both classes – all were either LCF or HCF.

The results from the experiments described point to the existence of a single major conversion control factor acting on *w-BHj*, which is designated *ccf-6*. Crosses of the type *w-BHj*, *ccf-6*(*A*) × +, *ccf-6*(*B*) are LCF and *w-BHj*, *ccf-6*(*A*) × +, *ccf-6*(*A*) are HCF.

(iv) *Conversion behaviour of* w-BHj *in* ccf-6(B) *homozygous crosses*

w-BHj strains carrying ccf-*6*(*B*) were obtained by isolating white spores from 2+ :6*w* octads from a *w-BHj*,*ccf-6*(*A*) × +,*ccf-6*(*B*) LCF cross (cross *w-BHj*− × 25-5R+ : Table 3). If *ccf-6* is closely linked to *w-BHj*, four white spore isolates per octad should carry *ccf-6*(*A*) and give LCF crosses with *ccf-6*(*B*) derived wild-type strains, while two white spore isolates should carry *ccf-6*(*B*) and hence may show altered conversion behaviour in crosses to the same tester strains. Accordingly, a ratio of 2:1 for LCF:altered CF might be expected if *w-BHj*, *ccf-6*(*A*) × +, *ccf-6*(*B*) and *w-BHj*, *ccf-6*(*B*) × +, *ccf-6*(*B*) crosses show different conversion frequencies.

Twenty-one white spore isolates gave fertile crosses with *ccf-6*(*B*) derived

wild-type strains. Sixteen crosses were LCF (with conversion frequencies of 5·00 % ± 0·37 to 8·39 % ± 0·32) while five crosses showed altered, elevated conversion frequencies of 11·18 % ± 0·12 to 14·45 % ± 0·17 (Howell, 1982). Those data closely fit the expected 2:1 ratio (χ^2, applying Yates' correction = 0·48, $P = 0{\cdot}30$–$0{\cdot}50$, 1 D.F.). It is almost certain that the isolates showing altered conversion frequencies arose by conversion of the *w-BHj* site and thus carry *ccf-6(B)*. So, *w-BHj* appears to show a high conversion frequency in *ccf-6(B)* homozygous crosses, very similar to that seen in *ccf-6(A)* homozygous crosses. Appreciable co-conversion of *w-BHj* and *ccf-6* is precluded, since it would result in fewer than expected segregants with altered conversion properties.

(v) *Conversion behaviour of* w-BHj *in* w-BHj, ccf-6(B) × +, ccf-6(A) *crosses*

From the previous experiment two *w-BHj* isolates known to carry *ccf-6(B)* were selected, while three derived wild-type isolates shown to carry *ccf-6(A)* were chosen from other experiments. All six possible crosses were fertile and showed low *w-BHj* conversion frequencies of 5·61 % ± 0·16 to 8·03 % ± 0·09 similar to those seen in + × *w-BHj* crosses, but with *ccf-6(A)* in cis to the mutation rather than in trans. The conversion spectrum was also similar in both types of cross. Thus there are no large cis/trans position effects of the *ccf-6* factors on *w-BHj* conversion.

(vi) *Linkage of* w-BHj *and* ccf-6

Wild-type spores were randomly isolated from 4+ : 4*w* octads from a *w-BHj*, *ccf-6(A)* × +, *ccf-6(B)* LCF cross (*w-BHj* − × 25-5R+ : Table 3).

Seventy-one random wild-type isolates gave fertile crosses with either *w-BHj* + or *w-BHj* − tester strains. Ten of these isolates were crossed to both *w-BHj*, *ccf-6(A)* and *w-BHj*, *ccf-6(B)* testers to provide additional confirmation of genotype. Sixty-six progeny appeared parental for the *w-BHj* and *ccf-6* sites, while five were recombinant (Howell, 1982), corresponding to a recombination frequency of 7 %. This can be regarded as an overestimate of map distance, since certain conversion octads (aberrant and correction 4+ : 4*ws*) can give rise to recombinant progeny as can false clusters of spores.

(vii) *Separate identity and specificity of* ccf-6

ccf-6 did not produce any effect on the conversion behaviour of mutations known to be affected by *ccfs -1*, *-2*, *-3*, *-4* and *-5*. So *ccf-6* appeared to be in no way associated with any known *ccf* and has a separate identity. Furthermore, *ccf-6* had no effect on the conversion behaviour of nine unlinked ascospore colour mutations of loci *wII*, *wIII* and *wIV* plus solitary mutations *w-9* and *NGw14* (Howell, 1982) and thus its effect appears to be strictly local and possibly specific to *w-BHj*.

A summary of the action of *ccf-6* on *w-BHj* conversion behaviour is given in Table 4. *w-BHj* conversion frequency is high in *ccf-6(A)* and *ccf-6(B)* homozygous crosses (13·38 % ± 0·11 and 12·28 % ± 0·41 respectively) and reduced by slightly less than 50 % in *ccf-6(A)* × *ccf-6(B)* heterozygous crosses to values of 7·29 % ± 0·14 with *ccf-6(A)* in cis to *w-BHj* and 7·68 % ± 0·08 with *ccf-6(A)* in trans. The conversion frequency differences between *ccf-6(A)* and *ccf-6(B)* homozygous crosses, although

slight, were significant ($\Sigma\chi^2 = 6{\cdot}62$, $P = 0{\cdot}01$–$0{\cdot}02$, 1 D.F.) as were the very slight differences between the two types of *ccf-6*(*A*) × *ccf-6*(*B*) heterozygous cross ($\Sigma\chi^2 = 6{\cdot}12$, $P = 0{\cdot}01$–$0{\cdot}02$, 1 D.F.). However, the main effect of *ccf-6* is to reduce *w-BHj* conversion frequency when heterozygous in a cross. Possible cis/trans position effects of *ccf-6* on *w-BHj* conversion frequency, although significant, are only slight. The relative frequency of PMS octads is about the same in crosses with all combinations of *ccf-6* factors, ranging from 20·00 to 26·57 %, while conversion is predominantly to wild-type, ranging from 67·14 to 77·27 %.

Table 4. *Conversion data from* + × w-BHj *crosses with different combinations of* ccf-6 *factors*

ccf-6 factor in		Octad classes (+ : *w*), %					Total	%NRCF	Relative frequency	% conversion
+	*w-BHj*	4:4	6:2	2:6	5:3	3:5	octads	±S.E.	of PMS, %	to +
A	A	86·33	7·68	2·51	1·73	1·47	97878	13·38±0·11	23·85	70·28
A	B	92·13	4·28	1·52	1·03	0·85	125102	7·68±0·08	24·51	69·12
B	A	92·52	3·91	1·45	0·99	0·95	36648	7·29±0·14	26·57	67·14
B	B	87·52	7·84	1·98	1·65	0·81	6556	12·28±0·41	20·00	77·27

4. DISCUSSION

The results described point to the existence of two hitherto unknown conversion control factors, *ccf-5* and *ccf-6*, which modify the conversion behaviour of closely linked ascospore colour mutations, giving higher conversion frequencies when either form of each factor is homozygous in a cross and reduced conversion frequencies for the affected mutations when heterozygous. *ccf-5* has a more drastic effect on *w-9* conversion behaviour than does *ccf-6* on *w-BHj*. The primary effect of both *ccf*s is on the overall conversion frequency of the affected mutation, with *ccf-6* having no apparent effect on the relative PMS segregation frequency of *w-BHj* in the various cross types. *ccf-5* may have some effect on *w-9* PMS, the latter being higher in *ccf-5*(*A*) homozygous crosses, but there is no consistent correlation between reduction in conversion frequency and reduction in PMS. Most probably other, minor, modifiers of gene conversion behaviour are involved. *ccf*s-*5* and -*6* only showed slight, but significant cis/trans position effects on the conversion frequency of the affected mutations while both *ccf*s appeared to act specifically on closely linked mutations.

The experimental system used has several advantages. The effects of all four possible combinations of the *ccf* factors on each mutant site could be studied, as could conversion to wild type and to mutant. Systems involving selective methods involve possible complications and biases. Furthermore, all the conversion classes were studied, giving a clearer indication of the mode of action of the factors.

*ccf*s-*5* and -*6* show close similarity in their mode of action and closely resemble certain other fungal conversion modifiers including *ccf-1* in the Pasadena strains of *Ascobolus* (Emerson & Yu-Sun, 1967), the *cv* conversion modifiers in the European strains of the same organism (Girard & Rossignol, 1974) and the *ss* recombination modulator in *Neurospora crassa* (Catcheside, 1981), while the

controls of intragenic recombination in the *adh-1* locus of *Zea mays* (Freeling, 1978) and in the *rosy* gene of *Drosophila melanogaster* appear broadly similar. Might all such factors operate by a common mechanism and if so what is their nature?

The range of possible different mechanisms by which the factors act is limited, since all act to reduce conversion/intragenic recombination when heterozygous. First, the *ccf*s could determine the pairing closeness of homologous DNA duplexes in their vicinity, after general synapsis of homologous chromosomes. The degree of this pairing closeness could determine the frequency of formation of hybrid DNA (H-DNA). Inclusion in a length of H-DNA is a pre-condition of the 'opportunity to convert' for any mutation, according to most recombination models (Holliday, 1964; Meselson & Radding, 1975).

Might one form of any such control factor consist of a chromosomal rearrangement with respect to the other form? Certainly heterozygosity for a chromosomal rearrangement might reduce gene conversion (and reciprocal recombination) in its vicinity by interfering with correct chromatid pairing, but this explanation seems unlikely since very high frequencies of ascospore abortion are expected in crosses heterozygous for a chromosomal rearrangement, unless it is very small. Such a phenomenon was not observed in crosses heterozygous for either *ccf-5* or *ccf-6* in this work, or in *ss* heterozygous crosses by Catcheside (1981). More probably the *ccf*s could consist of DNA sequences with smaller heterologies between the different forms of any factor, including small deletions or many individual sequence differences. Heterozygosity of these sequences could interfere with the binding of a DNA pairing protein (such as DNA synaptase reported in *E. coli* by Potter & Dressler, 1980), so reducing the frequency of intimately paired duplexes and hence H-DNA formation. Such DNA pairing proteins would act non-specifically, preferentially on regions of closely paired DNA duplexes, such as would occur when either form of a *ccf* was homozygous. Different forms of a particular *ccf* might result in different frequencies of intimately paired duplexes when homozygous in a cross, leading to different conversion frequencies of closely linked mutations. Such conversion frequency differences were slight but significant when considering homozygosity for the *A* and *B* forms of *ccf*s-*5* and -*6* and more pronounced for *ccf-1*, *ss* in *Neurospora* and *adh-1* in *Zea mays*. Comparable data do not exist for the *Ascobolus cv* modifiers or for the *Drosophila rosy* gene.

Secondly, the *ccf*s might be directly involved in H-DNA initiation, acting as recognition sites for an endonuclease. This seems unlikely, since it is difficult to see how an endonuclease, initiating the primary single (according to Meselson & Radding, 1975) or double strand break (Szostak *et al.* 1983) in one duplex and acting preferentially on identical sequences, could be aware of what is on the other chromatid at the corresponding position.

Thirdly, heterozygosity for the *ccf* factors could interfere with D-loop formation or with single- or double-strand branch migration (Catcheside, 1981). Interference with single-strand branch migration (strand assimilation) would reduce the frequency of H-DNA on the side of the *ccf* distal to its initiation point, while interference with double strand branch migration would specifically reduce symmetric H-DNA frequencies. The latter is more likely since on the Meselson–Radding (1975) model the propagation of the initial asymmetric H-DNA phase

(by single-strand branch migration) is enzyme driven and is thus likely to be less vulnerable to poor homology than symmetric H-DNA propagation, which is driven by rotary diffusion. It has been proposed that mutation *G234* in the *b-2* locus of *Ascobolus immersus* acts in just such a way (Hamza *et al.* 1981) although co-conversion studies (Leblon & Rossignol, 1973) indicate that heterozygosity for a single base pair does not prevent H-DNA propagation. Nevertheless, a longer region of poor homology would presumably do so.

In conclusion, it seems most likely that *ccf*s-*5* and *-6* correspond to DNA sequences which, when heterozygous, either interfere with the pairing closeness of DNA duplexes in their vicinity or reduce single- or double-strand branch migration after H-DNA initiation. Both models are equally in agreement with the existing data and it is quite probable that *ccf-1*, the *cv* modifiers, the *ss* recombination modulator, etc. act in the same way. If they do, can the differences between the systems in their relative effectiveness in reducing conversion of closely linked sites be explained? The greater the sequence differences between the alternative forms of any *ccf*, the more local intimate pairing or branch migration might be interfered with when the factor is heterozygous in a cross, resulting in more conversion reduction. On this basis, the alternative forms of *ccf-6* would be the most similar in sequence, while the forms of *cv-2* would show the greatest sequence divergence (heterozygosity for *cv-2* can reduce the conversion frequency of certain closely linked mutations several hundredfold). Alternative explanations are also possible, including the degree of linkage of the *ccf* and its 'target' mutation. More widely separated sites might contain additional sites of H-DNA initiation in the interval, masking interference with branch migration from the first H-DNA initiation site, or allow restoration of intimate pairing of the duplexes in that interval. The distance between the *ccf* and the H-DNA initiation site could also be important since in theory a *ccf* close to an initiation site (and hence in a region of asymmetric H-DNA according to the Meselson–Radding model) might have less effect than one further away (where the H-DNA would more often be symmetric) if the *ccf*s act by interfering with double-strand branch migration. The nature of the mutations themselves could also play some role, and does seem to do so in the case of *cv-2* and the *b-2* mutations in *Ascobolus* (Girard and Rossignol, 1974).

Sites which affect recombination in their vicinity when heterozygous seem to be very common, being found not only in fungi but also in higher eukaryotes. These local controls of recombination show much natural polymorphism, making their detection possible.

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