Time-dependent changes in gene conversion in *Ascobolus immersus*

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Summary

The stability of conversion frequencies with time was investigated in *Ascobolus immersus*. There were usually marked reductions in gene conversion frequencies at locus *w1* as crosses matured, to about one third of the initial values. This applied to all six *w1* alleles tested, irrespective of their molecular nature, and at all temperatures used. Asci dehiscing early from an apothecium had much higher conversion frequencies than those dehiscing late, but there were no differences between apothecia maturing at different times within a cross. Alleles at four loci unlinked to *w1* were also tested. All four showed significant changes, though not in all crosses; three loci showed decreases in conversion frequency with time, while one showed an increase. The relative frequencies of different conversion classes often changed with time. These changes appear to result from alterations in locus-specific recombination initiation and in repair of base mispairs in hybrid DNA, not from differential maturation rates of different ascal segregation classes. These effects could cause misinterpretation of quantitative tests of recombination models from gene conversion data.

1. Introduction

Gene conversion is an important phenomenon for studying mechanisms and controls of recombination (e.g. Holliday, 1964; Orr-Weaver & Szostack, 1985), for evolutionary and population genetics (e.g. Lamb & Helmi, 1982; Dover, 1982), and in other areas such as immunology (Wysocki & Gefter, 1989). As part of a wider study of recombination mechanisms and the controls of gene conversion, the stability of gene conversion frequencies with time has been investigated in the Pasadena strains of the Discomycete fungus, *Ascobolus immersus*. Work on recombination is normally based on the assumption that the meioses in a cross form a uniform population. Deviations from uniformity could affect the quantitative analyses of recombination data, as well as mapping. As crosses matured, there were large changes in gene conversion frequencies and in the relative proportions of different conversion classes. A technique for collecting dehisced meiotic octads from single apothecia at different periods enabled the cause of this phenomenon to be studied. Details are given of the nature, extent, generality, causes and importance of such time- and maturity-dependent changes.

Initial work was done with w^+ (wild-type, red ascospores) × w (white ascospores) crosses in Petri dishes, scoring ascospore colour segregation ratios in successive harvests of dehisced octads on sequentially replaced collecting lids. The generality of the changes was tested in a series of $w^+ \times w$ crosses with different w mutations, trying six different alleles (base-substitutions, frame-shifts of opposite sign, and a large deletion) at locus *w1*, and w alleles at four loci unlinked to *w1*. Three possible causes were investigated: (i) early maturing apothecia could give different conversion frequencies (CFs) from later maturing ones; (ii) asci produced earlier on an apothecium could give different CFs compared to asci produced later on the same apothecium; (iii) asci with gene conversion for the locus studied, or with certain conversion allele ratios, might ripen and dehisce earlier or later than those with no conversion (4+:4*w*) at the segregating locus. Possibilities (i) and (ii) were distinguished by using sequential collections of dehisced octads from individual apothecia in a cross; (iii) was studied from a number of crosses, mainly from whole lid collections, from changes in the relative frequencies of different ascal segregation classes.

In the present *Ascobolus* work, the distribution of

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conversion octads between different apothecia was tested for randomness. As very small numbers of conversion asci per apothecium make various tests difficult to apply, all the work on collection of asci from individual apothecia was done using cross $w^{+}(+) \times w1-78(-)$ (strains 3D2+; 3w1DEC−) as that gives a high conversion frequency, about 24%, relative to the usual range of 0·04% to over 28% in fungi (Lamb, 1984). The data presented are just from the experiments with the largest sample sizes, representing a very much larger body of similar data, some of which appeared in theses, e.g. Wickramaratne, 1974; Howell, 1982.

2. Methods, materials and strain designations

General methods, strains and media were as described by Emerson & Yu-Sun (1967), Lamb & Helmi (1978), Helmi & Lamb (1983), and references therein. Crosses were incubated at 17·5 °C unless otherwise stated. Crosses were made by placing inocula at opposite edges of 10 cm diameter glass Petri dishes containing crossing medium. 'Collecting lids' were bottoms of such Petri dishes, containing water agar with an inhibitor of spontaneous ascospore germination (17 g Difco bacto-agar, 7 ml of inhibitor stock (10 g methyl-*p*-hydroxybenzoate in 100 ml of 95% ethanol), distilled water to 1 l).

For individual apothecium analysis, young crosses were examined under a dissecting microscope in a laminar flow sterile chamber. Twenty-five yellow-brown young apothecia, widely distributed over the plate, were selected and numbered by engraving in the agar near them. The selected ones were individually enclosed in a sterile 20 × 2 mm piece of melting-point glass tubing, open at both ends, vertically inserting the lower end about 1 mm into the agar, so that the apothecium was not cut off from the mycelium in the agar to any great extent. A collecting lid was inverted to cover each plate, with the top end of each tube slightly inserted into the collecting agar surface. When dehiscence of asci began, the glass tubes were carefully replaced by new ones every eight hours through the whole period of dehiscence, with collecting lids being replaced daily. Dehisced octads inside the tubes, and on the surface of the collecting agar within the circle marked by the tube, were scored separately from those octads dehisced onto the collecting lids from apothecia not enclosed in the glass tubes.

Recombination properties in the Pasadena strains are often strongly influenced by genetic elements which have been called gene conversion control factors (*ccf*s). Conversion at locus *w1* is influenced by the closely linked *ccf2* (alleles *P*, *K* and *91*, named after the strains they were found in by Emerson & Yu-Sun, 1967) and by *ccf3* (alleles *E*, a dominant enhancer of conversion, and *e*) and *ccf4* (alleles *R*, a dominant reducer of gene conversion, and *r*), which are unlinked to each other or to *w1*. The combination *ccf3-E*, *ccf4-r* causes much higher conversion at locus *w1*, whatever alleles are present for *ccf2*: this phenotype with the higher conversion frequencies has been called 'super' (see Lamb & Helmi, 1978; Helmi & Lamb, 1983, and Lamb & Helmi, 1989, for details of *ccf* types, dominance and effects). The two main strains used here were 3D2(+), which is $w1^{+}$, *ccf2-P*, *ccf3-E*, *ccf4-r* (and hence 'super' in phenotype), of plus mating-type (mating-types are shown in parentheses), and 3w1DEC(−), which is *w1-78* (mutant allele 78 at locus *w1*), *ccf2-P*, 'non-super' for *ccf3* and *ccf4*, of minus mating-type. Alleles at white ascospore (*w*) loci have been named on different systems by different authors. Various alleles and loci have been renamed here to get a more uniform system, more in keeping with those for other organisms. New and old allele and locus names are shown in Table 1; existing strain designations have been kept, for direct comparability with previous papers.

3. Results

(i) *Changes in conversion frequency at locus* w1 *with time (overall cross maturity, from lids)*

Figure 1 shows the conversion results from successive collecting lids in a variety of $w^{+} \times w1^{-}$ crosses. *w1-10* and *w1-78* are spontaneous base-substitutions; *w1-5* is an NMG-induced base-substitution; *w1-2* is an EMS-induced frame-shift, isolated as an intragenic suppressor of ICR-170 induced frame-shift *w1-3*, and *w1-8* is a UV-induced large deletion. Crosses matured fastest at 22·5 °C, and slowest at 10 °C. The same trends were shown in replicates and repeats of each cross, with only minor variations. In all these crosses, there were changes in conversion frequency (CF) with time, usually significant at $P < 0.01$. The pattern was always for a decrease in conversion at locus *w1* in later harvests, with late collections generally having CFs of half to one quarter those of early collections. The

Table 1. *Old and new names for Pasadena strain alleles and loci*

Old:	wl	2w2	w3C1	NGw5	UVk8	w10	w78
New:	*w1*	*w1-2*	*w1-3*	*w1-5*	*w1-8*	*w1-10*	*w1-78*
Old:	NGw18	Sw26	Bw4·2	Bw3·10	Cw1·4	NGw11	NGw12
New:	*w2-1*	*w2-2*	*w3-1*	*w3-2*	*w3-3*	*w4-1*	*w4-2*
Old:	w62	BB9w9	BBw11	wBHj	BHo	BBm	BB5w
New:	*w5-1*	*w6-1*	*w6-2*	*w7-1*	*w8-1*	*w9-1*	*w10-1*

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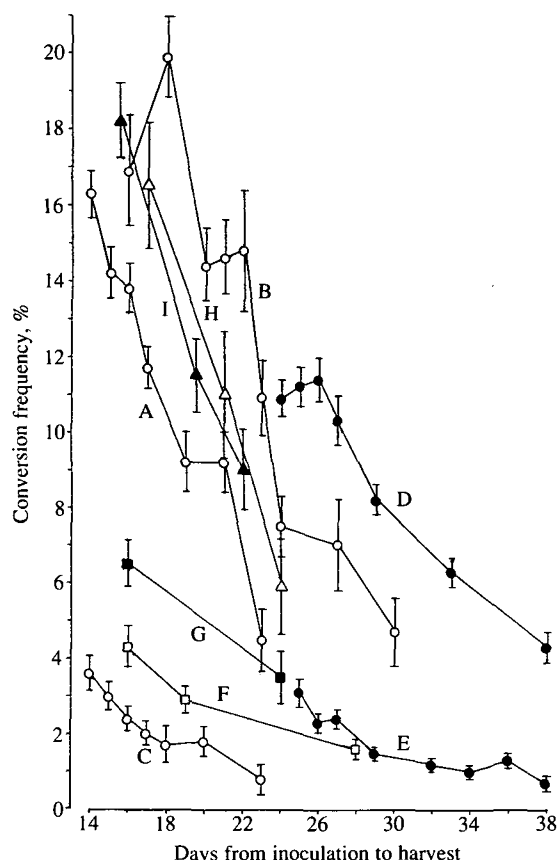

Fig. 1. Conversion frequencies for various alleles at locus *w1* in relation to maturity of cross, at different temperatures. Bars show standard errors. Cross details: (○, A, B, C) allele *w1-10*: (A), $w^+(-) \times$ *w1-10*(+) (strains P5−; 415-3-w10+), 22·5 °C; (B), same as (A), 17·5 °C; (C), $w^+(-) \times$ *w1-10*(+) (strains P5−; w10+), 22·5 °C; (●, D, E) allele *w1-78*: (D), $w^+(-) \times$ *w1-78*(+) (strains P5−; 577-6-w78+), 10 °C; (E), $w^+(-) \times$ *w1-78*(+) (strains P5-; w78+), 10 °C; (□, F), Allele *w1-5*: $w^+(-) \times$ *w1-5*(+) (strains P−; NGw5,13·3+), 17·5 °C; (■, G), allele *w1-2*: $w^+(+) \times$ *w1-2*(−) (strains 22-5R+, 2w2−), 17·5 °C; (△, H) allele *w1-8*: $w^+(+) \times$ *w1−8*(−) (strains 278+; UVk8.31−), 17·5 °C; (▲, I) allele *w1-3*: $w^+(-) \times$ *w1-3*(+) (strains 92−; w3C1+), 17·5 °C. $2 \times n$ χ^2 tests on each data set, (A–I), showed changes in conversion frequency with time significant at $P < 0{\cdot}01$.

reductions in CF with time were not totally linear, with the second or third collection sometimes having a higher CF than the initial one. The same general trend was shown for all six *w1* alleles, in spite of their different molecular natures, and was shown at all three temperatures tested.

The different wild-type and $w1^-$ strains used in Fig. 1 often differed in their conversion control factor alleles, so that the same *w1* allele gave quite different conversion frequencies in different crosses, e.g. compare *w1-10* crosses A and B, where all strains carried *ccf2-P*, and C, where one strain carried *ccf2-K*, or *w1-78* crosses D (both strains with *ccf2-P*) and E (one strain carrying *ccf2-K*). Irrespective of conversion frequency, a general reduction in CF with time was found. The slopes in Fig. 1 are generally steeper for crosses with high CFs than in crosses with low CFs.

Exactly the same trends were shown in the heteroallelic coupling and repulsion crosses of two locus *w1* alleles, *w1-5* and *w1-2*, as in Table 2, where the results include rare reciprocal recombinants as well as convertants. The effect was shown whether 0+:8*w* or 4+:4*w* was the majority class. Other heteroallelic crosses, not shown here, showed the same trend.

Tests for artefacts were carried out as described by Lamb & Wickramaratne (1973). Spontaneous reversions from w^- to w^+ were not found and spontaneous mutations from w^+ to w^- were too rare to affect these data. Phenocopies were very rare at all stages of apothecial development, as shown by germinating ascospores and testing the resultant colonies in crosses. If spore colour failed to develop properly in w^+ spores in early or late maturing asci, that could greatly increase the frequency of the normally very rare 0+:8*w* class in relation to other conversion classes, but such changes were not observed.

False clustering (formation of groups of eight ascospores from incomplete dehiscence of more than one ascus onto the same place on a collecting lid) would increase the frequencies of the rare conversion classes (8:0, 0:8, 7:1 and 1:7) far more than those of the common ones (6:2, 2:6, 5:3 and 3:5). The relative frequencies of rare and common conversion classes were roughly constant with time, ruling out changes in false cluster frequencies as the cause of time-dependent conversion frequency changes. Tests from germinated ascospores from octads with aberrant spore colour ratios, checking for 4(+):4(−) mating-type segregation, showed extremely low frequencies of false clusters. The time-dependence of CFs was found when collecting lids were changed daily and also when they were changed after a particular density of dehisced asci was observed, so changes in CF with time were not artefacts due to overcrowding on collecting lids causing false clusters at times of peak dehiscence. The fact that the same time-dependence was found in $w^+ \times w^-$ crosses irrespective of whether 6+:2*w*, 2+:6*w*, 5+:3*w* or 3+:5*w* was the most frequent conversion class, and in both coupling and repulsion heteroallelic crosses, is further evidence against artefacts being responsible for the changes found.

(ii) *Changes in conversion frequency at locus* w1 *with time, from individual apothecium collections*

All data analysed in Table 3 are from cross $w^+(+) \times$ *w1-78*(−) (strains 3D2+; 3w1DEC−), from 100 apothecia which produced 9 or more asci each. Fertile apothecia produced 5–21 asci, with most producing 12–20 asci. The time from inoculation to first ascal dehiscence varied between apothecia from 12 to 18 days, and the length of the dehiscence period varied from 6 to 9 days, with minor variations between replicates and repeats. Apothecia were classified according to time of first ascal dehiscence as:

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Table 2. *Changes in octad class frequencies with time in heteroallelic crosses involving alleles 5 and 2 at locus* w1

Days since inoculation	Total octads	'CF' (%)[a]	Octad class, +:w phenotype (%) 6:2	2:6	5:3	3:5	0:8	$2\times n$ χ^2
(i) Coupling phase, $w^+(+)\times w1\text{-}5, w1\text{-}2(-)$ (strains P5+; NGw5, 2w2−)[a]								
15	1101	4·8	0·1	4·6	0	0·1	0	24.8
21	796	2·5	0·1	2·4	0	0	0	**
29	913	1·1	0	0·9	0·1	0·1	0	
(ii) Repulsion phase, $w1\text{-}2(-)\times w1\text{-}5(+)$ (strains 2w2−; NGw5.13.3(91)+)[b]								
17	1037	5·7	0	5·7	0	0	94·3	30·2
18	1256	3·0	0	3·0	0	0	97·0	**
19	1058	3·5	0	3·5	0	0	96·5	
32	1066	1·4	0	1·4	0	0	98·6	

[a] In the coupling phase cross, this term includes conversions, coconversions and rare reciprocal recombinations. In the repulsion phase cross, 0+:8*w* includes the parental ditype octads, and the 'conversion frequency' (CF) column is for all non−0:8 octads, including rare reciprocal recombinations as well as conversions.
[b] Data of Mafi (unpublished).
** $P < 0.01$.

Table 3. *Changes in conversion frequency (CF) with time, in relation to ascus dehiscence period and apothecial maturity period, in cross* $w^+(+)\times w1\text{-}78(-)$ (*strains* 3*D*2+; 3*w*1*DEC*-)

(A) Individual apothecium collections, from individual tubes

Apothecial maturity period	Ascal dehiscence period: Early, Total asci	Early, CF (%)	Middle, Total asci	Middle, CF (%)	Late, Total asci	Late, CF (%)	Total over all ascal dehiscence periods, Total asci	Total, CF (%)	Number of apothecia
Early	235	35·7[a]	191	22·0[b]	158	5·1[c]	584	23·3	37
Middle	246	30·1[b]	258	26·0[c]	220	7·3[d]	724	21·4	46
Late	101	36·6[c]	92	20·7[d]	88	4·6[e]	281	21·4	17
Total over all apothecial maturities	582	33·5	541	23·7	466	6·0	1589	22·1	100

(B) Bulk collections from lids at corresponding periods

	Approximate number of days after first ascal dehiscence on the plate: 1-3[a]	3−6[b]	6-7[c]	7-9[d]	9-12[e]	Total
Total octads	1663	4288	4387	4040	764	15142
CF (%)	32·6	28·1	20·7	14·9	5·8	21·8

[a–e] In parts (A) and (B) indicate comparable periods of collection, allowing conversion results in part (A), from individual apothecial collections in tubes, to be compared with results from corresponding periods of bulk collection on lids (all apothecia apart from the 100 in collecting tubes) in part (B).

early, 12–14 days after inoculation; middle, 14–16 days; late, after 16 days. Octads from apothecia were classified according to their time of dehiscence from that apothecium: early, days 1 and 2 from first dehiscence; middle, days 3 and 4; late, after 4 days. Table 3 (A) shows the numbers of apothecia and asci in each category.

The apothecial maturity period clearly had no significant effect on CFs (averages for early, middle and late maturing apothecia were 23·3, 21·4 and 21·4%), but in each class of apothecium there was a dramatic and significant ($P < 0.01$) decrease in CF from early to middle to late dehiscing asci, the CF averages being 33·5, 23·7 and 6·0%, respectively

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(Table 3A). Table 3B gives the average CFs at successive intervals after first ascal dehiscence obtained from bulk collections from lids of the same plates (see methods). These CFs agree well with the corresponding averages from individual tubes [compare results *a–e* in Table 3(A) and (B)]. This shows that the use of individual collecting tubes did not introduce artefacts.

We conclude from Table 3 that the trends in CF with time shown in Fig. 1 and Table 2 are due to changes during the development of individual apothecia, with asci produced earlier having higher CFs than asci produced later on the same apothecium, and are not due to early maturing apothecia having higher CFs than later maturing apothecia. That is, possibility (i), Introduction, is wrong; possibilities (ii) and (iii) are distinguished in the Discussion.

The changes in CF with time are more extensive (4- to 8-fold, Table 3A) from single apothecia than from bulk collections from collecting lids (2- to 4·5-fold, Fig. 1). This is expected, as late collections from an apothecium will only include late asci, but a late collection lid (usually left on for several days as relatively few asci are dehiscing) could contain some late asci from middle matured apothecia, and early, middle and late asci from late maturing apothecia.

(iii) *Distribution of conversion asci between apothecia, and effects of apothecial fertility on conversion frequency at locus* w1, *from cross* w1⁺(+) × w1-78(−) (*strains* 3D2+ ; 3w1*DEC*-)

The numbers and types of conversion asci per apothecium were examined in data from individual collections from apothecia. In general, all distributions were random (fitting Poisson distributions), with no tendency for particular apothecia to produce or not produce particular types of ascus with respect to gene conversion classes. There was, however, some effect of apothecial fertility on conversion frequencies, as shown in Table 4. There was no significant difference between CFs of apothecia with small, below average or average numbers of asci, but apothecia with above average numbers of asci had a significant ($P < 0{\cdot}01$) increase in CF compared with the other types.

(iv) *How general are the effects of time on conversion frequency*?

Effects of time on conversion frequency were studied at four other loci, using alleles *w5-1*, *w6-1*, *w7-1* and *w2-1*, all of which are unlinked to *w1* or to each other: Fig. 2 shows the data. The dehiscence period for a

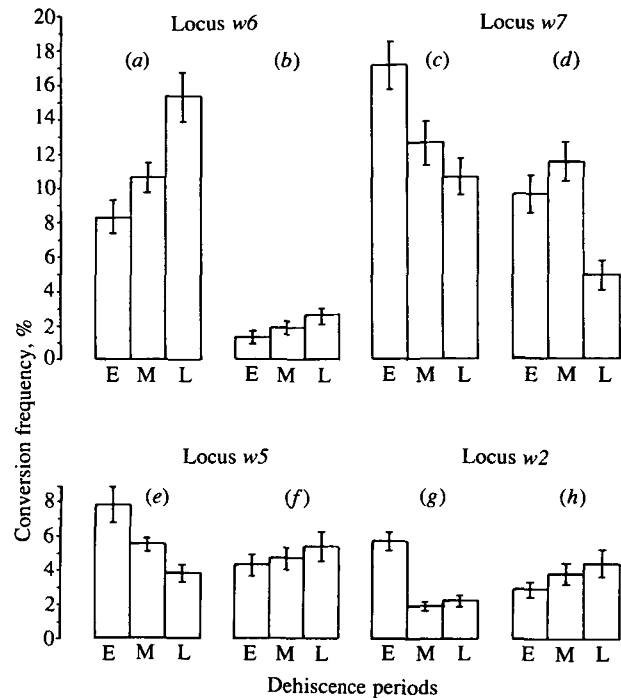

Fig. 2. Conversion frequencies for alleles of four different loci, not linked to each other or to *w1*, in relation to cross maturity periods. E, early; M, middle; L, late maturation; all crosses were made at 17·5 °C. Locus *w6*: (*a*) allele *w6-1*, $w^+(-) \times$ *w6-1*(+) (strains RS17-3 − ; BB9w9 +), $2 \times 3\chi^2 = 18{\cdot}0^{**}$; (*b*) allele *w6-2*, $w^+(+) \times$ *w6-2*(−) (strains 62 + ; BBw11 −) $2 \times 3\chi^2 = 3{\cdot}7$. Locus *w7*: (*c*) $w^+(-) \times$ *w7-1*(+) (strains BH3 − ; wBHj +), $2 \times 3\chi^2 = 14{\cdot}8^{**}$; (*d*) $w^+(-) \times$ *w7-1*(+) (strains BH9 − ; wBHj +), $2 \times 3\chi^2 = 19{\cdot}3^{**}$. Locus *w5*: (*e*) $w^+(-) \times$ *w5-1*(+) (strains 7114 − ; 207-5-w62 +), $2 \times 3\chi^2 = 13{\cdot}7^{**}$; (*f*) $w^+(-) \times$ *w5-1*(+) (strains 7114 − ; S62 +), $2 \times 3\chi^2 = 1{\cdot}1$. Locus *w2*: (*g*) $w^+(-) \times$ *w2-1*(+) (strains 92 − ; NGw18 +), $2 \times 3\chi^2 = 56{\cdot}4^{**}$; (*h*) $w^+(+) \times$ *w2-1*(−) (strains 257R-47 + ; 3NGw18 −), $2 \times 3\chi^2 = 2{\cdot}9$. ** $P < 0{\cdot}01$. Using regression analysis, positive slopes significant at $P < 0{\cdot}05$ were found for (*a*) but not for (*b*), (*f*) or (*h*), and a significant negative slope was found for (*c*) but not for (*e*).

Table 4. *Conversion frequencies for apothecia with different fertility, in cross* w⁺(+) × w1-78(−) (*strains* 3D2+ ; 3w1*DEC*−)

Relative number of asci/apothecium	Asci per apothecium	Number of conversion asci	Total asci	CF (%)
Small	9–11	15	73	20·5
Below average	12–14	71	353	20·1
Average	15–17	92	515	17·9
Above average	18–21	175	646	27·1

$2 \times n\chi^2$ over first three types, 0·83, D.F. = 2, $p = 0{\cdot}5$–$0{\cdot}7$.
$2 \times n\chi^2$ over all four types, 15·3, D.F. = 3, $P = < 0{\cdot}01$.

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cross was sub-divided into three equal periods, 'Early', 'Middle' and 'Late', often containing quite different numbers of asci. The results shown are a representative sample of a much larger body of data.

For all four loci, there were data sets with significant ($P < 0.01$) changes in CF with time, usually with decreases in CF with time, as for locus *w1*, but *w6-1* was an interesting exception, showing a rise in CF with time in a number of crosses, and never showing a significant decrease with time. A striking feature of these data was that, for each of these four loci, some crosses gave significant changes in CF with time, but other crosses did not give significant changes; these differences can not be explained purely in terms of different sample sizes. The different crosses for the same locus often differed in which conversion control factor alleles were present (*ccf5* for *w6-1*; *ccf6* for *w7-1*), which accounts for the large difference in CFs for *w6-1* in (*a*) and (*b*) in Fig. 2, but even crosses with the same *ccf* alleles differed in whether they gave significant CF changes with time, so some other type of genetic difference seems responsible. Individual crosses usually gave repeatable results for CF changes with time. All five loci tested gave significant changes in CFs with time, with nearly all locus *w1* crosses doing so, and roughly half of the crosses for the other four loci doing so.

4. Discussion

The nature of the effect is a decrease or, less often, an increase, in total conversion frequency with time (increasing maturity of each apothecium, as shown in Table 3). The extent of the effect depends partly on the method of measurement, with CF measurements from individual apothecia giving the biggest changes (Table 3); whole lid collections (which often include octads from asci of different maturation periods from apothecia of different maturation periods) for specific days or a very few days gave the next biggest changes. Most changes were about 2- to 4-fold, from early maturation to late maturation. The effect was very similar for all six alleles of locus *w1* and independent of the molecular nature of those mutations. A surprising finding for loci other than *w1* was that some crosses involving a particular allele may show clear CF changes with time, while other crosses (different strains, not just replicate crosses) do not. Temperature changed the absolute time of maturation of crosses, but did not affect the CF trend with time. Having different conversion control factor alleles in different crosses changed the absolute conversion frequencies for an allele, but did not change the general trend of CF with time.

Recording CFs from individual apothecia (Table 3) showed clearly that effects in whole lid collections were due to CF changes within apothecia during different ascal maturation periods [possibility (ii), Introduction], not to differences between apothecia maturing at different times. Several causes of these CF changes with time are possible. (a) The conversion frequency may change in successive meioses as individual apothecia mature, due to different concentrations of recombination-initiating factors or enzymes, or to different lengths of time being available for recombination events, or different proportions of recombination initiations giving gene conversions. (b) The conversion ascus phenotypes may result in a faster average maturation of the ascus after meiosis. (c) The occurrence of gene conversion could speed up ascal maturity, but that is unlikely: there are many different loci at which gene conversion could occur, with no more reason for conversion at one ascospore pigmentation locus to speed ascal maturation than conversion at any of the other types of loci.

Extensive data on changes in individual conversion classes give strong evidence against hypothesis (b). The only phenotypic differences between conversion and non-conversion asci are in the numbers of wild-type (red) and mutant (white) ascospores, with some conversion classes having more red spores than non-conversion asci, and others having fewer. Hypothesis (b) is tenable if mutant spores have less efficient metabolism round their nuclei in the developing ascus, with $6+:2w$ asci therefore maturing faster than $4+:4w$ asci, which themselves would mature faster than $2+:6w$ asci. A brief summary of relevant data is given here.

For alleles *w1-5*, *w1-8* and most of the *w1-10* and *w1-78* results, both $6+:2w$ and $2+:6w$ conversion classes decreased with time, in relation to non-convertant ($4+:4w$) asci, instead of showing opposite trends. The decrease in overall CF with time occurred both in crosses where $6+:2w$ was the most frequent conversion class, and where $2+:6w$ was the most frequent conversion class, e.g. for cross A in Fig. 1, the $6+:2w$ frequency reduced from 13·8 to 3·6% and the $2+:6w$ frequency reduced from 2·1 to 0·9% from first harvest to last, as total CFs reduced from 16·3 to 4·5% for *w1-10*. For cross $w1^{+}(-) \times w1\text{-}5(+)$ (strains P−; NGW5,P1+), the $6+:2w$ frequency reduced from 5·1 to 0·6% and the $2+:6w$ reduced from 9·5 to 1·3% from first harvest to last, as total CFs reduced from 14·7 to 1·9% for *w1-5*.

It is therefore most likely that CF changes with time come from changes in recombination conditions as the non-synchronous meioses occur during an apothecium's maturation. The fact that some crosses did not show significant changes of CF with time, and that locus *w6* showed an increase in CF with time, while other loci showed decreases with time, suggests that locus and cross-specific factors are responsible. Locus-specific recombination-initiation factors and compounds persisting from before meiosis have also been postulated for other phenomena in *Ascobolus immersus* by Helmi & Lamb (1983), and by Lamb (1971) to explain effects of temperature before meioses

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on meiotic recombination in *Neurospora*. Concentrations of such compounds could obviously change as an apothecium matured.

Conversion classes did not all change to the same extent with time. For *w1-10*, $w^{+}(-) \times$ *w1-10(+)* (strains P5−; 415-3w10+) crosses at 22·5 and 17.5 °C both showed very similar reductions with time in 6+:2*w* and 2+:6*w* asci, but not for 5+:3*w* asci; the 5+:3*w* class increased with time relative to the 6+:2*w* and 2+:6*w* classes. In contrast, for *w1-78*, in cross $w^{+}(-) \times$ *w1-78*(+) (strains P5−; 577-6-w78+) at 10·0 °C, with very large samples, the 5+:3*w* class did decrease sharply with time, from 1·19 to 0·13%, and the 3+:5*w* class also decreased. Examples were found of 5+:3*w* and 3+:5*w* classes changing in frequency in opposite directions with time, of 6+:2*w* and 2+:6*w* classes changing in opposite directions, or of one conversion class changing but not another, or of different conversion classes changing in parallel. These variations between classes make it clear that other parameters, such as the extent of mispair correction, and the relative frequencies of correction to wild-type and mutant, must also be able to change, as well as recombination initiation.

The evidence from changes in individual conversion classes strongly suggests that CF changes in time with *Ascobolus* are due to changes in production frequency of different segregation classes, not to differential maturation after production. Previously described changes, from fungi with ordered octads, have been ascribed to differential maturation and dehiscence, e.g. the decrease in second division segregation classes and in octad classes with wild-type spores near their base (Lamb, 1966, 1967; *Neurospora crassa* and *Sordaria fimicola*), and the decrease in ordered octads with 2:2:2:2 segregations (MacDonald & Bond, 1976, *Sordaria brevicollis*). Variations in mycological features during cross maturation, such as in basidiospore dimensions, have also been reported (e.g. Duncan & Galbraith, 1973).

A conclusion of practical importance is that crosses of comparable maturity must be scored for recombination research, as changes with time could result in quite different parameter values for recombination initiation and repair characteristics, if crosses were scored at different maturities. The most serious implications are for quantitative analyses of gene conversion data to find specific values for various parameters of hybrid DNA formation and mispair repair. Harvests at different periods of cross maturity could well require different sets of parameter values to get good fits to observed ascal class frequencies: a correct model of recombination might therefore be rejected on the grounds that no single set of parameter values based on the model fitted the experimental data.

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