Genet. Res., Camb. (2003), **81**, *pp.* 7–13. *With* 1 *figure.* © 2003 Cambridge University Press
DOI: 10.1017/S0016672302006006 Printed in the United Kingdom

Responses to selection for postmeiotic segregation frequencies in *Ascobolus immersus*

BERNARD C. LAMB* AND MUHAMMAD SALEEM†
Biological Sciences Department, Imperial College of Science, Technology and Medicine, London SW7 2AZ, UK

(*Received 27 May 2002 and in revised form 12 September and 7 October 2002*)

Summary

A composite cross was made between 12 strains of the fungus *Ascobolus immersus*, six with wild-type red ascospores (*w1*$^+$) and six with white ascospore mutation *w1-78*. A high postmeiotic segregation (PMS) frequency line was set up from colonies from ascospores from dehisced octads showing PMS, 5+ : 3*w* and 3+ : 5*w*. A low PMS line was started from ascospores from 4+ : 4*w* or 6+ : 2*w* octads, and a 'no selection' line was set up from ascospores from random octads. Colonies were crossed to tester strains to determine PMS frequencies and the selected lines were continued from ascospores of crosses of the red ascospore strain with the most extreme (e.g. high for the high line) PMS frequency with the white-ascospore strain of most extreme PMS frequency and of opposite mating type. Significant responses to selection were obtained for increased (+100%) and decreased (−58%) PMS, giving a 4·8-times difference in generation 4, with little change in the frequencies of conversion classes showing meiotic segregation (6+ : 2*w* and 2+ : 6*w*). The continuous, symmetrical, roughly normal distributions for PMS frequencies obtained when generation 5 strains were crossed to unselected tester strains are those expected if PMS frequencies are controlled by a number of polygenes, not major genes. Crosses of selected fifth-generation red-ascospore strains with extreme PMS values to base-substitution mutant *w1-78*, to frame-shift mutant *w1-3C1* and to white-ascospore mutants *w-BHj* and *w-9* at two loci unlinked to *w1* showed that the effects of selection were not allele specific, locus specific or mutation-type specific.

1. Introduction

Recombination between syntenic genes can occur by reciprocal crossing-over or gene conversion. Its molecular mechanisms have been studied in detail but much less is known about its natural controls. Selection for recombination or chiasma frequencies has been tried many times, as has modelling the effects of recombination frequencies on fitness (see references in Charlesworth & Charlesworth, 1985*a*, *b*; Zwolinski & Lamb, 1995; Korol & Preygel, 1994). The responses of recombination frequencies to selection in fungi were studied in *Neurospora crassa* by Cederberg (1985*a*, *b*). For reviews of the mechanisms, regulation, function and evolution of recombination, see Lamb (1996), Petes (2001) and Otto & Lenormand (2002).

* Corresponding author. Tel: +44 (0)20 7594 5382. Fax: +44 (0)20 7584 2056. e-mail: b.lamb@ic.ac.uk
† Present address: Government College, Lahore, Pakistan.

Ascobolus immersus is a very suitable fungus for studying gene conversion and its controls because several loci in the Pasadena strains have fairly high gene-conversion frequencies, and conversion and its controls have been extensively studied (e.g. Helmi & Lamb (1983)). Zwolinski & Lamb (1995) obtained significant responses to selection for gene-conversion frequencies in *A. immersus*, with the control of conversion frequencies by non-locus-specific polygenes. Selection for conversion frequency had little effect on the frequency of postmeiotic segregation (PMS) or on the relative frequencies of conversion to mutant or to wild type.

In the present work, we looked at the effects of selecting for increased or decreased frequencies of PMS, as measured by the frequency of 5+ : 3*w* and 3+ : 5*w* octads from wild-type red-ascospore strain mutant white-ascospore strain crosses, using *w1*$^+$ × *w1-78* crosses for the selection experiments; mating-type differences ensured that only the desired crosses

https://doi.org/10.1017/S0016672302006006 Published online by Cambridge University Press

occurred. Although one can detect aberrant 4 : 4 segregations in unordered octads in *A. immersus* using an unlinked second ascospore-pigmentation marker, such as *granular* (Ghikas & Lamb, 1977), these segregations were not scored here because no second marker was used. Conversion classes showing meiotic segregation (MS), 6+ : 2*w* and 2+ : 6*w*, were also scored to check whether any response of PMS frequencies to selection was due to a parallel change in all conversion classes (from a change in conversion frequencies generally) or just in PMS classes, from a response of mispair-correction systems.

The starting 'population' for selection was a composite cross between 12 strains of *A. immersus*, six with wild-type red ascospores (*w1*$^+$) and of (+) mating type, and six with white-ascospore mutation *w1-78* of (−) mating type. We looked at the responses of PMS frequencies to selection over four generations. The behaviour of PMS frequencies was looked at to see: (i) whether there was genetic variation; (ii) whether it was affected by the alleles present at the *conversion control factor* (*ccf*) *2* locus, which has major effects on conversion frequency at locus *w1* (Lamb & Helmi, 1978; Helmi & Lamb, 1983); (iii) whether it was affected by different mutational types of allele at locus *w1* (*w1-78* is a base substitution; *w1-3C1* is a frame shift); and (iv) whether *ccf-5* alleles affected the PMS frequencies of white mutations at two loci unlinked to *w1* (*w-BHj*; *w-9*) or to each other.

2. Materials and methods

(i) *Stocks, media and general methods*

All strains ultimately come from the original wild types P5(−) and K5(+), which were isolated from red ascospores from different asci in 1963 by Emerson & Yu-Sun (1967). The *w1-78* mutation (a spontaneous base substitution) was isolated by Emerson & Yu-Sun (1967); the other mutations were induced by Ghikas (Lamb & Ghikas, 1979). The red-ascospore strains used were derived from various crosses made over many years, because the two original strains became sterile. Stocks, media, general methods and control tests for reversions, phenocopies and false octads were as described by Emerson & Yu-Sun (1967), Ghikas & Lamb (1977), Lamb & Helmi (1978) and Zwolinski & Lamb (1995). All crosses were made at 17·5 °C in continuous light from a total of 48 W of daylight fluorescent tubes in each incubator.

(ii) *Initial composite cross and 'no selection' controls*

The initial composite cross was made with 12 strains on the same Petri dish, with a mixture of six *w1*$^+$ (+ mating type) red-ascospore wild-type strains and six *w1-78* (− mating type) white-ascospore mutant strains, with all strains carrying the same conversion control factor, *ccf-2*(*P*). This mixture allowed only *w1*$^+$(+) × *w1-78*(−) crosses, but any (+) strain could cross with any (−) strain, giving 36 possible crosses in the composite cross. To start the high-PMS line, on the assumption that 5+ :3*w* and 3+ :5*w* octads were more likely to have come from high-PMS than low-PMS crosses, >50 red and >50 white ascospores were picked up from such PMS octads and put on dung-extract germination medium (2 h heat shock at 50 °C for red ascospores, none for white ascospores), with overnight incubation of both spore types at 37 °C before picking up germlings into separate tubes. To start the low-PMS line, >50 red and >50 white ascospores were picked up from 4+ : 4*w* and 6+ : 2*w* octads (ignoring the 2+ : 6*w* octads because there was a very low chance of them arising from a new mutation to *white*, possibly at a different locus) and set up for germination and colony isolation as described above.

A 'no selection' line was set up as a control from the same composite cross. Red and white ascospores were taken at random, irrespective of + : *w* segregation ratio, and were germinated and colonies were isolated as described above. The red-ascospore-derived strains were crossed to a *w1-78*(−) tester to establish mating type, and the white-ascospore-derived strains were crossed to a *w1*$^+$(+) tester. A new composite cross was then made between six red-ascospore strains (*w1*$^+$) of (+) mating type with six white-ascospore strains (*w1-78*) of (−) mating type, irrespective of PMS frequency, and 2000 dehisced octads from the composite cross were scored for segregation ratios, and red and white ascospores were isolated for the next generation. The PMS frequencies for the 'no selection' line were scored from octads dehisced from the composite cross itself, not from crosses of individual colonies to tester strains. The main strains used as testers and/or in the composite cross were: (i) with red ascospores (genotype *w1*$^+$), 1WT3(+), 25-5R(+), 9RP(+), 2.2R(+), 595,P(+), Sk9R1(+), SkR7RP7(−), SKRP(−); with white ascospores (genotype *w1-78*): EC11w1-78,7P(−),Skw78P7(−),1w78.2P(−),EC11w98.7(−), w78,P(−), Sk2,w78,4(−), Skw78P7(+), 2.3Pw78(+).

(iii) *Selection in generations 1–4*

Within the high-PMS line, colonies from >50 red ascospores from 5+ : 3*w* and 3+ : 5*w* octads were crossed to tester strains of *w1-78*(−) to establish their mating type, and 25 fertile crosses (which must have been *w1*$^+$(+) × *w1-78*(−)) had 1000 dehisced octads scored from each for segregation classes. Similarly, colonies from >50 white ascospores from 5+ : 3*w* and 3+ :5*w* octads were crossed to tester strains of *w1*$^+$(+) to establish their mating type, and 25 fertile crosses (which must have been *w1*$^+$(+) × *w1-78*(−)) had 1000 dehisced octads scored from each. The red-ascospore-derived strain (*w1*$^+$(+)) giving the highest PMS

https://doi.org/10.1017/S0016672302006006 Published online by Cambridge University Press

Table 1. *Results of selection for increased or decreased frequency of postmeiotic segregation (PMS), with 'no selection' controls. In generations 1–4, high line, the results are from crosses of the red-ascospore strain* (w1$^+$(+), ccf-2(*P*)) *with the highest % PMS to the white-ascospore strain* (w1-78(−), ccf-2(P)) *with the highest % PMS, after testing each strain in crosses to standard tester strains. For the low line, the red- and white-ascospore strains with the lowest PMS frequencies were crossed*

	High-PMS line				'No selection' line			Low-PMS line			
Generation	Sample size	PMS ÷ MS	MS* (%)	PMS (%)	Sample size	PMS ÷ MS	MS (%)	PMS (%)	Sample size	PMS ÷ MS	MS (%)	PMS (%)
0					2000	0·15	8·2	**1·2**				
1	4000	0·17	8·2	**1·5**	2000	0·15	8·5	**1·3**	4000	0·12	8·4	**1·0**
2	4000	0·23	8·0	**1·9**	2000	0·13	8·2	**1·1**	4000	0·09	8·5	**0·8**
3	4000	0·25	7·5	**2·0**	2000	0·19	8·9	**1·7**	4000	0·07	8·4	**0·6**
4	4000	0·29	8·1	**2·4**	2000	0·16	8·5	**1·4**	4000	0·06	8·2	**0·5**
Proportional % change by generation 4 relative to the initial 'No selection' line value				+100				+17				−58

Comparison of PMS frequencies between generation 0, the 'no selection' line, and the three generation-4 lines. High line, $\chi^2 = 9{\cdot}8$, significant at $P = 0{\cdot}01$; 'no selection' line, $\chi^2 = 0{\cdot}3$, not significant at $P = 0{\cdot}05$; low line, $\chi^2 = 9{\cdot}0$, significant at $P = 0{\cdot}01$.
* MS, meiotic segregation.

frequency in the cross to the *w1-78*(−) tester strains was then crossed to the white-ascospore-derived strain (*w1-78*(−)) giving the highest PMS frequency in the cross to the *w1*$^+$ tester, and red and white ascospores from this highest-PMS × highest-PMS cross were used to obtain the next generation of isolates. For the low line, the lowest-PMS × lowest-PMS cross was used, taking spores only from 4+ : 4*w* and 6+ : 2*w* octads. In generations 2 to 4, 50 red and 50 white ascospores were taken for germination in each generation, and crossed to testers to establish mating type. However, only 15 (not 25) fertile crosses of each type were scored for PMS frequency.

(iv) *Generation 5*

In the high line, 200 red ascospores from the generation 4 highest PMS frequency *w1*$^+$(+) strain × highest PMS frequency *w1-78*(−) strain were isolated from 5+ : 3*w* and 3+ : 5*w* octads, were germinated, and the colonies were crossed to a *w1-78*(−) tester to establish mating type. From each fertile cross, 1000 dehisced octads were scored for segregation classes, to identify the selected strains with the highest PMS frequency for further experiments, and providing data for Table 2 and Fig. 1. In the low line, a similar procedure was used for 200 red ascospores from 4+ : 4*w* and 6+ : 2*w* octads from the generation-4 lowest-PMS-frequency *w1*$^+$(+) strain × lowest-PMS-frequency *w1-78*(−) strain cross in order to identify the red-ascospore-derived colonies with the lowest PMS frequency. In the 'no selection' line, 200 red ascospores from random asci were used from the generation-4 composite cross.

3. Results

The results of replicates (different Petri dishes) and repeats (the same crosses made at different times) were usually very similar for PMS and total conversion frequencies. Within a line, crosses of red-ascospore-derived strains with *w1-78*(−) testers gave similar PMS frequency results to those of white-ascospore-derived strains with *w1*$^+$(+) testers, suggesting that there is no close linkage of PMS-control genes to mating type or *w1*.

(i) *Changes in PMS frequency during selection*

The results are shown in Table 1. The 'no selection' control composite crosses showed some fluctuations in PMS between generations but no overall trend. Selection in the first generation changed the control value of 1·2% PMS in generation zero to 1·5% in the high-PMS line and to 1·0% in the low-PMS line, where these are (respectively) the PMS values of highest-red × highest-white ascospore strains and lowest-red × lowest-white strains. Selection in further generations gave further progressive responses in both

https://doi.org/10.1017/S0016672302006006 Published online by Cambridge University Press

Table 2. *Analysis of postmeiotic segregation (PMS) frequency data from crosses of generation five red ascospore strains, from high PMS, 'no selection' and low PMS lines, to* w1-78 *tester stocks. The red ascospore strains were* w1$^+$, ccf-2(P)*; tester stocks were* w1-78, ccf-2(*P*). *Results are based on 1000 octads per cross*

	High-PMS line	'No selection' line	Low-PMS line
Number of isolates tested	86	80	77
Mean PMS frequency (%)	**1·85**	**1·40**	**1·10**
Observed standard deviation (%)	0·59	0·46	0·56
Limits of PMS frequency (%)	0·9–3·0	0·4–2·3	0·3–2·0
Proportional change relative to the 'No selection line' (%)	+32		−21
Comparisons between lines			
Pair of lines	High and low	High and 'no selection'	'No selection' and low
d value*	8·42	5·54	3·68

* A method of comparing means from two large samples from normal populations. The *d* value is related to the percentage points of the normal distribution (Bailey, 1969). All three comparisons were significant at $P = 0{\cdot}001$.

the high and low lines. From generation zero to generation 4, the high line gave a doubling of PMS frequencies and the low line gave more than a halving of PMS frequencies, with a 4·8-times difference between these two lines in generation 4. This difference between the high and low lines was highly significant ($\chi^2 = 50{\cdot}5$, $P < 0{\cdot}001$). Even-more-extreme PMS values (0.3–3·0%, a tenfold difference) were found in generation 5 (Fig. 1, Table 2) among more isolates (77–86 per line) but in isolate × unselected tester crosses, not in highest × highest or lowest × lowest strains. The changes in PMS frequency from the generation 0 value to the generation 4 values were significant at $P = 0.01$ within the high and low lines, but were not significant for the 'no selection' line.

As shown in Table 1, there were only minor changes over five generations in the frequency of meiotic-segregation (MS) gene conversion classes, indistinguishable from random fluctuations. This shows clearly that these responses of PMS frequencies to selection were due to specific effects on PMS, not to a general selection for increased or decreased conversion affecting all conversion classes, although both kinds of response were possible from the selection system used here. The PMS/MS ratio therefore changed during selection. Selection responses were in the same direction and similar in amount for both PMS classes (5+ : 3*w* and 3+ : 5*w*).

(ii) *Differences in PMS frequencies between the high PMS, low PMS and 'no selection' lines in generation 5, in red-ascospore-derived colonies crossed to a* w1-78(−) *strain*

In this experiment, there was no selection for PMS within the generation-5 strains used, only selection for mating type so that all strains could be crossed to the same tester. Fig. 1 shows the distribution of PMS frequencies within and between the three lines in generation 5, with each point being the average value of two to 24 isolates with similar PMS frequencies. Each of the three lines showed a very approximately normal, symmetrical, distribution, but with no 'tails'. Table 2 shows the line averages, standard deviations and range limits, and a statistical analysis showing that all three lines had differences in PMS frequencies significant at $P = 0{\cdot}001$. PMS frequencies at a *w1*$^+$/*w1-78* heterozygosity for a base substitution were therefore under genetic control, with genetic variation for this existing in the initial composite cross and responding to selection for increased and for decreased PMS frequencies.

(iii) *How do the genes controlling PMS frequencies at* w1-78 *interact with conversion control factor* 2 *alleles* P, K *and* 91*?*

In this experiment, extreme high and extreme low PMS-selected strains were used, along with the 'no selection' control. These red-ascospore-derived strains in generation 5 all carried *ccf-2*(*P*). Table 3i shows the effects of crossing the generation-5 selected high line isolates, 'no selection' isolates and selected low line isolates to *w1-78*(−) tester strains carrying *ccf-2*(*P*), *ccf-2*(*K*) or *ccf-2*(*91*): *w1-78*(*P*)(−), *w1-78*(*P*)(+), *w1-78*(*K*)(−), *w1-78*(*K*)(+), *w1-78*(*91*)(−) and *w1-78*(*91*)(+). The red-ascospore-derived strains of generation 5 were two strains of each mating type from each line, with 2500 octads scored from each of these four crosses, giving 10 000 octads for each combination of line and *ccf* allele.

As expected from the work of Lamb & Helmi (1978), the *ccf-2*(*K*) and *ccf-2*(*91*) alleles in the white ascospore tester strains caused reduced PMS frequencies compared with the *ccf-2*(*P*) allele, even when heterozygous with *ccf-2*(*P*) in the red-ascospore generation-5 strains. Within each *ccf* allele's three crosses, the high line gave higher PMS frequencies than did the 'no selection' line, which in turn gave higher PMS frequencies than the low line, with the high line/low line difference being significant at $P = 0{\cdot}01$ or 0·001. The genetic controls of PMS frequency for *w1-78* selected in strains with

https://doi.org/10.1017/S0016672302006006 Published online by Cambridge University Press

Table 3. *The effect on postmeiotic segregation (PMS) and meiotic segregation (MS) frequencies, and other measures of crossing red-ascospore strains (all $w1^+$, ccf-2(P)) from high-PMS, 'no selection' and low-PMS lines of selection-generation five. These strains were crossed to tester strains carrying the* w1-78 *allele (i) or the* w1-3C1 *allele (ii), with different combinations of alleles at conversion control factor* ccf-2*; or to white-ascospore alleles at two loci unlinked to* w1: w-BHj *(iii) or* w-9 *(iv). Results are based on 10 000 octads per cross*

Line	*ccf-2* allele in mutant	PMS ÷ MS	MS (%)	**PMS (%)**	2×2 χ^2 for PMS frequency (high versus low lines)
(i) allele *w1-78*					
High	P	0·28	10·3	**2·8**	16·9***
'No selection'	P	0·25	10·1	**2·5**	
Low	P	0·22	9·0	**1·9**	
High	K	0·20	8·0	**1·6**	9·2**
'No selection'	K	0·18	7·3	**1·3**	
Low	K	0·13	8·5	**1·1**	
High	91	0·13	9·2	**1·2**	19·8***
'No selection'	91	0·11	8·3	**0·9**	
Low	91	0·11	5·5	**0·6**	
(ii) allele *w1-3C1*					
High	P	0·29	9·6	**2·8**	16·9***
'No selection'	P	0·27	9·3	**2·5**	
Low	P	0·23	8·7	**2·0**	
High	K	0·13	12·3	**1·6**	9·2**
'No selection'	K	0·16	8·2	**1·3**	
Low	K	0·15	7·3	**1·1**	
(iii) white allele at locus *w-BHj*					
High		0·30	9·7	**2·9**	30·6***
'No selection'		0·24	9·6	**2·3**	
Low		0·17	10·0	**1·7**	
(iv) white allele at locus *w-9*					
High		0·21	5·7	**1·2**	4·3*
'No selection'		0·17	5·9	**1·0**	
Low		0·13	6·9	**0·9**	

* Significant at $P = 0{\cdot}05$.
** Significant at $P = 0{\cdot}01$.
*** Significant at $P = 0{\cdot}001$.

the *ccf-2(P)* allele therefore still work, in the same direction, in the presence of different alleles at *ccf-2*. Lamb & Helmi (1978) showed that *ccf-2(K)* and *ccf-2(91)* were incompletely dominant to *ccf-2(P)* for conversion frequencies, giving lower values than *ccf-2(P)*; the present data are consistent with that. These genetic controls of PMS frequencies definitely affected the PMS frequencies in Table 3 but had rather varied effects on the frequencies of meiotic segregation conversion classes (6+ : 2*w* and 2+ : 6*w*), with minor or no effects on this in Table 3ii, iii and iv, but a larger effect in Table 3i in crosses involving *w1-78*(−) with the *ccf-2(91)* alleles.

(iv) *Are the genes controlling PMS frequencies at* w1-78 *allele specific or mutation-type specific?*

Table 3ii shows the results of crossing a generation 5 high line strain, a 'no selection' line strain and a low line strain to one *w1-3C1*(−) *strain* carrying *ccf-2(P)*, and to one carrying *ccf-2(K)*. As with crosses to *w1-78* (Table 3i), the high line strain gave higher PMS frequencies than the low line strain in crosses to *w1-3C1*(−) strains carrying *ccf-2(P)* or *ccf-2(K)*. The highly significant differences show that the genetic factors affecting PMS frequencies are not allele specific within the *w1* locus and are not mutation-type specific, because they affected a heterozygous frame-shift mutation, *w1-3C1*, as well a heterozygous base substitution, *w1-78*.

(v) *Are the genes controlling PMS frequencies at* w1-78 *and* w1-3C1 *locus specific?*

Table 3iii shows the results of crossing three generation-5 strains: a selected high line strain, a 'no selection' line strain and a selected low line strain to *white* mutations at two loci unlinked to *w1*, using

https://doi.org/10.1017/S0016672302006006 Published online by Cambridge University Press

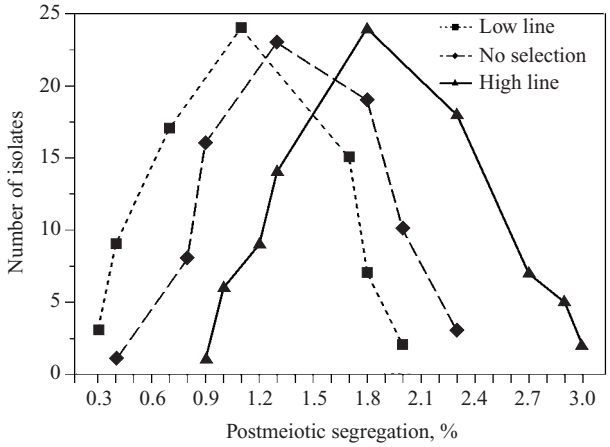

Fig. 1. The distribution of postmeiotic segregation (PMS) frequencies in the high-PMS line, 'no selection' line and low-PMS line in the fifth generation of selection. The generation-5 strains were unselected within that generation and were crossed to an unselected *w1-78* tester strain.

Gw-BHj 9·1(−), *Gw-BHj24.4*(−) and *Gw-9 17·1*(−). In the three types of cross to *w-BHj* and *w-9*, the high line strain gave a higher PMS frequency, significant at the $P = 0{\cdot}001$ or 0·05 levels, respectively, than did the low line strain. The genes controlling PMS frequencies at locus *w1* are therefore not locus specific. For both loci, there were no changes in MS frequencies so there were large changes in PMS/MS ratios.

4. Discussion

As with gene conversion frequencies in *A. immersus* (Zwolinski & Lamb, 1995), selection over five generations for increased or decreased values worked well for PMS frequencies, with little change in the 'no selection' line. In selected strain × tester strain crosses, the selected strains provide only half the genes at meiosis in the diploid fusion nucleus in the ascus. The effects of the selected strains will only show in such nuclei if they are dominant or partly dominant, or show additive action, in crosses to testers. Because of this, genetic differences between selected strains will, on average, be twice the phenotypic differences shown by PMS frequencies in crosses to unselected tester strains. The responses to selection were roughly the same in the upwards and downwards directions. Differences in the amount of response in two directions can sometimes just be problems of scale and can be dealt with by transformations, such as logs or arcsin-square root (Falconer & Mackay, 1996; Zwolinski & Lamb, 1995).

The more extreme high and low PMS values in generation 4 than in the generation 5 line average values (Tables 1, 2) are due to two factors. First, the generation 4 values are from highest PMS $w1^+(+)$ × highest PMS *w1-78*(−), or from lowest PMS $w1^+(+)$ × lowest PMS *w1-78*(−), whereas the generation-5 PMS values are from high or low PMS line strains crossed to unselected testers, not to selected strains within the same line. Second, the generation-4 results are from the most extreme strains in that generation, whereas the generation-5 results in Table 2 and Fig. 1 are population averages from all strains in each line.

The ranges of PMS frequencies within lines in generation 5 were large for all three lines, probably reflecting chance variation in the samples of 1000 octads per cross, some environmental variation even under lab conditions, and some remaining genetic variation within lines. The results in Table 1 and Fig. 1 do not suggest a levelling off of the response of PMS frequencies to selection even after four or five generations, so there was probably some genetic variation left in generation 5. The smooth changes in PMS frequency between generations (Table 1) and the approximately normal, not bimodal or multimodal, distributions of PMS frequencies within lines (Fig. 1) are consistent with polygenic control of PMS frequencies rather than control by segregating alleles of major effect. The persistence of genetic variation over five generations is more likely if there are several to many loci segregating, rather than one or two loci, because there would be more segregating loci to undergo fixation before genetic variation is exhausted. The distributions here are quite different from the discontinuous ones obtained for conversion frequencies when *conversion control factor* alleles with major effects were segregating (Helmi & Lamb, 1983 (Fig. 2); Howell & Lamb, 1984 (Fig. 1)).

Although the selection was made with $w1^+(+) \times$ *w1-78*(−) crosses, the results of crossing selected red-ascospore-derived strains ($w1^+(+)$) to another locus *w1* mutant allele, *w1-3C1*, showed that the genetic control factors for PMS frequency were not allele or mutation-type specific. Crosses to two mutations at other loci showed that the controls were not locus specific, either. It is therefore likely that the selection responses were caused by genetic differences affecting PMS frequencies generally, not just in one region or for one type of mispairing in hybrid DNA.

Some alleles at PMS control loci must increase PMS frequency, by decreasing the correction of mispairings, and the alternative alleles must increase correction frequency. Increases in correction could come from having higher concentrations or efficiencies of correction enzymes, or from having more time available for correction, or from some accessory compounds stimulating correction.

Although *A. immersus* is vegetatively haploid, the ascogenous hyphae in the apothecium are binucleate, with conjugate division of nuclei of opposite mating type. The fusion nucleus and young ascus before meiosis are diploid, so dominance could affect PMS frequencies. During the main selection stages in generations 1–4, standard *w1-78*(−) and $w1^+(+)$ testers

https://doi.org/10.1017/S0016672302006006 Published online by Cambridge University Press

were used to assess PMS frequencies of colonies derived from red ($w1^+$) and white (*w1-78*) ascospores, respectively, and the two types of tester might have differed in the alleles they carried for genes that affect PMS frequencies. The progress of selection depends on the number of segregating controlling loci, dominance relations between alleles, whether dominance at different loci was directional, and the alleles present in the testers and in the selection line strains. Selection would work best with recessive alleles in the testers, because dominance in the testers could mask differences between the selected strains.

All the strains used in this study came from two wild-type haploid ascospores from a canyon near Pasadena and are to some extent inbred, with a likelihood that some genetic variation between those original strains has already been lost, although there might have been some spontaneous mutation(s) increasing variation and there might have been some recombination producing new combinations of existing variations. Unless spontaneous mutation has occurred at PMS control loci during subsequent subculturing, there are unlikely to be more than two alleles at any locus even in the initial composite cross. The existence of substantial genetic variation for genes affecting PMS frequencies in the few strains studied here suggests that there is widespread genetic variation in natural populations for this character.

What are the practical consequences for the fungus of having genetically controlled PMS frequencies? In high-PMS lines, fewer mispairs in hybrid DNA are corrected than in low-PMS lines, so there will be more aberrant 4 : 4, 5+ : 3*m* (*m* = *mutant*) and 3+ : 5*m* octads, and fewer correction 4+ : 4*m*, 6+ : 2*m* and 2+ : 6*m* octads. This seems unlikely to have much effect on survival or fitness, or to have any selective value unless it reflects more general aspects of DNA correction, such as correction in replication, which could affect mutation frequencies. In another ascomycete, *Sordaria fimicola*, Lamb *et al*. (1998) found genetic variation in mutation frequencies, with different frequencies in different habitats, as if there had been selection for optimum mutation rates under different conditions. Saleem *et al*. (2001) found no correlation between the efficiency of correction enzymes in recombination and the overall mutation frequencies in strains of *S. fimicola* from different environments.

We thank the Education Ministry, Islamabad, Pakistan, for a scholarship for M.S.

References

Bailey, N. T. J. (1969). *Statistical Methods in Biology*. London: English University Press.

Cederberg, H. (1985*a*). Recombination in other chromosomal regions than the interval subjected to selection, in lines of *Neurospora crassa* selected for high and low recombination frequencies. *Hereditas* **103**, 89–97.

Cederberg, H. (1985*b*). Allelic recombination in the *his-1* locus of *Neurospora crassa* in isolates showing high and low frequencies of intergenic recombination. *Hereditas* **103**, 143–148.

Charlesworth, B. & Charlesworth, D. (1985*a*). Genetic variation in recombination in *Drosophila*. I. Responses to selection and preliminary genetic analysis. *Heredity* **54**, 71–83.

Charlesworth, B. & Charlesworth, D. (1985*b*). Genetic variation in recombination in *Drosophila*. II. Genetic analysis of a high recombination stock. *Heredity* **54**, 85–98.

Emerson, S. & Yu-Sun, C. C. C. (1967). Gene conversion in the Pasadena strain of *Ascobolus immersus*. *Genetics* **55**, 39–47.

Falconer, D. S. & Mackay, T. F. C. (1996). *Introduction to Quantitative Genetics*, 4th edn. Harlow, UK: Longman.

Ghikas, A. & Lamb, B. C. (1977). The detection, in unordered octads, of 6+ : 2*m* and 2+ : 6*m* ratios with postmeiotic segregation, and of aberrant 4 : 4s, and their use in corresponding-site interference studies. *Genetical Research* **29**, 267–278.

Helmi, S. & Lamb, B. C. (1983). The interactions of three widely separated loci controlling conversion properties of *w* locus I in *Ascobolus immersus*. *Genetics* **104**, 23–40.

Howell, W. M. & Lamb, B. C. (1984). Two locally acting genetic controls of gene conversion, *ccf-5* and *ccf-6*, in *Ascobolus immersus*. *Genetical Research* **43**, 107–121.

Korol, A. B. & Preygel, I. A. (1994). *Recombination Variability and Evolution*. Andover, UK: Chapman & Hall.

Lamb, B. C. (1996). Ascomycete genetics: the part played by ascus segregation phenomena in our understanding of the mechanisms of recombination. *Mycological Research* **100**, 1025–1059.

Lamb, B. C. & Ghikas, A. (1979). The intergradation, genetic interchangeability and interpretation of gene conversion spectrum types. *Genetics* **92**, 49–65.

Lamb, B. C. & Helmi, S. (1978). A new type of genetic control of gene conversion, from *Ascobolus immersus*. *Genetical Research* **32**, 67–78.

Lamb, B. C., Saleem, M., Scott, W., Thapa, N. & Nevo, E. (1998). Inherited and environmentally induced differences in mutation frequencies between wild strains of *Sordaria fimicola* from 'Evolution Canyon'. *Genetics* **149**, 87–99.

Otto, S. P. & Lenormand, T. (2002). Evolution of sex: resolving the paradox of sex and recombination. *Nature Reviews Genetics* **3**, 252–261.

Petes, T. D. (2001). Meiotic recombination hot spots and cold spots. *Nature Reviews Genetics* **2**, 360–369.

Saleem, M., Lamb, B. C. & Nevo, E. (2001). Inherited differences in crossing over and gene conversion frequencies between wild strains of *Sordaria fimicola* from 'Evolution Canyon'. *Genetics* **159**, 1573–1593.

Zwolinski, S. A. & Lamb, B. C. (1995). Non-locus-specific polygenes giving responses to selection for gene conversion frequencies in *Ascobolus immersus*. *Genetics* **140**, 1277–1287.

https://doi.org/10.1017/S0016672302006006 Published online by Cambridge University Press