Inherited Differences in Crossing Over and Gene Conversion Frequencies Between Wild Strains of *Sordaria fimicola* From "Evolution Canyon"

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ABSTRACT

Recombination generates new combinations of existing genetic variation and therefore may be important in adaptation and evolution. We investigated whether there was natural genetic variation for recombination frequencies and whether any such variation was environment related and possibly adaptive. Crossing over and gene conversion frequencies often differed significantly in a consistent direction between wild strains of the fungus *Sordaria fimicola* isolated from a harsher or a milder microscale environment in "Evolution Canyon," Israel. First- and second-generation descendants from selfing the original strains from the harsher, more variable, south-facing slope had higher frequencies of crossing over in locus-centromere intervals and of gene conversion than those from the lusher north-facing slopes. There were some significant differences between strains within slopes, but these were less marked than between slopes. Such inherited variation could provide a basis for natural selection for optimum recombination frequencies in each environment. There were no significant differences in meiotic hybrid DNA correction frequencies between strains from the different slopes. The conversion analysis was made using only conversions to wild type, because estimations of conversion to mutant were affected by a high frequency of spontaneous mutation. There was no polarized segregation of chromosomes at meiosis I or of chromatids at meiosis II.

TO understand biodiversity, adaptation, and evolution, one must explore the causes of variation and the controls of processes causing genetic variation. Most natural inherited variation arises through mutation and recombination, and Zhuchenko and Korol (1985) concluded that in higher Eukaryotes, recombination plays a greater role in the short-term release of variability than does mutation. We have studied the variation in recombination properties—crossing over and gene conversion—between different wild strains of the coprophilous Ascomycete fungus, *Sordaria fimicola*, from different microscale environments in "Evolution Canyon," Lower Mount Carmel, Israel. We used 11 ascospore color mutations that occurred spontaneously in three strains isolated from the wild, with six mutations from two south-facing slope (SFS) strains and five from one north-facing slope (NFS) strain.

Our aims were to see whether there were significant differences in recombination properties between the strains overall, between strains from the two different slopes (SFS and NFS), or between strains from the same slope; to determine whether two different aspects of recombination—crossing over in the gene-centromere intervals and gene conversion—showed similar or different changes between strains; and to see whether recombination differences were consistent in amount and direction for different intervals (crossing over) or for different mutation sites (gene conversion). We were particularly interested in whether any differences were environment related and possibly adaptive. The working hypothesis was that the harsher, more changeable, and more diverse SFS environment might favor processes increasing genetic variation, including a higher mutation rate and more recombination through crossing over and/or gene conversion, compared with the milder NFS environment. Our mutation studies (Lamb *et al.* 1998) showed that three south-facing slope wild-type strains had significantly higher mutation frequencies than five from the north-facing slopes, with much of the difference being inherited through two generations of selfing.Three different aspects of meiotic gene conversion were studied: conversion frequencies, conversion direction (the relative frequencies of conversion to wild type and to mutant), and the frequency of correction of mispairs in hybrid DNA. The mutations were also mapped from crosses between them. For a review of recombination in Ascomycete fungi, including crossing over, gene conversion, and interference, see Lamb (1996).

The large samples of ordered octads needed for the recombination studies provided extensive data on whether there was polarized segregation (Lamb 1966), that is, whether there was biased segregation with respect to the top and bottom poles of the spindles for homologous

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chromosomes at meiosis I and/or for chromatids at meiosis II. This was of interest in its own right and because apparent polarized segregation is an indicator of the differential maturation and dehiscence of different ordered octad classes, which can bias recombination results (Lamb 1967).

The south-facing slope of Evolution Canyon, Lower Mount Carmel, Israel, bears African and Asian xeric tropical species, has up to 300% more solar radiation (the total solar radiation on a horizontal surface at the site is 700–725 gram-calories per square centimeter per day), and is warmer, drier, more fluctuating, and more heterogeneous than the lush "temperate European" north-facing slope, with only 100–400 m between the slopes (see Nevo 1995, which includes a color photograph of Evolution Canyon; Nevo 1997). The increased solar radiation acts through soil, air moisture, and temperature on living organisms, but both slopes are of geologically identical eroded Upper Cenomanian limestone. The SFS has Lithic Rossa soils, the NFS has reddish-brown forest soils, and the valley bottom has alluvial soils (Nevo *et al.* 1998). Wild strains of *S. fimicola* were isolated from three different levels (60, 90, and 120 m above sea level) on each of the SFS and NFS. The Evolution Canyon project aims to study, at a microscale, biodiversity (*i.e.*, the patterns and dynamics of genes, species, and ecosystems) across different groups of organisms from bacteria through fungi, plants, and animals. It attempts to assess the relative importance of the driving evolutionary forces caused by interslope differential solar radiation, which gives physically and biotically different organisms on the opposite slopes (reviewed by Nevo 1995, 1997). *S. fimicola*, a homothallic, self-fertile fungus, is vegetatively haploid, with meiosis plus two mitoses giving asci containing eight haploid black binucleate ascospores (Webster 1980). The ascospore color mutations, where genes for wild-type black can mutate to give white or other colors (Olive 1956; Kitani and Olive 1967), were isolated as spontaneous mutants during our studies of mutation (Lamb *et al.* 1998). There are ~16 known loci for ascospore pigmentation (Catalogue of Strains 1996) in Olive's strains in several linkage groups, but strains of different origin are often not interfertile, so it is difficult to compare mutations from different strains. We crossed our Evolution Canyon strains to Olive's strains but they were not interfertile (Lamb *et al.* 2000).

The wild isolates were obtained vegetatively, not from ascospores, and were subcultured as little as possible, using large inocula to minimize genetic drift. From the SFS, S1 was isolated from dung near the top, S2 from dung near the middle, and S3 from soil near the bottom. From the NFS, N5 was isolated from soil near the bottom, N6 from soil near the middle, and N7 from dung near the top. Selfed generation 1 strains were isolated by germinating black ascospores from the original wild-type strains, and selfed generation 2 strains were obtained from selfed generation 1 black ascospores. Because there can be some effects of premeiotic conditions (such as temperature) on recombination at meiosis in Sordaria (Lamb 1969b), and because we wanted to study inherited recombination frequencies, only strains of selfed generations 1 and 2 were used in the recombination experiments. They had existed only under our standard lab conditions.

For comprehensive reviews of stress and evolution, see Hoffmann and Parsons (1991) and Parsons (1994). For variability of recombination and evolution, and for computer simulations of the effects of changing environments and other factors such as linkage on selection for recombination modifiers, see Korol *et al.* (1994) and Charlesworth (1993). See Bell (1982) for theories of sex and recombination and their evolutionary and ecological advantages and disadvantages. While there has been a lot of modeling of theoretical situations, and some studies of specific recombination mutations, there have been few experimental studies on the genetic control of recombination in wild organisms in relation to their environments. The theoretical studies have usually been of sexually reproducing diploids, but the haploidy of Sordaria changes aspects of selection because deleterious recessive alleles are not protected from selection in heterozygotes.

MATERIALS AND METHODS

Isolation of strains: Strains were isolated between August 1994 and February 1995; see Lamb *et al.* (1998) for details.

Procedures, strains, and controls: Crosses were made at 17.5° in the light by inoculation of one strain in the center (selfing), or two strains on opposite sides, of 9-cm petri dishes of minimal medium (Olive 1956) with at least four replicates or repeats of each cross. The light was from two 30 W fluorescent tubes running the height of the incubator, with internally reflective incubator surfaces. Growth and ascospore germination were on cornmeal agar with sodium acetate (Kitani and Olive 1967) at 18° for germination. Dehisced ascospores for scoring or germination were collected on plates of 1.7% water agar with 0.7 g/liter methyl-*p*-hydroxybenzoate to inhibit spontaneous ascospore germination. Black ascospores from selfed perithecia of the original wild strains were germinated to obtain the selfed generation 1 strains, which were used in turn to get selfed generation 2 wild-type strains from their black ascospores. The term "wild type" will usually be used for an allele or phenotype here, as opposed to using it for an original strain from the wild. The ancestries of the selfed generation 1 and 2 strains are shown in Tables 3 and 4.

Each ascospore color mutant, obtained from an original SFS or NFS wild strain, was crossed to different strains that were wild type for spore color, two derived from SFS strains and two from NFS strains. Perithecia were dissected in 2 M sucrose solution to display the linear ordered octads. The six ordered tetrad classes (numbered as in Lamb 1996) were recorded for the determination of centromere distances, and gene conversions were noted. Ascospores from >100 asci with gene conversion patterns were isolated and germinated and the colonies were allowed to self-fertilize to test for phenocopies. Controls for reversion were set up, scoring selfed crosses of ascospore color mutations for asci with black ascospores;

controls for new spontaneous ascospore color mutations consisted of scoring selfed crosses of different strains wild type for spore color and looking for octads with some nonblack ascospores, usually four black:four nonblack.

Estimation of crossing over: Crossing over was measured in gene-centromere intervals from the percentage of asci showing second-division segregation patterns; scoring was visual under the microscope. Centromere distances in centimorgans are half the percentage of asci with second division segregation (DS) in crosses of mutant (*m*) × wild type (+). Interpreting differences in the percentage of asci with second division segregation in terms of differences in crossing-over frequencies in the gene-centromere interval is complicated because of the upper limit of 66.7% on the percentage of second DS. Differences in percentage of second DS will be less than the differences in crossing-over frequency, especially for loci far from their centromeres.

Estimation of gene conversion properties: Gene conversion was studied from the visually observed frequencies of asci with particular non-4+:4*m* segregations in + × *m* crosses. Conversion direction (measured by the percentage of conversion asci with conversion to wild type, out of all conversion ratio asci) and a measure of correction inefficiency from hybrid DNA (the percentage of conversion asci with postmeiotic segregation among conversions to wild type) were determined to test whether hybrid DNA correction was affected as well as conversion initiation. Correction direction was measured by (8:0 + 7:1 + 6:2 + 5:3) × 100/(8:0 + 7:1 + 6:2 + 5:3 + 3:5 + 2:6 + 1:7 + 0:8), where each octad ratio is given as + (black ascospore):*m* (color mutant ascospore). Correction inefficiency in asci with conversion to wild type was measured by (7:1 + 5:3) × 100/(8:0 + 7:1 + 6:2 + 5:3). When controls for spontaneous mutation to ascospore color mutants (Lamb *et al.* 1998) showed that estimates of conversion to mutant were biased by new mutations, it was decided to use only the unbiased measure of conversion to wild type for the between- and within-slope analyses of conversion.

Changing the percentages of second division segregation into map distances based on calculated crossover frequencies: If there is no chromosome interference or chromatid (strand) interference, then the expected upper limit for percentage second division segregation is 66.7% (see Barratt *et al.* 1954). One cannot use Haldane's map function to transform recombination percentages from tetrad data into true map units, because his formula treats four-strand double crossovers as being detected (they give 2 *Ab*, 2 *aB* in random spores from an *AB/ab* heterozygote). In tetrad mapping, four-strand double crossovers are not detected; they just give a first division segregation when occurring between a locus and its centromere. The zero-interference tetrad mapping function is $y_t = \frac{2}{3}(1 - e^{-3x})$, where y_t is the observed fraction of second division segregations or of tetratype segregations, and x is the map units/100 (see Barratt *et al.* 1954). Chromatid interference is generally low or absent in fungi (see Lamb 1996) but chromosome interference may be positive. Barratt *et al.* (1954) used the term k as proportional to the chromosome interference coincidence, varying from 0 (complete positive interference) to 1.0 (no interference).

Mapping: Recombination frequencies for mapping ascospore color mutants were obtained from the numbers of parental ditype, nonparental ditype, and tetratype asci from repulsion-phase dihybrid crosses, with ~800 asci per cross. Allelism is extremely difficult to determine for closely linked ascospore color mutations, which are expressed only in haploid ascospores, as the *cis/trans* test needs diploids or partial diploids. The 11 color mutants are therefore known by their strain designations rather than by locus designations.

Nomenclature of strains and mutations: S is for a strain used by Saleem, followed by R for black ascospores, or by *w* (white), *lg* (light gray), or *g* (gray) for ascospore color mutations, then a number for the octad, a full stop, and a number for the spore within the octad from which the strain was isolated. Thus, strain S*lg*60.3 is a strain of Saleem with light gray ascospores, from octad 60, ascospore 3, obtained when new mutants were being isolated from an original wild-type strain (S2); the tables carry the information that this is a selfed generation 1 strain from the original S2 wild strain and so is of SFS ancestry.

Statistical tests: At the suggestion of an anonymous referee, for the significant values of χ^2, the usual probability values of 0.05 and 0.01 have been divided by the number of tests in each table, to make the Bonferroni correction for multiple tests. For example, in Tables 1 and 2, with 10 tests each, the level required for a significant difference (*) is $P = 0.005$ instead of 0.05, and $P = 0.001$ instead of 0.01 for a highly significant difference (**). The two-tailed test of equal binomial proportions was also carried out, but the significance results were so similar to the χ^2 results that they are not given here.

Another approach used was the analysis of deviance (Hosmer and Lemeshow 2000), based on a logistic regression form of a general linear model, to base inferences on parameter estimates and standard errors. The analysis of deviance allows comparison between different models without using a normality assumption. The goodness of fit of a proposed model is traded off against the number of parameters (explanatory variables) that are incorporated, as measured by the degrees of freedom, which are given by the number of data minus the number of fitted parameters. The lower the deviance, the better the model fits the data.

RESULTS

Tables 1–4 show the results for six color mutations from SFS strains and for five from NFS strains, with each mutation crossed to two selfed generation 1 or 2 SFS wild types (*i.e.*, two strains of wild-type genotype for spore color, derived from an original SFS strain by selfing) and to two selfed generation 1 or 2 NFS wild types. For the four or more replicates or repeats of each cross, there was generally good agreement for the percentage of second DS and for gene conversion frequencies, with no more significant differences than expected by chance. Agreement between replicates was better than in the corresponding mutation work (Lamb *et al.* 1998). There were highly significant differences between different fungal strains in crossing-over frequencies in gene-centromere intervals and in gene conversion frequencies. There were prominent between-slope differences and lesser within-slope differences.

Controls for crossing-over experiments: absence of polarized segregation, spindle overlap, and differential dehiscence biases: As shown in Table 3, the two first division classes for ordered octads generally showed the expected 1:1 ratio and the four second division classes generally showed the expected 1:1:1:1 ratio. There was no consistent trend for class I > class II nor for class V > class VI and class IV > class III, so there was no genuine polarized segregation and no apparent polar-

TABLE 1

Summary of recombination results from centromere distances for color mutants crossed to wild types from SFS or NFS

Color mutations and the kinds of wild type (wt) to which they were crossed, including the slope (SFS or NFS) and the generation (selfed generation 1, SG1, or selfed generation 2, SG2)	Total asci	% second division segregation asci	χ^2 value, SFS wt pooled *vs.* NFS wt pooled	Centromere distance, map units, no interference, $k = 1$[a]	Centromere distance, map units, if chromosome interference is positive, $k = 0.2$[b]
(i) South-facing slope color mutant strains					
S*w*18.2, selfed generation 1 from the original S3 strain					
SFS wt strains, SG1	57077	47.9	75.9**	42.2	26.2
NFS wt strains, SG1	80674	45.5		38.2	24.7
S*w*24.3, selfed generation 1 from the original S3 strain					
SFS wt strains, SG1	74049	51.6	59.5**	49.6	28.6
NFS wt strains, SG1	70671	49.6		45.3	27.4
S*w*92.1, selfed generation 1 from the original S3 strain					
SFS wt strains, SG1	36061	62.0	1061.5**	88.8	36.1
NFS wt strains, SG1	30657	49.5		45.1	27.3
S*lg*60.3, selfed generation 1 from the original S2 strain					
SFS wt strains, SG1	53945	62.9	72.7**	96.0	36.7
NFS wt strains, SG1	49261	60.3		78.2	34.8
S*w*67.1, selfed generation 1 from the original S3 strain					
SFS wt strains, SG2	34484	66.6	77.6**	242.2	39.8
NFS wt strains, SG2	44997	63.6		102.8	37.4
S*g*4.3, selfed generation 1 from the original S2 strain					
SFS wt strains, SG2	31531	69.1	23.8**	Infinity	42.1
NFS wt strains, SG2	34814	67.3		Infinity	40.3
(ii) North-facing slope color mutants					
S*w*17.2, selfed generation 1 from the original N5 strain					
SFS wt strains, SG1	51885	49.7	132.0**	45.7	27.5
NFS wt strains, SG1	50458	46.1		39.2	25.1
S*lg*14.3, selfed generation 1 from the original N5 strain					
SFS wt strains, SG1	58405	52.1	216.0**	50.7	29.1
NFS wt strains, SG1	43480	47.5		41.5	26.0
S*w*41.3, selfed generation 1 from the original N5 strain					
SFS wt strains, SG1	51799	55.2	107.5**	58.7	31.2
NFS wt strains, SG1	47059	51.9		50.3	28.9
S*g*16.2, selfed generation 1 from the original N5 strain					
SFS wt strains, SG1	51998	65.0	176.2**	123.8	38.6
NFS wt strains, SG1	52636	61.1		82.6	35.2
S*g*21.3, selfed generation 1 from the original N5 strain					
SFS wt strains, SG1	Not fertile				
NFS wt strains, SG1	47371	64.3		107.7	37.8

See Table 3 for details, including within-slope variation. Significant (*) or highly significant (**) χ^2 values, after making the Bonferroni correction for multiple tests; because there are 10 tests, the usual significance levels of 0.05 and 0.01 are each divided by 10. *$P < 0.005$; **$P < 0.001$.

[a] Value obtained from a tetrad mapping function: see MATERIALS AND METHODS.

[b] Value obtained from a tetrad mapping function: see MATERIALS AND METHODS.

TABLE 2

Summary of gene conversion results from color mutants crossed to wild types from SFS or NFS, using unbiased data from conversions to wild type only

Color mutations and the kinds of wild type (wt) to which they were crossed, from south- or north-facing slopes	Total asci with conversion to wild type	Asci with conversion to wild type		% of asci with postmeiotic segregation among asci with conversion to wild type	
		%	χ^2 value for SFS wt *vs.* NFS wt	%	χ^2 value for SFS wt *vs.* NFS wt
(i) South-facing slope color mutations					
S*w*18.2, selfed generation 1 from the original S3 strain					
SFS strains, SG1	394	0.68	39.9**	53.0	0.3
NFS strains, SG1	349	0.43		51.0	
S*w*24.3, selfed generation 1 from the original S3 strain					
SFS strains, SG1	789	1.04	52.7**	52.6	2.5
NFS strains, SG1	493	0.69		48.1	
S*w*92.1, selfed generation 1 from the original S3 strain					
SFS strains, SG1	672	1.82	3.0	46.7	2.8
NFS strains, SG1	523	1.65		41.9	
S*lg*60.3, selfed generation 1 from the original S2 strain					
SFS strains, SG1	1390	2.43	67.2**	44.5	0.1
NFS strains, SG1	871	1.72		45.1	
S*w*67.1, selfed generation 1 from the original S3 strain					
SFS strains, SG2	278	0.79	1.3	50.4	0.8
NFS strains, SG2	393	0.86		46.8	
S*g*4.3, selfed generation 1 from the original S2 strain					
SFS strains, SG2	213	0.67	50.7**	44.6	0.5
NFS strains, SG2	102	0.29		49.0	
(ii) North-facing slope color mutations					
S*w*17.2, selfed generation 1 from the original N5 strain					
SFS strains, SG1	280	0.53	12.7**	57.1	0.9
NFS strains, SG1	195	0.38		51.3	
S*lg*14.3, selfed generation 1 from the original N5 strain					
SFS strains, SG1	527	0.89	24.0**	52.6	0.2
NFS strains, SG1	271	0.62		50.9	
S*w*41.3, selfed generation 1 from the original N5 strain					
SFS strains, SG1	523	0.99	1.6	51.1	0.4
NFS strains, SG1	437	0.91		49.0	
S*g*16.2, selfed generation 1 from the original N5 strain					
SFS strains, SG1	175	0.33	0.5	58.3	0.4
NFS strains, SG1	164	0.31		54.9	
S*g*21.3, selfed generation 1 from the original N5 strain					
SFS strains, SG1	Not fertile				
NFS strains, SG1	176	0.37		52.3	

See Table 4 for details, including within-slope variation. Significant (*) or highly significant (**) χ^2 values, after making the Bonferroni correction for multiple tests; because there are 10 tests, the usual significance levels of 0.05 and 0.01 are each divided by 10. *$P < 0.005$; **$P < 0.001$.

ized segregation that would have indicated preferential dehiscence of certain octad classes with possible bias in the percentage of second DS (Lamb 1966, 1967). These are the most extensive data in any studies giving evidence against polarized segregation of chromosomes at meiosis I and/or of chromatids at meiosis II. There was also no tendency for the second division asymmetric classes (V and VI) to exceed the second division symmetric classes (III and IV), so partial spindle overlap at the second division (see Lamb 1996) did not bias the percentage of second DS values by interconverting first division asci and second division asymmetric asci.

Crossing over: between-slope differences between wild-type strains: Table 1 shows the percentage second division segregation pooled for crosses of color mutants to two SFS wild types and pooled for crosses to two NFS wild types. For all six SFS mutations, and for all four NFS color mutations that were fertile with NFS and SFS wild types, there were completely consistent, highly significant differences, with the crosses to SFS wild types

TABLE 3

Recombination data for gene-centromere intervals for crosses of ascospore color mutants to wild types from the south- or north-facing slopes of Evolution Canyon

Color mutants, wild types (wt) to which they were crossed, and their origins	Ordered octad ascal classes						Total asci	% second division segregation asci	Heterogeneity χ^2 for same slope strains[a]
	I	II	III	IV	V	VI			
(i) Color mutants from south-facing slope strains, all selfed generation 1									
Mutant S*w*18.2, selfed generation 1 from the original S3 strain									
SR2.3 (SG1 from S3)	9768	9781	4786	4709	4294	4193	37531	47.9	0.0
SR4.2 (SG1 from S2)	5128	5068	2527	2484	2167	2172	19546	47.8	
SR1.4 (SG1 from N5)	13092	13059	6203	6101	5283	5217	48955	46.6	57.8**
SR5.3 (SG1 from N7)	8910	8899	3559	3536	3418	3397	31719	43.9	
Mutant S*w*24.3, selfed generation 1 from the original S3 strain									
SR2.3 (SG1 from S3)	7878	7859	4576	4567	4157	4127	33164	52.6	21.9**
SR4.2 (SG1 from S2)	10043	10064	5371	5338	5034	5035	40885	50.8	
SR1.4 (SG1 from N5)	9576	9459	5189	5044	4773	4633	38674	50.8	50.5**
SR5.3 (SG1 from N7)	8346	8262	4153	4040	3626	3570	31997	48.1	
Mutant S*w*92.1, selfed generation 1 from the original S3 strain									
SR2.3 (SG1 from S3)	3531	3631	2926	2870	2794	2815	18567	61.4	5.8
SR4.2 (SG1 from S2)	3268	3265	2878	2845	2664	2574	17494	62.7	
SR1.4 (SG1 from N5)	3437	3489	1856	1888	1854	1936	14460	52.1	76.1**
SR5.3 (SG1 from N7)	4278	4288	1862	1921	1902	1946	16197	47.1	
Mutant S*lg*60.3, selfed generation 1 from the original S2 strain									
SR2.3 (SG1 from S3)	5074	5171	4410	4310	4407	4455	27827	63.2	2.4
SR4.2 (SG1 from S2)	4936	4848	4135	4099	4066	4034	26118	62.5	
SR1.4 (SG1 from N5)	4989	4933	3993	3925	3783	3756	25379	60.9	8.3
SR5.3 (SG1 from N7)	4827	4814	3626	3656	3488	3471	23882	59.6	
Mutant S*w*67.1, selfed generation 1 from the original S3 strain; the wild types are selfed generation 2 strains									
SR2.3i (SG2 from S3)	3066	3071	3247	3239	3129	3102	18854	67.5	12.6*
SR4.2i (SG2 from S2)	2685	2686	2624	2587	2529	2519	15630	65.6	
SR1.4i (SG2 from N5)	4029	3997	3562	3526	3500	3448	22062	63.6	0.0
SR5.3i (SG2 from N7)	4187	4157	3716	3704	3625	3546	22935	63.6	
Mutant S*g*4.3, selfed generation 1 from the original S2 strain; the wild types are selfed generation 2 strains									
SR2.3i (SG2 from S3)	2179	2172	2541	2530	2303	2339	14064	69.1	0.0
SR4.2i (SG2 from S2)	2694	2709	3160	3138	2860	2906	17467	69.1	
SR1.4i (SG2 from N5)	3219	3216	3402	3350	3269	3226	19682	67.3	0.0
SR5.3i (SG2 from N7)	2474	2476	2593	2574	2518	2497	15132	67.3	
(ii) Color mutants from north-facing slope strains, all selfed generation 1									
Mutant S*w*17.2, selfed generation 1 from the original N5 strain									
SR10.2 (SG1 from S3)	6024	5971	2946	2952	2969	2977	23839	49.7	0.1
SR1.3 (SG1 from S2)	7022	7057	3510	3490	3472	3495	28046	49.8	
SR1.4 (SG1 from N5)	7252	7218	3018	3048	3095	3120	26751	45.9	0.3
SR2.3 (SG1 from N7)	6398	6371	2664	2711	2800	2763	23707	46.1	

(*continued*)

TABLE 3

(Continued)

Color mutants, wild types (wt) to which they were crossed, and their origins	Ordered octad ascal classes						Total asci	% second division segregation asci	Heterogeneity χ^2 for same slope strains
	I	II	III	IV	V	VI			
Mutant S*lg*14.3, selfed generation 1 from the original N5 strain									
SR8.4 (SG1 from S3)	6951	7048	3879	3832	3760	3750	29220	52.1	0.0
SR2.2 (SG1 from S2)	7014	6958	3794	3778	3811	3830	29185	52.1	
SR3.3 (SG1 from N5)	5878	5900	2673	2646	2659	2663	22419	47.5	0.0
SR4.1 (SG1 from N7)	5539	5530	2524	2491	2490	2487	21061	47.4	
Mutant S*w*41.3, selfed generation 1 from the original N5 strain									
SR4.2 (SG1 from S3)	5780	5803	3586	3590	3589	3661	26009	55.5	1.2
SR2.2 (SG1 from S2)	5736	5872	3504	3559	3526	3593	25790	55.0	
SR4.5 (SG1 from N5)	5672	5629	2995	2989	3049	3070	23404	51.7	0.9
SR2.1 (SG1 from N7)	5695	5622	3031	3121	3096	3090	23655	52.2	
Mutant S*g*16.2, selfed generation 1 from the original N5 strain									
SR6.3 (SG1 from S3)	4646	4673	4329	4330	4298	4328	26604	65.0	0.1
SR4.3 (SG1 from S2)	4429	4430	4158	4166	4092	4119	25394	65.1	
SR1.1 (SG1 from N5)	5025	4902	3945	3241	4058	3937	25108	60.5	7.7
SR2.1 (SG1 from N7)	5239	5320	4234	4270	4235	4230	27528	61.6	
Mutant S*g*21.3, selfed generation 1 from the original N5 strain. It was infertile with SFS strains									
SR3.3 (SG1 from N5)	4007	4090	3769	3764	3716	3819	23165	65.0	20.1**
SR4.1 (SG1 from N7)	4455	4485	3788	3785	3801	3892	24206	63.1	

In heterogeneity χ^2 tests on numbers of first and second division segregation asci for crosses to two south-facing slope strains or to two north-facing slope strains, significant χ^2 values are shown by * and **; no asterisk indicates homogeneity. The significance values are the normal ones of 0.05 and 0.01 divided by 21, the number of tests, in the Bonferroni correction for multiple tests. *$P < 0.0024$; **$P < 0.00048$.

always (10 cases) giving a higher percentage of second DS than crosses of the same mutation to NFS wild types.

The magnitude of the between-slope differences varied for different mutations, being greatest for S*w*92.1, where the difference between 49.5% second DS in crosses to NFS wild types and 62.0% in crosses to SFS wild types is proportionately larger when these values are converted to 45.1 and 88.8 map units, respectively, if there is no chromosome interference. In *S. fimicola*, Perkins *et al.* (1963) found no chromatid interference. S*g*4.3, with 67.3 and 69.1% second DS, gave more than the theoretical upper limit of 66.7% second DS, which demonstrates some positive chromosome interference if chromatid interference is absent, which it usually is in fungi (references in Lamb 1996).

By what proportion did the frequency of crossing over increase in crosses to SFS wild types compared to crosses to NFS wild types? Table 1 shows the centromere distances in calculated map units (MU, calculated by allowing mathematically for nondetection of certain crossovers in double or multiple crossovers) for each mutation in pooled cross-values under two conditions: no chromosome or chromatid interference ($k = 1$) or with strong positive chromosome interference within chromosome arms at a level common in *Neurospora crassa* and *Drosophila melanogaster*; $k = 0.2$. See Barratt *et al.* (1954) for theory and formulas. From their tetrad mapping graph for $k = 0.2$ (their Figure 7), the maximum percentage of second DS expected is ~83.5 for loci far from their centromere. Using the standard formula, centromere distance in centimorgans equals second DS/2 and is equivalent to assuming that $k = 0$, complete positive chromosome interference.

With no chromatid interference, the proportional increase in crossing over (measured by changes in the calculated mutation-to-centromere map distances) in crosses to SFS wild types compared with crosses to NFS wild types varied from <10%, *e.g.*, from 45.3 to 49.6 MU for S*w*24.3, to >100%, *e.g.*, from 102 to 242.2 MU for S*w*67.1. With positive interference, the calculated pro-

TABLE 4

Gene conversion data for crosses of ascospore color mutants to wild types from the south- or north-facing slopes of Evolution Canyon

Octad class (+:*m*)	Wild type crossed to:	Octad nos. SR2.3 (SG1 from S3)	Octad nos. SR4.2 (SG1 from S2)	Heterogeneity χ^2 for the two south-slope strains[b]	Octad nos. SR1.4 (SG1 from N5)	Octad nos. SR5.3 (SG1 from N7)	Heterogeneity χ^2 for the two north-slope strains[b]
		S*w*18.2, selfed generation 1 strain from the original S3 strain					
8:0		7	2		1	3	
0:8[a]		6	2		2	3	
7:1		10	4		6	7	
1:7		5	4		3	3	
6:2, 6:2(2)		114	62		110	57	
2:6, 2:6(2)[a]		191	93		74	29	
5:3, 5:3(3)		125	70		103	62	
3:5, 3:5(3)		152	84		51	32	
4:4, 4:4(4)		37531	19546		48955	31719	
ab4:4		17	7		10	8	
Total octads		38158	19874		49315	31923	
Total conversion frequency (TCF) %[a]		1.64	1.65	0.0	0.73	0.64	2.3
Total conversion to wild type (%)		**0.67**	**0.69**	**0.1**	**0.45**	**0.40**	**1.1**
Total % conversion to mutant[a]		0.90	0.92		0.26	0.21	
Relative % of conversions to wild type[a]		42.0	43.0	0.1	62.9	65.8	0.5
% postmeiotic segregation[a]		49.3	51.5	0.4	48.1	54.9	2.4
		S*w*24.3, selfed generation 1 from the original S3 strain					
8:0		5	5		3	3	
0:8		4	5		4	2	
7:1		4	4		5	1	
1:7		4	7		3	1	
6:2, 6:2(2)		188	176		146	102	
2:6, 2:6(2)		247	254		114	75	
5:3, 5:3(3)		208	199		131	102	
3:5, 3:5(3)		226	219		80	53	
4:4, 4:4(4)		33164	40885		38674	31997	
ab4:4		11	10		10	8	
Total octads		34061	41764		39170	32344	
TCF %		2.63	2.10	23.0**	1.27	1.07	5.7
Total conversion to wild type (%)		**1.19**	**0.92**	**13.1****	**0.73**	**0.64**	**2.0**
Total % conversion to mutant		1.41	1.16		0.51	0.41	
Relative % of conversions to wild type		45.7	44.2	0.4	58.6	61.4	0.6
% postmeiotic segregation		50.5	49.9	0.1	46.2	47.6	0.2

(*continued*)

TABLE 4

(Continued)

Octad class (+:*m*)	Wild type crossed to:	Octad nos. SR2.3 (SG1 from S3)	Octad nos. SR4.2 (SG1 from S2)	Heterogeneity χ^2 for the two south-slope strains[b]	Octad nos. SR1.4 (SG1 from N5)	Octad nos. SR5.3 (SG1 from N7)	Heterogeneity χ^2 for the two north-slope strains[b]
		S*w*92.1, selfed generation 1 from the original S3 strain					
8:0		6	5		8	10	
0:8		5	5		7	10	
7:1		4	2		10	10	
1:7		8	6		9	19	
6:2, 6:2(2)		170	177		149	137	
2:6, 2:6(2)		235	187		147	108	
5:3, 5:3(3)		152	156		82	117	
3:5, 3:5(3)		196	155		86	69	
4:4, 4:4(4)		18567	17494		14460	16197	
ab4:4		17	30		15	14	
Total octads		19360	18217		14973	16691	
TCF %		4.10	3.97	2.0	3.43	2.96	5.6
Total conversion to wild type (%)		**1.71**	**1.87**	**0.3**	**1.66**	**1.64**	**0.0**
Total % conversion to mutant		2.29	1.94		1.66	1.23	
Relative % of conversions to wild type		42.8	49.1	5.8	50.0	57.1	4.9
% postmeiotic segregation		47.5	48.3	0.1	39.4	46.4	5.0
		S*lg*60.3, selfed generation 1 from the original S2 strain					
8:0		7	13		11	6	
0:8		3	4		6	2	
7:1		3	9		7	7	
1:7		5	15		8	7	
6:2, 6:2(2)		468	284		240	221	
2:6, 2:6(2)		512	438		184	160	
5:3, 5:3(3)		335	271		189	190	
3:5, 3:5(3)		403	358		139	119	
4:4, 4:4(4)		27909	26118		25379	23882	
ab4:4		14	14		15	18	
Total octads		29659	27524		26178	24612	
TCF %		5.9	5.10	17.2**	3.05	2.97	0.3
Total conversion to wild type (%)		**2.74**	**2.10**	**25.0****	**1.71**	**1.72**	**0.0**
Total % conversion to mutant		3.11	2.96		1.29	1.17	
Relative % of conversions to wild type		46.8	41.5	9.1*	57.0	59.6	1.0
% postmeiotic segregation		43.4	47.4	5.1	44.8	46.7	0.6

(*continued*)

TABLE 4

(Continued)

Octad class (+:*m*)	Wild type crossed to:	Octad nos. SR2.3i (SG2 from S3)	Octad nos. SR4.2i (SG2 from S2)	Heterogeneity χ^2 for the two south-slope strains[b]	Octad nos. SR1.4i (SG2 from N5)	Octad nos. SR5.3i (SG2 from N7)	Heterogeneity χ^2 for the two north-slope strains[b]
		S*w*67.1, a white ascospore mutation from original S3 strain					
8:0		4	3		4	4	
0:8		2	4		4	4	
7:1		4	6		5	3	
1:7		4	4		4	5	
6:2, 6:2(2)		73	58		103	98	
2:6, 2:6(2)		126	101		56	56	
5:3, 5:3(3)		81	49		93	83	
3:5, 3:5(3)		115	71		54	54	
4:4, 4:4(4)		18854	15630		22062	22935	
ab4:4		5	6		9	12	
Total octads		19268	15932		22394	23254	
TCF %		2.15	1.90	2.8	1.48	1.37	1.0
Total conversion to wild type (%)		**0.84**	**0.73**	**1.3**	**0.92**	**0.81**	**1.5**
Total % conversion to mutant		1.28	1.13		0.53	0.51	
Relative % of conversions to wild type		39.6	39.2	0.0	63.5	61.2	0.3
% postmeiotic segregation		50.5	45.0	2.1	49.7	49.2	0.0
		S*g*4.3, a gray ascospore mutation from original S2 strain					
8:0		1	3		1	2	
0:8		3	2		3	2	
7:1		2	3		4	1	
1:7		1	1		2	2	
6:2, 6:2(2)		45	69		25	24	
2:6, 2:6(2)		37	47		28	22	
5:3, 5:3(3)		43	47		23	22	
3:5, 3:5(3)		46	57		29	21	
4:4, 4:4(4)		14064	17467		19682	15132	
ab4:4		4	5		4	5	
Total octads		14247	17701		19801	15233	
TCF %		1.28	1.32	0.1	0.60	0.66	0.5
Total conversion to wild type (%)		**0.64**	**0.69**	**0.3**	**0.27**	**0.32**	**1.0**
Total % conversion to mutant		0.61	0.60		0.31	0.31	
Relative % of conversions to wild type		50.8	53.3	0.2	46.1	51.0	0.5
% postmeiotic segregation		53.0	48.3	0.9	52.1	50.5	0.1

(*continued*)

TABLE 4

(Continued)

Octad class (+:*m*)	Wild type crossed to:	Octad nos. SR10.2 (SG1 from S3)	Octad nos. SR1.3 (SG1 from S2)	Heterogeneity χ^2 for the two south-slope strains[b]	Octad nos. SR1.4 (SG1 from N5)	Octad nos. SR2.3 (SG1 from N7)	Heterogeneity χ^2 for the two north-slope strains[b]
S*w*17.2, a white ascospore mutation from original N5 strain							
8:0		2	4		5	4	
0:8		3	2		4	4	
7:1		4	5		5	6	
1:7		4	3		5	6	
6:2, 6:2(2)		56	69		45	41	
2:6, 2:6(2)		67	84		35	40	
5:3, 5:3(3)		67	73		49	40	
3:5, 3:5(3)		43	59		23	16	
4:4, 4:4(4)		23839	28046		26814	23707	
ab4:4		50	76		37	52	
Total octads		24135	28421		27022	23916	
TCF %		1.23	1.32	2.2	0.77	0.87	1.7
Total conversion to wild type (%)		**0.53**	**0.53**	**0.1**	**0.38**	**0.38**	**0.2**
Total % conversion to mutant		0.48	0.52		0.25	0.28	
Relative % of conversions to wild type		52.4	50.5	0.2	60.8	58.0	0.3
% postmeiotic segregation		56.8	57.6	0.0	57.2	57.4	0.0
Octad class (+:*m*)	Wild type crossed to:	Octad nos. SR8.4 (SG1 from S3)	Octad nos. SR2.2 (SG1 from S2)	Heterogeneity χ^2 for the two south-slope strains[b]	Octad nos. SR3.3 (SG1 from N5)	Octad nos. SR4.1 (SG1 from N7)	Heterogeneity χ^2 for the two north-slope strains[b]
S*lg*14.3, a light gray ascospore mutation from original N5 strain							
8:0		4	3		2	2	
0:8		2	2		0	2	
7:1		6	5		4	4	
1:7		7	4		3	3	
6:2, 6:2(2)		125	118		66	63	
2:6, 2:6(2)		120	128		55	61	
5:3, 5:3(3)		138	128		72	58	
3:5, 3:5(3)		115	118		38	34	
4:4, 4:4(4)		29220	29185		22419	21061	
ab4:4		55	58		51	35	
Total octads		29792	29749		22710	21323	
TCF %		1.92	1.90	0.1	1.28	1.23	0.3
Total conversion to wild type (%)		**0.92**	**0.85**	**0.7**	**0.63**	**0.60**	**0.3**
Total % conversion to mutant		0.82	0.85		0.42	0.47	
Relative % of conversions to wild type		52.8	50.2	0.7	60.0	55.9	0.8
% postmeiotic segregation		56.1	55.5	0.0	57.7	51.1	2.4

(*continued*)

TABLE 4

(Continued)

Octad class (+:*m*)	Wild type crossed to:	Octad nos. SR4.2 (SG1 from S3)	Octad nos. SR2.2 (SG1 from S2)	Heterogeneity χ^2 for the two south-slope strains[b]	Octad nos. SR4.5 (SG1 from N5)	Octad nos. SR2.1 (SG1 from N7)	Heterogeneity χ^2 for the two north-slope strains[b]
S*w*41.3, a white ascospore mutation from original N5 strain							
8:0		3	4		2	2	
0:8		3	4		2	1	
7:1		5	4		6	5	
1:7		3	4		4	4	
6:2, 6:2(2)		137	112		117	102	
2:6, 2:6(2)		144	148		91	81	
5:3, 5:3(3)		155	103		104	99	
3:5, 3:5(3)		140	136		76	76	
4:4, 4:4(4)		26009	25790		23404	23656	
ab4:4		63	49		54	47	
Total octads		26662	26354		23860	24073	
TCF %		2.45	2.14	5.7	1.91	1.73	2.2
Total conversion to wild type (%)		**1.13**	**0.85**	**10.6***	**0.96**	**0.86**	**1.2**
Total % conversion to mutant		1.09	1.11		0.73	0.67	
Relative % of conversions to wild type		50.8	43.3	6.3*	57.0	56.2	0.0
% postmeiotic segregation		56.0	52.5	1.6	53.5	55.4	0.3

Octad class (+:*m*)	Wild type crossed to:	Octad nos. SR6.3 (SG1 from S3)	Octad nos. SR4.3 (SG1 from S2)	Heterogeneity χ^2 for the two south-slope strains[b]	Octad nos. SR1.1 (SG1 from N5)	Octad nos. SR2.1 (SG1 from N7)	Heterogeneity χ^2 for the two north-slope strains[b]
S*g*16.2, a gray ascospore mutation from original N5 strain							
8:0		3	3		2	3	
0:8		2	1		1	1	
7:1		5	4		4	3	
1:7		2	1		3	1	
6:2, 6:2(2)		37	33		34	35	
2:6, 2:6(2)		67	60		19	22	
5:3, 5:3(3)		49	44		43	40	
3:5, 3:5(3)		63	55		15	14	
4:4, 4:4(4)		26604	25394		25108	27528	
ab4:4		75	71		74	73	
Total octads		26906	25668		25303	27720	
TCF %		1.13	1.06	0.6	0.77	0.69	1.4
Total conversion to wild type (%)		**0.35**	**0.33**	**0.1**	**0.33**	**0.29**	**0.8**
Total % conversion to mutant		0.50	0.46		0.15	0.14	
Relative % of conversions to wild type		41.2	41.8	0.0	68.6	68.1	0.0
% postmeiotic segregation		64.0	64.3	0.0	71.3	68.2	0.4

(*continued*)

TABLE 4

(Continued)

Octad class (+:*m*)	Wild type crossed to:	Octad nos. SR6.3 (SG1 from S3)	Octad nos. SR4.3 (SG1 from S2)	Heterogeneity χ^2 for the two south-slope strains[b]	Octad nos. SR3.3 (SG1 from N5)	Octad nos. SR4.1 (SG1 from N7)	Heterogeneity χ^2 for the two north-slope strains[b]
*Sg*21.3, a gray ascospore mutation from original strain N5							
8:0		—	—		5	3	
0:8		—	—		2	2	
7:1		—	—		5	6	
1:7		—	—		3	4	
6:2, 6:2(2)		—	—		38	38	
2:6, 2:6(2)		—	—		24	31	
5:3, 5:3(3)		—	—		39	42	
3:5, 3:5(3)		—	—		22	19	
4:4, 4:4(4)		—	—		23165	24206	
ab4:4		—	—		57	59	
Total octads		—	—		23360	24410	
TCF %		—	—		0.83	0.84	0.0
Total conversion to wild type (%)					**0.37**	**0.36**	
Total % conversion to mutant					0.22	0.23	0.0
Relative % of conversions to wild type		—	—		63.0	61.4	0.1
% postmeiotic segregation		—	—		64.6	63.7	0.0

[a] For all color mutants listed, these ascal classes or conversion measures have been affected by a high frequency of spontaneous new mutations to mutant.

[b] In testing the two crosses to different SFS wild-type strains or to two different NFS wild-type strains, significant χ^2 values are shown by *, $P < 0.0024$; **, $P < 0.00048$; no asterisk indicates homogeneity. These values are the normal ones of 0.05 and 0.01 divided by 21, the number of tests, in the Bonferroni correction for multiple tests.

portional changes are smaller. Whatever the level of interference, the direction of the between-slope difference was absolutely consistent, with significantly more crossing over in crosses to SFS wild types than to NFS wild types.

Crossing over: within-slope differences between wild-type strains: For crossing over in the locus-centromere intervals, there were some highly significant between-strain differences within slopes when using selfed generation 1-derived wild types (the majority of cases) or selfed generation 2-derived wild types (for crosses of color mutation S*w*67.1). One can therefore conclude that there are genetic differences for recombination control genes between some SFS strains, *e.g.*, between SR2.3 (from original strain S3) and SR4.2 (from original strain S2) and between some NFS strains. These within-slope differences can be attributed to genetic differences between the different original isolates obtained from the wild in Evolution Canyon, *e.g.*, between S2 and S3, inherited by selfed generation 1 and 2 strains obtained from self-fertilized perithecia.

For the six south-facing slope color mutations, results were very diverse. Four mutations showed no significant differences between crosses to two different SFS wild types, and two showed significant differences. For the same six SFS mutations in crosses to two different NFS wild types, three showed no significant differences and three showed highly significant differences. For the five north-facing slope color mutations in crosses to two different SFS wild types, four showed no significant differences and one was not fertile. For the same five NFS color mutations in crosses to two different NFS wild types, four showed no significant differences and one showed a highly significant difference.

From Table 3 it can be seen that selfed generation 1 strains SR1.4 and SR5.3, respectively, derived from original wild strains N5 and N7, gave highly significant differences in the percentage of second DS with three out of four of the color mutations to which they were crossed. Each time, SR1.4 gave higher values than did SR5.3, showing a consistent within-slope difference in crossing over. Selfed generation 1 strains SR2.3 and SR4.2, derived, respectively, from original wild strains S3 and S2, gave a highly significant difference in the percentage of second DS with S*w*24.3, but not with three other color mutants (S*w*18.2, S*lg*60.3, and S*w*92.1), although the genetic intervals overlap. There could therefore be some degree of separate control for different linked loci, even for S*w*18.2 and S*w*24.3, which are only 2.1 cM apart, in their different responses to two SFS wild types, SR2.3 and SR4.2.

In the case of SFS color mutations, where there were significant within-slope differences, S3-derived wild types twice gave higher values than S2-derived wild types; N5-derived wild types gave a higher percentage of second DS than N7-derived wild types in all three cases. For the NFS color mutation with a significant within-slope difference, the N5-derived wild type gave a higher value than the N7-derived wild type. The significant within-slope differences were thus consistent in direction.

Gene conversion: controls for new ascospore color mutations, reversions to wild type, and phenocopies: Spontaneous reversions from spore color mutations to black wild-type ascospores were not found for any mutations in samples for each mutant of ~20,000 asci from selfed perithecia, so asci with apparent conversion to wild type were genuine, especially as phenocopies were not found (see below). In samples of >38,000 asci, each from selfed wild-type strains of selfed generation 1 (the same generation as that used in the recombination experiments), asci with four black:four nonblack ascospores occurred with frequencies of 0.66% (for strain N7), 0.84% (N5), 1.71% (S1), and 2.06% (S3; Lamb *et al.* 1998). Those frequencies of aberrant ratio asci from mutation are sufficiently high in relation to the conversion frequencies to bias the apparent frequency of conversions to mutant and hence the total conversion frequencies.

The classes affected by mutations to mutant in the $+ \times m$ crosses are the 2+:6*m* and 0+:8*m* patterns, with lesser effects on the 4+:4*m* patterns. The effects on different ascal classes depend on whether the new mutation arose in the wild type or in the known color mutant strain and whether the new mutation is linked to the known one and on the centromere distances of the two mutations. Comparisons between strains for conversion frequency are therefore based here entirely on conversions to wild type, because these were unbiased by mutation or reversion. Other measures, such as apparent overall conversion frequencies, are given for completeness in Table 4, but are not discussed. The important unbiased measure, the frequency of conversion to wild type, is given in boldface type in Table 4.

Ascospores from >100 asci with gene conversion patterns were isolated, germinated, and allowed to self-fertilize to test for phenocopies such as black spores that were genotypically color mutant, *e.g.*, in a 6 black:2 mutant octad, or for color mutant spores that were genotypically wild type, *e.g.*, in a 2 black:6 color mutant octad. That is, we tested for false gene conversions caused by phenocopies, but found none.

Conversion analysis based only on unbiased conversions to wild type: between-slope differences in conversion frequency: Of 10 between-slope comparisons in pooled data in Table 2, six had highly significant differences, always with the crosses to the SFS wild type giving higher conversion frequencies than crosses to NFS strains, and four had no significant differences.

The magnitude of the significant between-slope differences varied for different mutants, being greatest at 2.3-fold for S*g*4.3 and least at 1.3-fold for S*w*41.3, but as explained in the DISCUSSION, the genetic differences are underestimated by a factor of 2. Conversion frequen-

cies therefore follow a very similar pattern of between-slope differences as for the percentage of second DS, with SFS wild types giving higher values than NFS wild types.

There was also an effect of the color mutant strain on conversion frequencies to wild type, which were generally higher in color mutants from SFS strains (average 1.24% in crosses to SFS wild types, 0.94% in crosses to NFS wild types) than those from NFS strains (0.69% in crosses to SFS wild types, 0.52% in crosses to NFS wild types); these figures include all color mutants, not just those showing significant differences. Crosses of SFS-derived color mutations averaged 1.09% conversion to wild type, compared to 0.61% for crosses of NFS-derived color mutations, a 1.8-fold difference for SFS/NFS. This is higher than the 1.32-fold difference for SFS color mutants crossed to SFS *vs.* NFS wild types and the 1.33-fold difference for NFS color mutations crossed to SFS *vs.* NFS wild types.

Conversion analysis based only on unbiased conversions to wild-type: within-slope differences: Of 21 cases in which a color mutant was crossed to two different SFS wild types or to two different NFS wild types, three cases gave significant or highly significant within-slope heterogeneity, but differences were not significant in the other 18 cases (Table 4). All three significant cases had higher frequencies of conversion to wild type in the cross to the S3-derived wild type rather than to the S2-derived one.

Analysis of deviance results for percentage of second division segregation and the frequency of conversion to wild type: Table 5 shows the results of this analysis. Dramatic decreases in deviance, and hence increases in goodness of fit, were achieved by incorporating more terms into the model. The best-fitting models (4, 6, and 7) include differences between mutations and between slopes and/or origin interactions involving slopes (see Table 5). The best-fitting model tried, model 7, includes differences between mutations and slopes and different mutation/slope interactions for each mutation. Checks of residuals and fitted values revealed that the best model represented a good fit to the data. The analysis of deviance results therefore agree with the χ^2 and equal binomial proportions test results in showing differences in recombination properties between strains from different slopes.

The frequency of asci with conversion to wild type that have postmeiotic segregation: There were no significant differences for this character between strains from the two different slopes (Table 2). As expected, there were clear differences in this measure between different color mutants, ranging from 41 to 58%, as different heterozygous mutations had different levels of correction of hybrid DNA, but they were not dependent on which wild-type strain the mutant was crossed to, nor on whether the color mutant was in a SFS or NFS strain.

Mapping: The results (Figure 1), with recombination distances between mutations of 0.0–10.1 cM for 9 of the 11 mutations, and some larger distances for the remaining 2, showed that a number of different ascospore color loci were involved, although the mutations cannot be assigned to distinct loci because the standard *cis/trans* allelism test is not possible in haploid ascospores. No rigid genetic distance would conclusively define 2 mutations as being alleles or nonalleles, but 2 mutations are increasingly likely to be at different loci as they get farther apart. Mutations 0.1 cM apart could be alleles or in adjacent loci, but 2 mutations ≥5 cM apart are most unlikely to be alleles: S*w*17.2 and S*w*67.1 are 8.8 cM apart (see Figure 1) and so are presumably at different loci, leading to the conclusion that a number of different loci must be involved. S*w*18.2 and S*lg*14.3

TABLE 5

Analysis of deviance to test various models

Model	Deviance	d.f.
	% second division segregation	
1	24682.7	43
2	2280.9	31
3	904.5	30
4	265.9	21
5	901.4	29
6	265.9	21
7	201.2	20
	% of asci with conversion to wild type	
1	3769.4	43
2	854.6	31
3	463.8	30
4	73.9	21
5	421.4	29
6	73.9	21
7	49.1	20

Model 1. No differences between mutations, slopes, or strains.

Model 2. Difference between mutations; no differences between slopes or strains for each mutant.

Model 3. Differences between mutations and slopes; no differences between strains within slopes.

Model 4. Differences between mutations and slopes, and different responses of different mutations to slope types; no differences within slopes.

Model 5. Differences between mutations and different origin interactions (*i.e.*, differences according to whether the mutation and the wild type originate from the same or different slopes, with four combinations possible: wild type from SFS with mutation from SFS, wild type from NFS with mutation from NFS, wild type from SFS with mutation from NFS, and wild type from NFS with mutation from SFS); no differences between strains or slopes for each mutation.

Model 6. Differences between mutations and different origin interactions for each mutation; no differences between strains or between slopes except for the origin interactions.

Model 7. Differences between mutations and slopes, and different mutation/slope interactions for different mutations; differences between strains within slopes, but the model assumes the same differences between strains for all mutations.

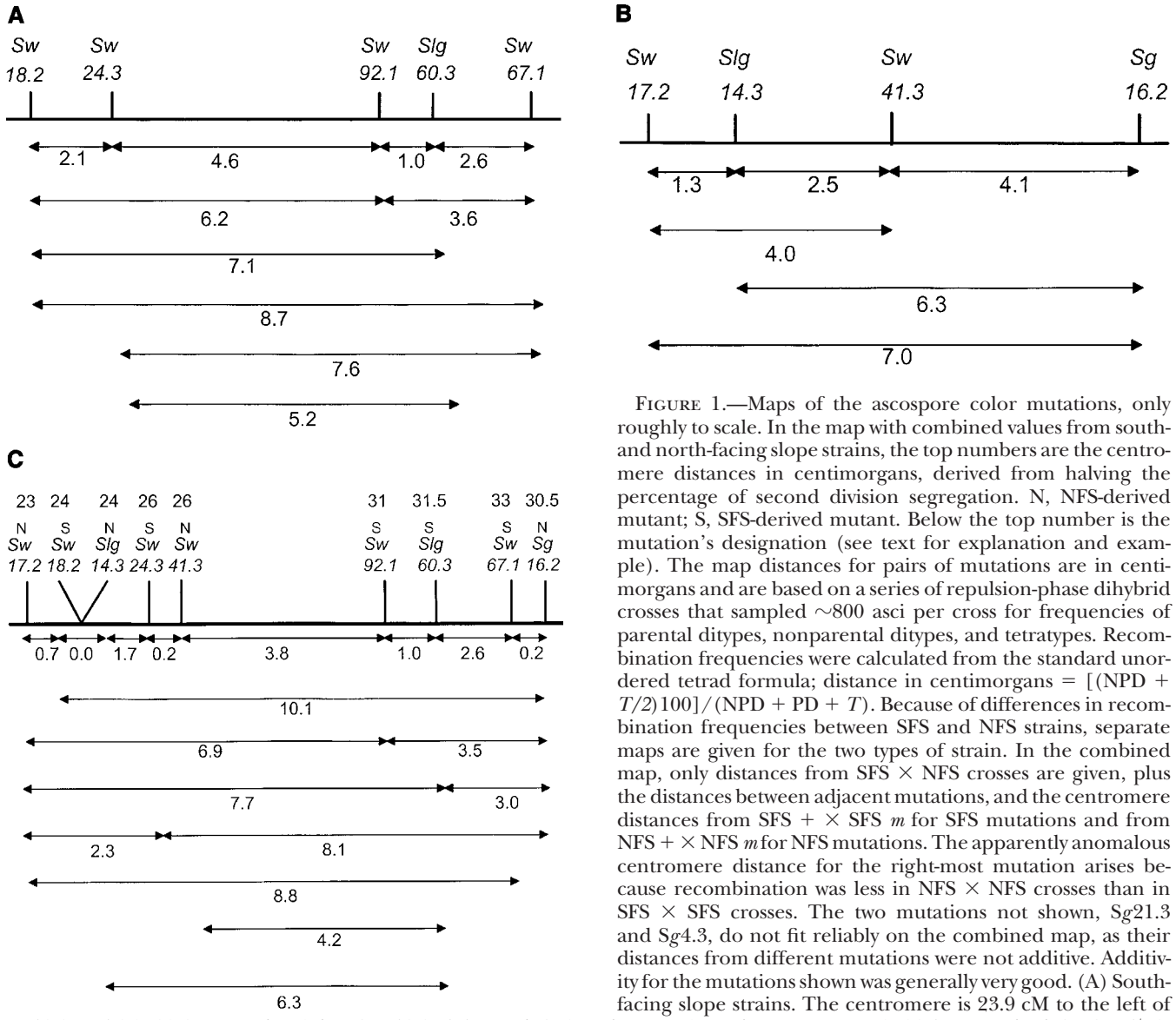

FIGURE 1.—Maps of the ascospore color mutations, only roughly to scale. In the map with combined values from south- and north-facing slope strains, the top numbers are the centromere distances in centimorgans, derived from halving the percentage of second division segregation. N, NFS-derived mutant; S, SFS-derived mutant. Below the top number is the mutation's designation (see text for explanation and example). The map distances for pairs of mutations are in centimorgans and are based on a series of repulsion-phase dihybrid crosses that sampled ~800 asci per cross for frequencies of parental ditypes, nonparental ditypes, and tetratypes. Recombination frequencies were calculated from the standard unordered tetrad formula; distance in centimorgans = $[(\text{NPD} + T/2)100]/(\text{NPD} + \text{PD} + T)$. Because of differences in recombination frequencies between SFS and NFS strains, separate maps are given for the two types of strain. In the combined map, only distances from SFS × NFS crosses are given, plus the distances between adjacent mutations, and the centromere distances from SFS + × SFS *m* for SFS mutations and from NFS + × NFS *m* for NFS mutations. The apparently anomalous centromere distance for the right-most mutation arises because recombination was less in NFS × NFS crosses than in SFS × SFS crosses. The two mutations not shown, S*g*21.3 and S*g*4.3, do not fit reliably on the combined map, as their distances from different mutations were not additive. Additivity for the mutations shown was generally very good. (A) South-facing slope strains. The centromere is 23.9 cM to the left of *Sw*18.2; *Sg*4.3 is 30.6 cM to the right of *Sw*18.2. (B) North-facing slope strains. The centromere is 23.1 cM to the left of *Sw*17.2; *Sg*21.3 is 22.7 cM to the right of *Sg*16.2. (C) Combined values from south- and north-facing slope strains.

showed no recombination in >700 asci and so may well be alleles. They are definitely different mutations as they were isolated independently, one from a SFS strain and one from a NFS strain, and they differed in phenotype, one being white and the other being light gray, which allowed crossed perithecia to be identified from selfed ones for mapping in the cross between them. If both had been white, that would have been very difficult.

All 11 mutants tested were in the same linkage group, which was unexpected because spore color loci occur in several different linkage groups in Olive's strains (CATALOGUE OF STRAINS 1996). Any loci for which color mutants give poor ascospore germination would be scored in the mutation work but would be underrepresented in mapping, crossing over, and gene conversion data, as isolation of color mutant strains requires ascospore germination. From Figure 1, the additivity can be seen to be very good, with no major map expansion or contraction effects with increasing distance. Mutations from the original SFS and NFS wild-type strains were intermingled on the map.

Crosses with Olive's strains: A range of wild-type selfed generation 1 and two strains from S1, S3, N5, and N7 were crossed to Olive's spore color mutant strains such as C7 *hyaline* and *gray*, and Olive's wild-type strains were crossed to spore color mutant strains from Evolution Canyon. Although various crossing methods were tried, including inoculating strains on opposite sides of petri dishes, inoculating them together at the side of the dish, and inoculating them on solid medium after growing them together as hyphal fragments in liquid crossing medium, no crossed perithecia were found, even from very self-fertile strains.

In which regions of the chromosome are the changes

in recombination occurring? In Tables 1 and 2, the color mutants are listed in map order, going away from the centromere (see Figure 1). There is a gap of ~23 cM between the centromere and the most proximal site and then most mutations are within 10 cM of the most proximal site, S*w*17.2. Any change in crossover frequency in the interval between the centromere and the most proximal site would affect that site and all sites distal to it: all sites showed significant between-slope differences, with more crossing over in crosses to SFS strains than to NFS strains. If there were also parallel differences in crossing over within the ~10 cM region within which most sites occurred, the more distal sites should show proportionally larger changes in crossover frequency than the more proximal sites. Unfortunately, because of the maximum theoretical value of 66.7% second DS, having proportionally more crossing over between a distal site and the centromere would not show as a proportionally greater change in percentage of second DS compared with changes for a more proximal site. In Table 1, if one takes the centromere distance in map units with no interference, instead of as percentage of second DS, then the proportional changes in crossing over between SFS and NFS strains in successive intervals going away from the centromere are 1.1, 1.1, 2.0, 1.2, and 2.4 for SFS color mutants and 1.2, 1.2, 1.2, and 1.5 for NFS color mutants, with some indication of larger changes for more distal sites.

The best evidence on regions of change in recombination between slopes comes from the unbiased results on gene conversion to wild type (Table 2), which are unaffected by theoretical upper limit problems and unaffected by the problem of having some intervals in common. For SFS color mutations, sites with highly significant between-slope conversion differences were proximal, medial, and distal, with the largest proportional change being for the most distal site. For NFS color mutants, the two proximal sites showed highly significant between-slope differences, but the two distal sites did not. Taking the crossing over and conversion data together, the changes in recombination are seen to have occurred in proximal, medial, and distal regions of the chromosome, not just in any one region such as the immediately centromeric region, but more data are needed to test whether these different regions differed in the amount of change.

Were any of the original strains from the wild (S1, S2, S3, N5, N6, N7) heterokaryotic for recombination control factors? If there were genetic heterogeneity for recombination-control genes within single colonies obtained as multinucleate, haploid, and possibly heterokaryotic vegetative cultures from the wild, it would show up as differences in recombination between different selfed generation 1 strains derived from the same wild type, *e.g.*, selfed generation 1 wild types SR2.3 and SR10.2, which were obtained from different asci from selfed perithecia from original wild strain S3. In the present work, color mutants were always crossed to wild types derived from different original strains, but the different selfed generation 1 color mutants derived from the same original wild isolate provide examples of strains that could differ in any recombination-control genes heterokaryotic within their original wild strain. For example, all five north-slope color mutants used came from original strain N5, and S*w*18.2, S*w*24.3, and S*lg*60.3 all came from S3. The fact that, for percentage of second DS, S*w*18.2 showed no differences when crossed to SR2.3 and SR4.2, while S*w*24.3 showed highly significant differences in the two crosses, could well be due to different alleles for recombination controls in the two color mutants, rather than to separate controls for different intervals, especially as those two mutations share most of their locus-centromere intervals. It therefore seems likely that there was heterokaryosis for different recombination-control alleles in one original strain from the wild, S3. The nuclear ratios in the heterokaryons for the different recombination control types would determine how often the different selfed generation 1 strains from the same original strain showed differences for recombination measures.

DISCUSSION

Magnitude of recombination effects: The genetic differences for recombination controls between two strains (*e.g.*, two wild types) are twice as great as measured from their crosses to a common tester strain (*e.g.*, an ascospore color mutant) because the tested strain provides only half the genes in the diploid fusion nucleus that undergoes meiosis in these crosses. This underestimation factor of two applies to crossing over and to gene conversion and could be affected by the dominance relations of control factor alleles.

Crossing over in the locus-centromere intervals: The biggest and most consistent differences for percentage of second DS were between slopes, not within slopes. For S*w*92.1, for example, within-slope differences were 61.4 *vs.* 62.7% second DS for SFS wild types and 47.1 *vs.* 52.1% for NFS wild types, but the between-slope difference was 62.0% for SFS *vs.* 49.5% for NFS strains. Both types of difference, within-slope and between slope, must have a genetic basis from genes controlling crossing-over frequencies, as all selfed generation 1 and 2 strains were raised and crossed in the same lab constant environment.

The percentage of second DS for mutations isolated from SFS strains was generally higher (average, 60.0% in crosses to SFS wild types, 56.0% in crosses to NFS wild types) than those from NFS strains (55.5% in crosses to SFS wild types, 54.2% in crosses to NFS wild types), although their sites are intermingled along the chromosome. This fits with recombination-controlling gene differences between SFS and NFS color mutation strains, as well as between the SFS and NFS wild types, as might

be expected.The percentage of second DS for some loci could be of some survival value, as the ascospores from a single meiosis usually dehisce in a compact group and so often land on the same substrate. If several spores germinate together, they could form vegetative heterokaryons, with possibly beneficial interactions (*e.g.*, heterosis) between different alleles at the same locus. One often observes incompletely dehisced asci (Webster 1980), where perhaps the four apical spores dehisce as a group and the four lower spores get stuck within the perithecium. In first division segregation asci, the ordered patterns are 4:4, *e.g.*, *A A A A a a a a*, while second division patterns are 2:4:2, *e.g.*, *A A a a a a A A*, or 2:2:2:2, *e.g.*, *A A a a A A a a*. A higher frequency of second division segregation would therefore give more groups of genetically diverse (*e.g.*, having *A* and *a*) spores when, say, only three or four spores dehisce together. In fungi with different mating types, it could also affect the chance of one substrate receiving spores of opposite mating types. That could be important for sexual reproduction in isolated substrates, especially if there are no conidia, as in *Ascobolus immersus*.

Gene conversion: The best estimate of overall between-slope differences in conversion to wild type comes from comparing SFS color mutations × SFS wild types with NFS color mutations × NFS wild types, where the 1.24 *vs.* 0.52%, respectively, gives a 2.4-fold difference based on very large sample sizes (Tables 2 and 4). One cannot repeat these calculations to get a comparable estimate for crossing over, because of the already described problem of changing the percentage of second DS into crossover units, but the direction of change was the same.

Genetics of the recombination-control factors: The data do not allow an estimate of the number of different recombination-control factors. Taking average values for conversion to wild type over all mutations studied, for different types of cross with respect to different slopes, we get SFS + × SFS *m*, 1.24%; SFS + × NFS *m*, 0.69%; NFS + × SFS *m*, 0.94%; NFS + × NFS *m*, 0.52%. With SFS × SFS giving the highest conversion frequencies, NFS × NFS giving the lowest values, and SFS × NFS giving intermediate values, additive action or partial dominance is seen, not complete dominance. While there could be complete dominance for some recombination factors and additive action for others, these results fit an overall pattern of additive action in the diploid ascus cell undergoing meiosis. It is more difficult to do a comparable test for additive action for percentage of second DS, because three mutations from SFS strains are in the group farthest from the centromere, with only one from NFS strains (Figure 1). Average percentages of second DS values were 60.0% for SFS + × SFS *m*, 54.2% for NFS + × NFS *m*, 55.5% for SFS + × NFS *m*, and 56.0% for NFS + × SFS *m*. The results are compatible with overall additive action.

General discussion: As the recombination differences were found under constant lab conditions between strains in both selfed generations 1 and 2, none of which had been exposed to growth in Evolution Canyon, they were heritable. Although the results for conversion and crossing over were not identical, they were sufficiently similar, especially in direction, for the main between-slope differences for these two different aspects of recombination to have been caused largely by the same genetic controls. The results for centromere interval crossing over and gene conversion fitted the working hypothesis very well, with more recombination in the strains from the harsher, more stressful, and more varied south-facing slope than in strains from the lusher north-facing slope.

The percentage of conversion asci with postmeiotic segregation among conversions to wild type was looked at because the mispair or nonpair correction enzymes for hDNA in recombination might be related to those involved in repair of premutational lesions involved in mutation. Lamb *et al.* (1998) found much higher mutation frequencies in the SFS strains than in the NFS strains. As shown in Table 2, there were no significant differences for the percentage of conversion asci with postmeiotic segregation between crosses to SFS and NFS wild type. There was therefore no correlation between the efficiency of repair enzymes in recombination and overall mutation frequencies in those strains. The direction of conversion could not be measured accurately because the spontaneous mutations to mutant affected estimates of conversion to mutant.

There were some recombination differences between strains from the same slope, but the between-slope differences were much more consistent and usually larger. There was evidence that one strain isolated from the wild was heterokaryotic for recombination control factors, but the experiments were not set up to test that rigorously. The fact that genetic heterogeneity for recombination has been found between strains in testing only a few isolates from the wild suggests the presence of a large amount of genetic variation in the wild for genes controlling recombination frequencies. Selection could act on that variation to give optimum levels for this variation-generating process in any particular environment. See Houle (1992) for a consideration of the relation between the variability of quantitative traits and their ability to respond to selection.

There are no asexual reproductive propagules for dispersal in *S. fimicola*, and the ascospores usually dehisce over distances of less than a meter (Webster 1980). Some gene flow between SFS and NFS populations could occur for this coprophilous fungus, as browsing goats and cattle can move between the slopes. Movement of ascospores or hyphal fragments in rainwater is unlikely between the two slopes, as gravity would give transport only from the upper levels of one slope to the lower levels of the same slope. Any selection pressure for more recombination on the southern slope must

therefore be strong enough to overcome equalizing effects from any gene flow between the slopes. Gene flow between and within populations would be restricted by genes limiting fusion of different isolates (Carlile and Watkinson 1994); such genes are known in *S. fimicola* (Olive 1956). He found that strains from different areas were often not cross-fertile. We found that some Evolution Canyon SFS strains would not cross with any NFS strains, and vice versa, and that some strains from one slope crossed with some but not all strains from the opposite slope, so there are some barriers to gene flow. Our strains did not cross with Olive's strains. Lamb *et al.* (1998) found that the SFS strain geographically nearest to the NFS strains was most different from them in mutation frequencies, which would not be expected if there was much gene flow between slopes.

Derzhavets *et al.* (1996) found about four times as much (0.29%) male recombination in *D. melanogaster* flies from the SFS than in ones from the NFS (0.07%) from Evolution Canyon, even after many generations in lab culture. That suggests that selection for recombination properties was sufficiently strong to overcome equalizing effects of migration even for such mobile organisms as fruit flies.

The value to an organism of being able to adapt its recombination frequencies to its environment, either by mutational changes in recombination controls or by physiological adaptation (*e.g.*, genetically predetermined responses of recombination frequencies to temperature effects), is limited by the following considerations. Recombination controls are irrelevant to nonsyntenic loci and to syntenic loci so far apart that they show 50% recombination. They are also irrelevant unless two syntenic loci are both heterozygous, as crossing over has no effect on allele arrangements where only one locus is heterozygous. The only cases where changing crossover frequencies will have any effect are where two or more syntenic loci are all heterozygous and are not so far apart as to show independent assortment.

Because *S. fimicola* is homothallic and self-fertile with no mating types, many—probably most—perithecia from sexual reproduction in the wild will be selfed, when all chromosomes are homozygous in the diploid fusion nucleus, and recombination frequencies from independent assortment, crossing over, and gene conversion are irrelevant. Recombination frequency importance is possible only for syntenic loci in crossed perithecia. As there are no gametes, getting crossed perithecia depends on getting colonies that are heterokaryotic from vegetative fusion of genetically different colonies, or if colonies have become heterokaryotic through internal mutation. There are several well-established genetic systems controlling recombination in heterothallic fungi such as *A. immersus* and *N. crassa* (*e.g.*, see Helmi and Lamb 1983), but there have been few, if any, reported from homothallic fungi.

The present correlation between an organism's recombination frequencies and its different environments supports the idea that recombination between loci can be beneficial for survival. Crossing over produces new variants that may be able to colonize new habitats or be more successful in the existing environment or respond to a changing environment, but many new variants will be less successful than an already well-adapted parent, especially if there are clusters of co-adapted genes. Both reciprocal crossing over and nonreciprocal gene conversion can produce new alleles in a doubly heterozygous locus, *e.g.*, giving 1^-, 2^- and/or 1^+, 2^+ from 1^-, $2^+/1^+$, 2^-. The functions of recombination include the production of new alleles as well as new combinations of existing alleles at different loci.

The recombination differences between strains from the south- and north-facing slopes may have a selective value in adapting the strains to their particular microscale environment, with more recombination in the harsher, more variable, and fluctuating environment where more variation may assist survival even if many variants are less successful than their parents. The results agree well with other findings from Evolution Canyon, especially those of Nevo (1995, 1997) on genetic diversity in diverse unrelated taxa from multilocus allozymes and DNA diversities, and with those of Derzhavets *et al.* (1996) on recombination frequency in male *D. melanogaster*. In general, the evolutionary forces causing and maintaining genetic diversity are more pronounced in Evolution Canyon on the ecologically more heterogeneous and stressful south-facing slope.

The between-slope differences were clear and completely consistent in direction, affecting a number of different gene intervals in crossing-over and mutational sites in gene conversion. The within-slope differences were sometimes significant, sometimes not, and were not consistent in direction, so it is not worth speculating on possible ecological factors involved in within-slope differences. Finding recombination-control differences between the descendants of just four original wild-type strains (S2, S3, N5, and N7) suggests that genetic differences for recombination are very frequent in Sordaria in wild strains.

In locusts, Bugrov and Vysotskya (1988) found that species with large variations in recombination (chiasma frequency) were ecologically flexible and less specialized, while species with stable chiasma frequencies occupied narrow ecological niches. There therefore seems to be selection on recombination variability as well as for particular recombination levels in particular circumstances. The efficiency of recombination as a source of variability depends on the organism's breeding system, life cycle duration, ploidy level, and chromosome numbers, as well as on crossover frequency and distribution, and on their genetic and environmental variation (Korol *et al.* 1994). Because the north-facing slope in Evolution Canyon is a more constant and more uniform environment than the south-facing slope, the present

finding of lower recombination frequencies from NFS strains than from SFS strains is consistent with the theory of Fisher (1930) that a constant environment favors tighter linkage between genes (lower recombination frequencies).

The genetic control of gene conversion may be partly related to the control of crossing over, as the same initiating mechanisms are probably responsible for both processes. There could also be a different selection for particular levels of gene conversion, as conversion can have several different roles, including recombination of alleles within genes if they do not co-convert. Biased gene conversion (say with conversion favoring the wild-type allele in some cases or the mutant allele in other cases; see Lamb 1988) in heterozygotes can change allele frequencies in a population (Lamb 1985a, 1986, 1996). It may also change base ratios and/or total amounts of DNA (Lamb 1985b).

Genetic variation in a population is reduced by stabilizing and directional selection, and by genetic drift, and is increased by mutation and recombination. In fungi like Sordaria with haploid vegetative stages and haploid sexual spores, selection against deleterious recessives is much stronger than in diploids where they are protected in heterozygotes. Similarly, the concept of additive genetic variation does not really apply in haploids, although aspects of dominance do apply to recombination controls in the diploid fusion nucleus that undergoes meiosis.

S. fimicola has overlapping generations, vegetative reproduction by haploid growth and fragmentation of colonies, and sexual reproduction by ascospores from selfed or crossed perithecia, being homothallic but capable of crossing with other strains. Because of its colonial nature, fragmentation, different survival bodies (ascospores, perithecia, and hyphae), and hyphal fusions between different colonies, one cannot easily define an individual or estimate the number of individuals in a population, with populations not being discrete. On dung, the fungus usually appears as part of a succession of different genera, not independently (Webster 1980), and can also live saprophytically on plant remains. Its biology, especially haploidy and frequent self-fertilization, makes it doubtful whether many of the classic evolutionary and population genetics considerations, equations, and simulations based on two sexes of diploids can be applied to Sordaria.

Lande and Shannon (1996), considering sexual diploids, found that genetic variation could be beneficial or detrimental, depending on the pattern of evolutionary change. They found that it could play a decisive role in allowing a population to persist and adapt to a changing environment, especially if the population is tracking an optimum that has some predictability, but that in a constant environment it was disadvantageous. Although in Evolution Canyon the NFS is not a constant environment, it is much more uniform than the SFS.

Bell (1982) reported that measures of asexuality (*e.g.*, parthenogenesis) tend to increase at high elevations and in disturbed habitats and that in some harsh environments there was less recombination. It is therefore relevant to mention direct effects of temperature on recombination in *S. fimicola*, although the present experiments were done at a constant 17.5° to show up genetic differences in recombination between strains. In Olive's strains, Lamb (1969a) found that fertile crosses could be made between most strains over the range 10°–31°. For *hyaline*, the percentage of second DS showed a U-shaped response to temperature, with maxima of 57% at 10° and 63% at 31° and a minimum of 46% at 20°. For *gray*, there was a much flatter inverted U-curve, with a maximum of 67% at 17.5°–22.5°, which is just above the expected maximum value of 66.7% if there is no interference. Gene conversion also showed a U-curve, with maxima at 10° and 30° and a minimum at 15°, while *gray* showed an almost linear increase from 10° (0.9%) to 30° (10.6%) and then a slight drop at 31°. Lamb (1969b) also studied the effects of temperature on recombination in *S. brevicollis*, where crossing over generally increased with temperature over the range of 12.5°–30° in all four short intervals studied. There was thus some trend toward more recombination at the higher temperature extreme and sometimes also at the lower temperature extreme.

In the natural Evolution Canyon environment, there are large diurnal fluctuations in temperature, especially on the SFS, as well as seasonal fluctuations. The effects on recombination of fluctuating temperature, as opposed to different constant temperatures, have rarely been studied. Lamb (1969b) made *S. fimicola* crosses outdoors, with temperatures typically varying between ~5° and ~22° every 24 hr. The percentage of second DS and conversion frequencies for *gray* were within the range of those obtained by continuous incubation at 10° (the lowest continuous temperature for fertility) and 22.5°, but the conversion frequency for *hyaline* was the lowest recorded. Transfers of crosses at different stages of development between two constant temperatures sometimes gave percentages of second DS values significantly outside the range of the two constant values and also showed that recombination is sensitive to temperature at premeiotic stages, as well as during meiosis.

It could be argued that on the milder, more populated, and more uniform NFS, biological competition between species might be more intense than on the harsher, more variable SFS, where survival might be more of a struggle against extinction from physical factors, so that genetic variation might be valuable in both environments. Genetic variation is produced in both environments, from mutation and recombination, both of which produce loads on fitness if the parents are well adapted. It would be harder, however, to have a single ideal strain on the SFS, which is much more varied and patchy, than on the NFS. Our data do not distinguish

between the effects of greater stress on the SFS than on the NFS and the effects of more patchiness and variability, but they do show very consistent differences between strains and slopes in inherited recombination controls, with more recombination in SFS strains.

CONCLUSION

There was heritable genetic variation in recombination between the original *S. fimicola* wild strains, as shown by the observed differences in crossing over and gene conversion between selfed generation 1 and 2 strains derived from four of them, S2, S3, N5, and N7. There were differences within slopes and particularly between slopes, on which natural selection could act in adaptation. There were consistently higher recombination frequencies in strains from the harsher, more variable south-facing slope environment than in strains from the milder north-facing slope. Of the 20 recombination comparisons between SFS and NFS strains, 16 gave highly significant differences, always with higher values from the SFS strains. The consistency of the higher recombination from the SFS strains matched that found earlier (Lamb *et al.* 1998) for higher mutation frequencies in the SFS strains. Crossing over and gene conversion varied within a species, on a microscale, in the direction predicted when the harsher environment selects for higher recombination frequencies.

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