*THE RELATIONSHIP OF GENE CONVERSION TO CROSSING OVER IN NEUROSPORA**

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The production of infrequent wild-type progeny in crosses between strains carrying apparently allelic mutants of similar phenotype has been termed gene conversion. Mitchell[1] demonstrated in a cross between two pyridoxine mutants that conversion differed from crossing over in two ways: (1) the convertants did not have the expected array of closely linked markers; (2) the event was non-reciprocal; the ascus in which a *pdx*+ spore pair occurred did not contain a pyridoxine double mutant spore pair.

In spite of these differences, gene conversion and crossing over were correlated. The frequency of crossing over between the markers embracing the pyridoxine locus was only about 5 per cent in the general population, while 14 of the 32 *pdx*+ convertants had such crossing over. Mitchell suggested that gene conversion and crossing over were separate events and that they were correlated because both were favored by the same local conditions, as perhaps, especially close pairing of the homologous chromosomes.

A hypothesis whereby a single event results in both gene conversion and crossing over was formulated by Freese,[2] and this scheme was designed to account for the apparent differences between the two types of recombination reported by Mitchell. Freese states that gene conversion is observed for segregating genes *at the site* of the event which may result in crossing over for the surrounding markers. It is proposed that the recombination event is not a single exchange, but a switching of the replicating strands back and forth between the two templates, two or three or more times in a short interval. An odd number of switches results in crossing over with regard to the surrounding markers, while an even number of switches does not.

If conversion resulted from the same event as crossing over, it might be expected to have an effect associated with crossing over: interference with crossing over in neighboring regions. An experiment has been performed to study the relationship of gene conversion to crossing over in a separate but nearby chromosome region. The single event hypothesis would predict interference in all the convertants, even those with no recombination for the markers embracing the conversion locus. The separate events hypothesis would predict no interference in this group.

Materials and Methods.—Strains *lys*+ *cys-t ylo*+ *ad cot a* and *lys cys-c ylo ad*+ *cot A* were crossed on two sets of plates for which the procedures were identical except that they were allowed to mature at 25° in one case and at 18° in the other. The experiment was designed in this way in order to observe how the frequencies of gene conversion and of crossing over varied at the two temperatures.

Lys (lysine-requiring mutant DS6-85), *cys-t* and *cys-c* (cysteine mutants 80702 and 48401), *ylo* (yellow conidia, Y30539y), and *ad* (adenine mutant 3254) are all in the left arm of linkage group VI, and the order of the loci is *lys...cys...ylo...ad...*centromere.[3] *Cys-t* and *cys-c* are functionally allelic in that they do not complement each other in heterocaryons; however, crosses between them yield 2-3 *cys*+ asco-

spores per thousand by gene conversion.[4] The two cysteine mutants can be distinguished by nutritional responses at 25°C; *cys-t* will grow on either cysteine or thiosulfate, while *cys-c* will grown only on cysteine. The temperature colonial gene *cot*, present in both parents, results in the formation of small, dense colonies on agar plates at temperatures above 31°C, while growth at lower temperatures appears identical to wild-type.[5]

Random ascospores were suspended in 0.1 per cent agar and filtered through surgical gauze to remove clumps and debris. The suspension was then added to liquid 1 per cent agar at 60°C and held at this temperature for 25 minutes to accomplish activation of the spores. Four milliliter aliquots of the suspension (containing 1,000 to 5,000 ascospores) were poured over plates of minimal medium supplemented with lysine and adenine. Only the *cys*+ convertants were capable of growth on this medium. The plates were incubated at 34° for six days, at which time all colonies were transferred to slants of complete medium and allowed to mature at 25°. Classification for *ylo* was noted, and growth tests were performed to score for *lys* and *ad*.

A *ten-fold* dilution of the ascospore suspension was plated over minimal medium supplemented with lysine, cysteine, and adenine. These plates were incubated six days at 34° and colonies were counted to determine the number of viable spores in the original suspension. Colonies from these plates were picked up and fully classified to determine crossing over frequencies in the general population.

TABLE 1

FREQUENCIES OF CYS+ CONVERTANTS

....lys+....cys-t....ylo+....ad.....
....lys.....cys-c....ylo.....ad+....

Genotype	25°	18°	Per 10^6 germinated spores 25°	18°
+ + + ad	112	52	42.6	30.4
lys + ylo +	201	147	77.0	85.8
+ + ylo +	155	98	59.4	57.2
lys + + ad	116	74	44.5	43.2
+ + + +	14	17	5.4	9.9
lys + ylo ad	34	24	13.0	14.0
+ + ylo ad	6	4	2.3	2.3
lys + + +	5	12	1.9	7.0
Total	643	428	246.0	250.0
Total germinated spores	260,800	171,200		

Results.—The frequencies of *cys*+ convertants with the different combinations of linked markers are shown in Table 1. A comparison of the results of the cross which matured at 25° with those from the 18° cross reveals that the total conversion frequencies are very similar, and there are no striking differences in the frequencies of the various marker combinations.

The genotypes of randomly selected progeny are listed in Table 2. There is a sharp disparity between the numbers of complementary types in several classes; this results from the relative inviability of ascospores carrying the *cys-c* gene (particularly at 18°). There are no marked differences in crossing over frequency at the two temperatures, in contrast to other studies[3,6] which showed more crossing over at 18° than at 25°. One noteworthy aspect of the present data, however, is

TABLE 2

CROSSING OVER FREQUENCIES AMONG RANDOMLY SELECTED GERMINATED ASCOSPORES

....lys+....cys-t....ylo+....ad.....
I II III
....lys.....cys-c....ylo.....ad+....

Genotype	Region of Crossover	25°	18°
lys+ cys-t ylo+ ad	None	405	303
lys cys-c ylo ad+	None	79	20
lys+ cys-c ylo ad+	I	13	0
lys cys-t ylo+ ad	I	17	22
lys+ cys-t ylo ad+	II	103	62
lys cys-c ylo+ ad	II	18	3
lys+ cys-t ylo+ ad+	III	64	42
lys cys-c ylo ad	III	14	1
lys+ cys-c ylo+ ad	I, II	2	0
lys cys-t ylo ad+	I, II	7	1
lys cys-t ylo+ ad+	I, III	0	3
lys+ cys-t ylo ad	II, III	1	6
lys+ cys-c ylo+ ad+	I, II, III	1	0
Total		724	463

25° map: lys..5.5..cys..18.3..ylo..11.1..ad
18° map: lys..5.6..cys..15.5..ylo..11.2..ad

that the two crosses differ in patterns of multiple crossing over. Double exchanges involing the *lys-cys* and *cys-ylo* regions are more frequent in the 25° cross, while doubles in which one crossover takes place between *ylo* and *ad* occur more often at 18°.

The results are summarized with respect to interference with crossing over in the *ylo-ad* region in Table 3. The critical question is whether gene conversion results in such interference even in the absence of crossing over between *lys* and *ylo*. The answer is that it does not. In the 25° cross, the frequency of *ylo-ad* recombination among spores with parental combinations of *lys* and *ylo* is much the same (13.3% to 13.8%) whether or not gene conversion to *cys*+ has taken place. Among the corresponding types from the 18° cross the frequency of *ylo-ad* recombination among convertants is somewhat *higher* (less interference) than among non-convertants.

The results indicate that conversion differs from crossing over in that it does not interfere with crossing over in a nearby region. Is it possible that this is an erroneous conclusion resulting from some ambiguity of the experiment? Two aspects of the experimental setup may be suspect: the relative inviability of spores carrying the *cys-c* gene and the considerable crossing over distance between the conversion locus and the test region (*ylo-ad*).

Is the apparent interference among random spores a spurious result of the inviability of *cys-c*? No. In Table 4 the 25° data on interference are broken down into two groups according to which *cys* gene is present, and there is no apparent disparity between the two.

Does interference in the *ylo-ad* region take place only with crossing over to the right of the *cys* locus, which is itself too far away for such interference? This is plausible, as the map distance from *cys* to *ylo* is quite large (18.3% recombination at 25°). Were this the case, the absence of interference among *cys*+ convertants would not conflict with the single event hypothesis. In such a situation, however, this hypothesis would predict no interference in any of the convertants, whether the

TABLE 3

EFFECT OF GENE CONVERSION AT THE CYSTEINE LOCUS ON CROSSING OVER BETWEEN YLO AND AD

	ylo-ad recombination frequency	
	25°	18°
Cys+ convertants		
Parental combination of lys and ylo	48/361 (13.3%)	41/240 (17.1%)
non-parental combination of lys and ylo	11/282 (3.9%)	16/188 (8.5%)
Random spores		
parental combination of lys and ylo	79/572 (13.8%)	43/367 (11.7%)
non-parental combination of lys and ylo	1/152 (0.7%)	9/96 (9.4%)

TABLE 4

EFFECT OF THE CYSTEINE GENOTYPE ON INTERFERENCE (25° cross)

	ylo-ad recombination frequency		
	cys-c	*cys-t*	*total*
Parental combination of lys and ylo	15/96	64/476	79/572
Non-parental combination of lys and ylo	0/31	1/121	1/152

switching at the *cys* locus resulted in recombination for the surrounding markers or not. Clearly this prediction is not fulfilled (see Table 3).

Evidence that the *cys* locus is close enough to the *ylo-ad* interval for crossing over interference would be provided by a demonstration that such interference is manifest with crossing over in the *lys-ylo* region, which is even farther away. This appears to be the case, although the data are scant. In the 25° data of Table 2, the predicted number of coincident crossovers in regions I and III would be 4.4 in the absence of interference, while only one such event was observed.

The results of the 18° cross are less clear-cut, because there is only weak interference in the *ylo-ad* region even among *lys-ylo* recombinants. To the extent that they can be evaluated, these data tend to confirm the conclusion drawn from the 25° results.

Discussion.—Two general types of hypothesis have been advanced regarding the relationship of gene conversion to crossing over. (1) the two are separate events; (2) they are both results of a single recombination event. Several studies have resulted in findings which are difficult to explain by the single event hypothesis. Roman[7] observed mitotic conversion to wild-type in an adenine-requiring diploid yeast; the event was not accompanied by crossing over. Heat shock in Neurospora[8] and ultraviolet treatment in yeast[9] caused changes in conversion frequency without parallel changes in crossing over. Changing the genetic background resulted in a threefold increase in crossing over around the *cys* region in Neurospora without significant changes in the frequency or pattern of gene conversion at this locus.[4]

The present study has shown that gene conversion differs from crossing over in that it does not interfere with crossing over in a neighboring region. The single event hypothesis would not have predicted such a result, although the hypothesis could be readily modified in such a way as to account for this finding. It would seem, however, that the simpler assumption at the present time is that gene conversion and crossing over result from different events.

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*CONFORMALLY INVARIANT METRICS ON RIEMANN SURFACES**

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The object of this note is to assert the existence of two conformally invariant metrics, give the definitions, and describe the principal results with some indications of the proofs. By a metric d we shall mean a metric function in the topological sense so that d is a non-negative function on the product $X \times X$. Here the underlying set X is a Riemann surface and so it is a topological space with a locally Euclidean topology $\mathfrak{I}$. If we speak about a metric d for X we shall always tacitly assume that d is compatible with $\mathfrak{I}$, that is, the topology generated on X by d is $\mathfrak{I}$.

We shall define two pseudo-metrics d^i $(i = 1,2)$ on any Riemann surface X and save for a few exceptional types these functions d^i will turn out to be proper metrics compatible with the topology $\mathfrak{I}$. The definitions will be intrinsic and hence these metrics d^i will be invariant under conformal mappings: If the points x_1, x_2 are mapped into y_1, y_2 under a conformal map of X onto Y then $d^i_X(x_1, x_2) = d^i_Y(y_1, y_2)$ where d^i_X and d^i_Y denote the metrics given on X and Y, respectively.

The pseudo-metrics d^i will be defined by extending the notion of extremal length due to Ahlfors and Beurling (see ref. 1, pp. 114–115). The *extremal length* λ of a family Γ of curves γ on a Riemann surface X is defined as follows: Let ω be a pure first order differential on X, that is let $^*\omega + i\omega = 0$ where $^*\omega$ is the star conjugate of ω. Since ω is pure its modulus $|\omega|$ can be integrated along the curves γ of Γ. We let