Recombination at *his-3* in Neurospora Declines Exponentially With Distance from the Initiator, *cog*

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ABSTRACT

By deletion of 1.8 kb of sequence between cog^L and *his-3* and replacement with sequences of different lengths, we have generated a set of Neurospora strains in which the distance between cog^L and the site at which recombination is selected varies from 1.7 to nearly 6 kb. Each of the manipulated strains includes cog^L, a highly active recombination hotspot, and *rec-2*, thus allowing high-frequency recombination. In addition, each is a *his-3* mutant, either K26 or K480. The frequency of His^+ recombinants in progeny of these crosses is inversely proportional to the distance between *his-3* and *cog*. Specifically, there is a linear relationship between $\log_{10}$ (recombination frequency) and the distance in base pairs, indicating that as distance decreases, the rate of interallelic recombination increases exponentially. An exponential relationship between distance separating markers and the chance of co-conversion has been found in both Drosophila and fission yeast, indicating that the extension of recombination events may be a stochastic process in most organisms. On the basis of these and additional data presented in this article, we conclude that recombination is initiated at cog^L in >17% of meioses, that most conversion tracts are very short, and that few extend >14 kb.

MEIOTIC recombination occurs more often in some regions of eukaryotic chromosomes than in others (Lichten and Goldman 1995). Regions in which recombination is more frequent are assumed to include recombination hotspots (Holliday 1968).

Recombination hotspots have been studied in most detail at the *HIS4* (Nicolas *et al.* 1989) and *ARG4* (Detloff *et al.* 1992) loci of *Saccharomyces cerevisiae*, at the *his-3* locus of *Neurospora crassa* (Angel *et al.* 1970; Catcheside and Angel 1974; Yeadon and Catcheside 1995, 1998, 1999; Yeadon *et al.* 2001), and at the *ade6* locus of *Schizosaccharomyces pombe*, where generation of a mutation, M26, fortuitously resulted in hotspot activity (Gutz 1971).

Interallelic recombination in Neurospora has been shown to be due primarily to gene conversion (Mitchell 1955; Case and Giles 1958; Stadler 1958; Suyama *et al.* 1959; Bowring and Catcheside 1996), the nonreciprocal transfer of DNA sequence information that leads to non-Mendelian segregation of the sequence (Fogel and Hurst 1967). Conversion events are initiated close to recombination hotspots (de Massy *et al.* 1995; Liu *et al.* 1995; Xu and Kleckner 1995), which are usually located at the 5′ end of a coding sequence (Catcheside and Angel 1974; Nicolas *et al.* 1989; Detloff *et al.* 1992; Wu and Lichten 1994; Zahn-Zabal *et al.* 1995; Bowring and Catcheside 1998; Yeadon and Catcheside 1998, 1999). Conversion tracts usually extend in both directions from the initiation site (Schultes and Szostak 1990; Malone *et al.* 1994; Bowring and Catcheside 1998; Yeadon and Catcheside 1998), although there are exceptions to bidirectionality (Porter *et al.* 1993).

Lengths of meiotic conversion tracts have been measured in *S. cerevisiae* (Judd and Petes 1988; Borts and Haber 1989), *S. pombe* (Grimm *et al.* 1994), Neurospora (Bowring and Catcheside 1998; Yeadon and Catcheside 1998), and *Drosophila melanogaster* (Hilliker *et al.* 1994). However, a mathematical relationship between the number of nucleotides separating two sites in a gene and the chance of co-conversion has been described in only two species, *D. melanogaster* (Hilliker *et al.* 1994) and *S. pombe* (Grimm *et al.* 1994).

At *ade6* of *S. pombe*, the frequency of co-conversion of a silent marker with a selected mutant site decreased exponentially with increasing distance from the mutation (Grimm *et al.* 1994). The minimum average tract length was estimated at ~1 kb. Similarly, co-conversion data at the *rosy* locus of Drosophila revealed an exponential relationship between the distance between two sites and the chance that both sites would experience conversion (Hilliker *et al.* 1994), with an average length of 352 bp for unselected and 706 bp for selected tracts. Hilliker *et al.* (1994) suggested that the probability that a conversion tract will be at least a particular length x (in nucleotides) is ϕ^x, where ϕ is the probability at each nucleotide that a tract will continue. This relation-

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ship was shown to be a good fit to the co-conversion data and generated an estimate of $\phi = 0.99717$, with a standard error of 0.00026.

Interallelic recombination at the *his-3* locus of Neurospora is initiated by the recombination hotspot *cog* in the absence of the dominant allele of the *trans*-acting *rec* gene *rec-2*$^+$ (Angel *et al.* 1970; Catcheside and Angel 1974; Yeadon and Catcheside 1998). *cog* is located centromere-distal (Angel *et al.* 1970) of the 3′ end of the *his-3* locus (Bowring and Catcheside 1991). *rec-2*$^+$ reduces recombination in *his-3* ~30-fold below that observed in a *rec-2* homozygote (Angel *et al.* 1970). Recombination at *cog* is turned off by *rec-2*$^+$ (Catcheside and Angel 1974) and any residual recombination events are initiated close to the 5′ end of *his-3* (Yeadon and Catcheside 1998).

The *cog* initiation site is >2.2 kb from the 3′ end of *his-3* (Yeadon *et al.* 2001), probably at or close to the 10-bp sequence required for the high-frequency recombination phenotype of *cog*^{L} (Yeadon and Catcheside 1998), which is located near the 5′ end of the *lpl* gene at the centromere-distal end of the *cog* region (Yeadon and Catcheside 1995; Yeadon and Catcheside 1999).

By deletion of 1.8 kb of sequence between *cog*^{L} and *his-3* and replacement with sequences of different lengths, we generated a set of Neurospora strains in which the distance between *cog*^{L} and the site at which recombination is selected varies from 1.7 to nearly 6 kb. Each of the altered strains includes *cog*^{L}, the more active allele of *cog*, and *rec-2*, which in combination ensure a high frequency of recombination in the vicinity of *cog* (Angel *et al.* 1970). In addition, each is a *his-3* mutant (K26 or K480). Since the manipulated strains that have not been outcrossed are, except for the modification, genetically identical to the transfection recipients, this eliminates other genetic influences (Catcheside 1970) on recombination frequency. Since we know that most interallelic recombination in *his-3* is initiated by *cog* (Angel *et al.* 1970; Catcheside and Angel 1974; Yeadon and Catcheside 1998) at positions located centromere-distal of the site of our modification (Yeadon *et al.* 2001), the distance travelled by a recombination event that yields a His$^+$ recombinant depends on the number of nucleotides between *his-3* and the presumptive initiation site within the *cog* region. Thus, by altering the distance between *cog* and *his-3* we can investigate the relationship between the distance travelled by a recombination event and the resultant frequency of recombinant progeny. We can then assess whether the exponential relationship between distance separating markers and the chance of co-conversion in Drosophila (Hilliker *et al.* 1994) and *S. pombe* (Grimm *et al.* 1994) holds for heteroallelic recombination in Neurospora.

MATERIALS AND METHODS

Culture methods and media: Except that crosses were supplemented with 200 μg/ml histidine, 500 μg/ml alanine, 500 μg/ml arginine, 200 μg/ml adenine, and 400 μg/ml lysine as required, culture methods and media were as described by Bowring and Catcheside (1996). Vegetative cultures were supplemented with 200 μg/ml histidine, 500 μg/ml alanine, 500 μg/ml arginine, 400 μg/ml adenosine, and 400 μg/ml lysine as required.

Molecular methods: Restriction enzymes, Klenow, and T4 DNA ligase were supplied by New England Biolabs (Beverly, MA) and were used according to the manufacturer's instructions. Electrophoresis used TAE and 1% agarose. The polymerase used for PCR was RedHot supplied by Abgene. PCR amplification used a Perkin-Elmer (Norwalk, CT) 2400 thermal cycler. Each 50-μl reaction included 0.5 units RedHot polymerase (Abgene), 200 ng of each primer, 1.75 μM $MgCl_2$, and 0.8 mM dNTP mixture (equal concentration of each). Cycling conditions were 94° for 1 min, then 30 cycles of 94° for 15 sec, 52° for 30 sec, and 72° for 3 min, followed by 72° for 7 min, using a Perkin-Elmer GeneAmp 2400 thermal cycler. Primers used were NEWXF-GGCACATAGAGTAATGATAACG and NEWC8R-ACTACAAGCAAAGTCGGG.

Construction of plasmid vectors and strains: p(1-16)X was made by insertion of a 197-bp synthetic oligonucleotide (1-16) into the *Spe*I site (Rasmussen *et al.* 2002) of pJY64 (Yeadon *et al.* 2001) between *his-3* and the *arg-2-TK* construct (Sachs *et al.* 1997). p(17-32)X was made by deletion of 4 bp at position 77 in the *TK* coding sequence in pJY65 (Yeadon *et al.* 2001), followed by insertion of a slightly different 197-bp synthetic oligonucleotide (17-32) into the *Spe*I site. pJY64, pJY65, pDV2-B5, and pDV3-B5 were made as described previously (Yeadon *et al.* 2001; Rasmussen *et al.* 2002).

The *his-3* K458 strains T11630, T11631, and T11644 were made as described previously (Yeadon *et al.* 2001).

Strains T11783 and T11785 (Tables 1 and 2), in which 1.8 kb between *his-3* and *cog* was deleted (Figure 1), were made by transfection of T11644 with pDV3 and of T11630 with pDV2, respectively. The distance between *his-3* and *cog* in the resultant monokaryotic strain (Yeadon *et al.* 2001) was determined to be the same as that in the DV plasmids by electrophoresis of PCR products generated using the primers NEWXF and NEWC8R.

Strains T11738 and T11739 (Tables 1 and 2), with a human immunoglobulin κ light chain gene inserted between *his-3* and *cog* (Figure 1), were made as described previously (Yeadon *et al.* 2001), by transfection of T11644 with pDV3-B5 and T11631 with pDV2-B5, respectively. Additionally, T11740, T11741, and T11742 were made by transfection of T11631 with pDV2-B5 (Yeadon *et al.* 2001).

T11782 (Tables 1 and 2) was made by transfection of T11644 with pDV2 (Yeadon *et al.* 2001; Rasmussen *et al.* 2002). By electrophoresis of PCR products generated using the primers NEWXF and NEWC8R, the distance between *his-3* and *cog* in the resultant monokaryotic strain (Yeadon *et al.* 2001) was determined to be the same as that in T11644.

T11686 and T11690 (Table 1), strains with the *arg-2-TK* construct (Sachs *et al.* 1997) inserted between *his-3* and *cog* (Figure 1), were made as described previously (Yeadon *et al.* 2001). All other *arg-2-TK* strains (Tables 1 and 2) were extracted from crosses between each of the primary transformants T11686 and T11690 and either T11668 or T11670 (Table 1).

Strains with the *arg-2-TK* construct (Sachs *et al.* 1997) and a 197-bp oligonucleotide inserted between *his-3* and *cog* (Figure 1) were made in a similar way to T11686 and T11690 (Yeadon *et al.* 2001). T11768 was made by transfection of p(1-16)X into T11630. T11769, T11770, and T11806 were made by transfection of p(17-32)X into T11644. Constructs were verified by Southern analysis (data not shown).

Strains T12002–T12004, with the *egl3* constructs inserted between *his-3* and *cog* (Figure 1), were supplied by Neugenesis (Yeadon *et al.* 2001; Rasmussen *et al.* 2002).

TABLE 1

Neurospora strains

Stock no.	Genotype
F10292	*arg-1 a his-3*(K480) cog^E; *rec-2*
T6275	*a his-3*(K874) cog^L; *rec-2*
T9149	*a his-3*(K874) cog^L *ad-3; am rec-2*
T11317	*A his-3*(K1201) cog^L; *am rec-2*
T11630/631	*a his-3*(K458) cog^L; *rec-2*
T11644	*A his-3*(K458) cog^L; *rec-2*
T11668	*a lys-4* cog^E *ad-3; am rec-2*
T11670	*a lys-4* cog^E *ad-3; rec-2*$^+$
T11681	*a lys-4 his-3*(K480) cog^E *ad-3; am rec-2*
T11686	*A his-3*(K26) (*arg2-TK*) cog^L; *rec-2*
T11690	*A his-3*(K480) (*arg2-TK*) cog^L; *rec-2*
T11703–09/T11718–25	*A his-3*(K480) (*arg2-TK*) cog^L; *rec-2*
T11710/712/726/727	*a his-3*(K480) (*arg2-TK*) cog^L; *rec-2*
T11730/734	*A his-3*(K26) (*arg2-TK*) cog^L; *rec-2*
T11732/736	*a his-3*(K26) (*arg2-TK*) cog^L; *rec-2*
T11738	*A his-3*(K480) (IGκB) cog^L; *rec-2*
T11739/740/741/742	*a his-3*(K26) (IGκB) cog^L; *rec-2*
T11759	*a his-3*(K480) cog^E; *am rec-2*
T11760/764	*a his-3*(K480) cog^L; *am rec-2*
T11768	*a his-3*(K26) (1-16; *arg2-TK*) cog^L; *rec-2*
T11769/770/806	*A his-3*(K480) (17-32; *arg2-TK*-FS) cog^L; *rec-2*
T11772/773	*A his-3*(K26) cog^E; *am rec-2*
T11782	*A his-3*(K26) cog^L; *rec-2*
T11783	*A his-3*(K480) (del. 1.8) cog^L; *rec-2*
T11785	*a his-3*(K26) (del. 1.8) cog^L; *rec-2*
T12002/3	*A his-3*(K480) (*egl3*) cog^L; *rec-2*
T12004	*a his-3*(K26) (*egl3*) cog^L; *rec-2*

The *am* allele is K314, *lys-3* is STL4, *arg-1* is K166, and *ad-3* is K118. All strains include the colonial temperature-sensitive mutation *cot-1* C102t.

TABLE 2

Interallelic recombination in crosses in which we have altered the distance between *cog* and K480

Cross	*x* (bp)	His$^+$/10^5
T11783 × T11785	1704	624, 383, 461, 404, 371, 369
T11738 × T11739	2454	370, 298, 346
T11738 × T11740		498, 226, 301
T11738 × T11741		483, 350, 357
T11738 × T11742		407, 315, 297
T11759 × T11782	3504	281
T11681 × T11782		228, 170
T11760 × T11772		116, 105
T11760 × T11773		72, 105
T11764 × T11772		101, 130
T11764 × T11773		73, 111
T11703 × T11732	3904	84
T11704 × T11732		75
T11707 × T11732		75
T11708 × T11732		55
T11709 × T11732		92
T11710 × T11730		117
T11712 × T11730		93
T11718 × T11736		69
T11721 × T11736		59
T11725 × T11736		73
T11726 × T11734		70
T11727 × T11734		56
T11768 × T11806	4101	121, 131, 189
T11768 × T11769		124, 115, 138
T11768 × T11770		127, 117, 152
T12003 × T12004	5956	78, 87, 97, 78, 62, 68
T12002 × T12004		79, 49, 64, 80, 58, 56, 54

x is the distance between *cog* and the K480 mutant site.

Location of the position of the *his-3* K480 mutant site: The position of K480 was determined in a similar manner to those of K874, K26, and K1201 (Yeadon and Catcheside 1999), that is, by comparing the *his-3* sequence from F10292 with that of the cosmid G3:6F (Yeadon and Catcheside 1999) made from the His$^+$ strain ST74-OR23-IVA (Orbach 1994).

Recombination assays: Recombination frequency between *his-3* alleles was measured by the method of Angel *et al.* (1970). "Total A counts" (Tables 2 and 3) reflects the number of His$^+$ colonies generated by 75% of the spores from a cross. "Total C counts" (Tables 2 and 3) reflects the number of colonies, both *his-3* and His$^+$, generated by 0.056% of the spores from a cross.

Calculation of distance (*x* bp) between K480 and the initiator in each strain: Assuming that initiation occurs at the 10-bp sequence motif necessary for the high-frequency recombination phenotype of cog^L (Yeadon and Catcheside 1998), the initiation site is 3432 bp from the 3′ end of the *his-3* coding sequence in an untransformed cog^L strain. The *his-3* sequence is 2676 bp long in total (Yeadon and Catcheside 1999), so that with knowledge of the position of K480, the value of *x* for an untransformed strain can be calculated. For each modified strain, *x* was determined arithmetically.

Statistical analysis of recombination data: *y*, the $\log_{10}$ of each recombination frequency (His$^+$/10^5 viable spores; Table 2), was plotted against *x* (Figure 2). The line of best fit was plotted, together with the equation describing the line and the r^2 value. This process was repeated (Figure 3) using only values of *x* for which the crosses involve only transformed strains that have not been outcrossed (1704, 2454, 4104, and 6054). The equation for the exponential relationship was calculated and used to predict the frequency of interallelic recombination at a theoretical distance of $x = 0$ and to predict the distance from *cog* at which the hotspot can no longer influence recombination within *his-3*.

RESULTS

Positions of *his-3* mutant sites and distances between mutant pairs: The K874 mutant site is at position +1717, K1201 is at position +115, and the K26 mutant site is at position +1502 (Yeadon and Catcheside 1999). The K480 mutant site is an A → T transversion at +2605 in the *his-3* coding sequence, resulting in a replacement of a lysine with a stop codon at codon 848. Thus the distance between K26 and K480 is 1103 bp and the distance between K1201 and K874 is 1602 bp. K874 is therefore 888 bp farther from the initiation site than is K480, so in K1201/K874 crosses the distance *x* is 4392

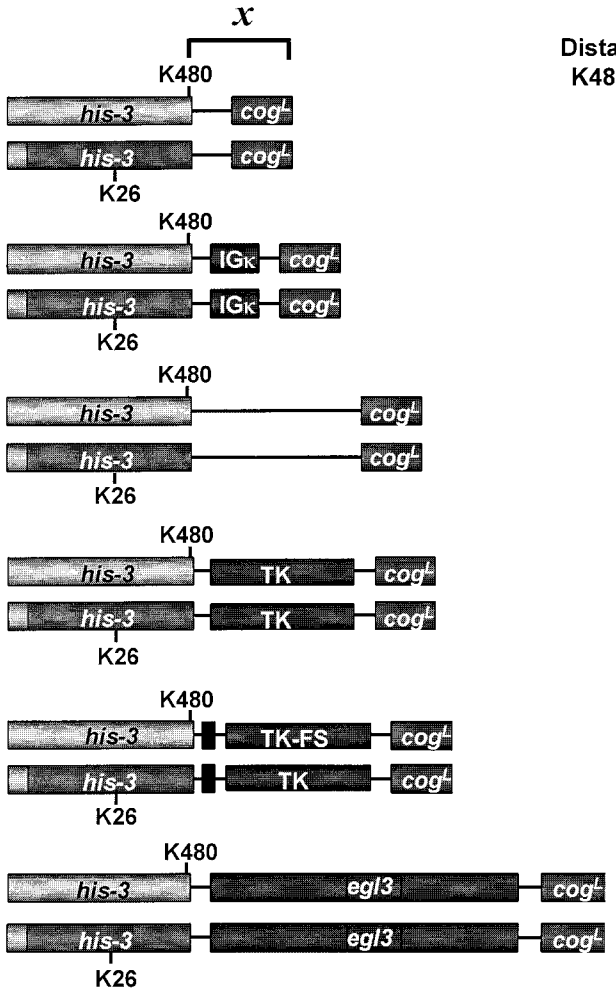

FIGURE 1.—Crosses in which we have altered the distance between K480 and *cog*. Light shading within *his-3* indicates the sequence of Emerson *a* origin while dark shading indicates that of Lindegren Y8743 origin. Unmodified strains are depicted in the third diploid from the top. The centromere is to the left. Not to scale.

bp, 888 bp greater than the 3504 bp present in untransformed K480 strains (Figure 1).

The relationship between recombination and distance from the initiator: Recombination frequencies (Table 2) increase exponentially with decrease in distance from *cog* ($r^2 = 0.67$; Figure 2). To obtain a better estimate of the effect that distance from *cog* has on recombination frequency in *his-3*, we considered which data sets might be influenced by factors other than this distance.

The untransformed strains ($x = 3504$ bp; Table 2) have different genetic backgrounds and it is likely that different alleles of genes with small effects on recombination (CATCHESIDE 1970) are present in each. In addition, T11681 and T11759, unlike all other strains used in this analysis, are cog^E (Table 1). Although the more active hotspot allele cog^L is ostensibly dominant to cog^E (ANGEL *et al.* 1970), the presence of cog^E in some of the crosses may contribute to the greater variability (Table 2 and Figure 2) in this data set ($x = 3504$ bp).

The TK^+ strains ($x = 3904$ bp; Table 2) are all progeny of T11644 (Table 1), transformed with pJY64 or pJY65, and crossed to either T11668 or T11670 (Table 1). Thus alleles of genes with small effects on recombination (CATCHESIDE 1970) are likely to be segregating in the TK^+ strains.

The data sets in which $x = 1704$, 4101, or 6054 bp (Table 2) are all from crosses between strains genetically identical to T11630 or T11644, except for the sequences within *his-3* and those inserted into the interval between *cog* and *his-3*. The data set in which $x = 2454$ bp (Table 2) is similar, except that T11631, of the same parentage as T11630 and T11644, is substituted for T11630. Thus, in these crosses very little is variable except the distance *x* between *cog* and *his-3*, which has been altered deliberately.

Data from crosses in which the only difference is our modification between *his-3* and *cog* ($x = 1704$, 2454, 4101, and 6054; Table 2) demonstrate clearly (Figure 3) that interallelic recombination increases exponentially as *x* decreases. The relationship is described by the equation $\log_{10} y = 2.9836–0.0002x$ [or $y = 963(0.99954)^x$] with $r^2 = 0.928$. Thus, when $x = 0$ (Figure 3) and K480 is adjacent to *cog*, $y = 963/10^5$.

In the presence of $rec\text{-}2^+$, which turns off initiation of recombination at cog^L (ANGEL *et al.* 1970), the frequency of His^+ progeny from K26/K480 crosses is $1.5/10^5$ viable spores (data not shown). When $y = 1.5$ (Figure 3) and no detectable influence of recombination is initiated at cog^L, $x = 14{,}038$ bp.

Extrapolating from our data (Figure 3), we conclude

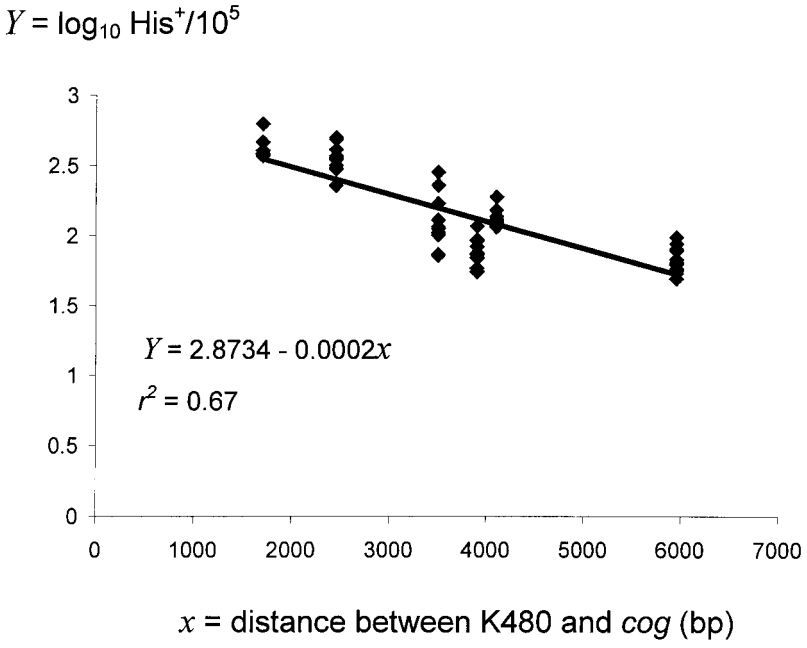

FIGURE 2.—The relationship between distance from *cog* and recombination frequency at *his-3* in all crosses. Recombination frequency is measured as histidine prototrophs per 10^5 viable spores. Columns of data points from left to right, respectively, correspond to the crosses depicted in Figure 1 from top to bottom.

that, if recombination were initiated adjacent to K480, the theoretical interallelic recombination frequency from a cross heteroallelic for *his-3* K480/K26 would be ~960 His^+ progeny in 100,000 viable spores. In addition, if cog^L were 14 kb away from *his-3*, the effect of the hotspot would no longer be detectable at *his-3*, suggesting that the frequency of events initiated at cog^L that are longer than 14 kb is negligible.

Estimation of minimum frequency of recombination initiation at cog^L: Because an initiation event in a single meiosis can result in two His^+ and six His^- spores and because as calculated above it is theoretically possible to obtain 0.96% His^+ progeny from a K26 × K480 cross, recombination must be initiated in at least 3.84% (4 × 0.96%) of meioses. This must be an underestimate, as it does not consider the chance that a recombination event may reach K480 without yielding a His^+ spore.

Crosses heteroallelic for K874/K1201 yield His^+ progeny at a frequency of ~550/10^5 viable spores (Table 3). The K874 mutant site is 888 bp farther from the initiation site than is K480, so for K874/K1201 heterozygotes, x = 4392 bp. Extrapolating from Figure 3 with an x

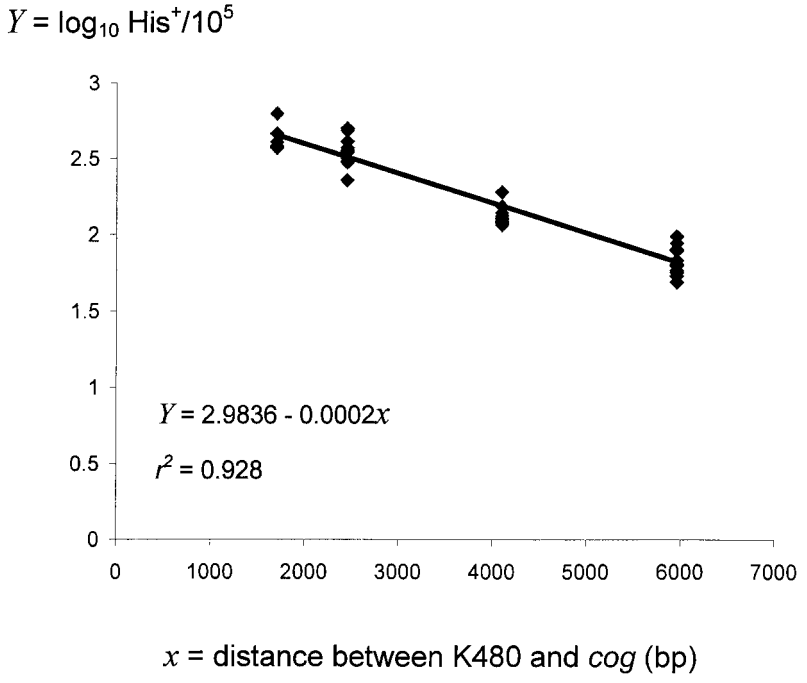

FIGURE 3.—The effect of distance from *cog* on recombination in *his-3* in diploids that differ only in the size of the *his-3*-to-*cog* interval. As in Figure 2, recombination frequency is measured as histidine prototrophs per 10^5 viable spores. However, in these crosses any influence of genes with small effects on recombination has been removed since diploids are genetically identical except for the modification between *his-3* and *cog*.

TABLE 3

Interallelic recombination in crosses heteroallelic for *his-3* K1201/K874 and homozygous for cog^L

Cross	$His^+/10^5$	SE
T11317 × T9149	541	41
T11317 × T6275	560	52

SE is the standard error of the recombination frequency.

value of 4392 bp, we predict 127 His^+ progeny per 10^5 viable spores. The recombination frequency in K874 × K1201 crosses (Table 3) is 4.3-fold higher than this estimate, probably reflecting the greater distance between K1201 and K874 (1602 bp, 599 bp more than the 1103 bp between K480 and K26). Since the yield of His^+ progeny from crosses heteroallelic for K26/K874, in which only 214 bp separate the mutant sites, is substantially reduced (Angel *et al.* 1970) and, in our system, the choice of the *his-3* allele is unlikely to influence initiation frequency, 3.84% must be an underestimate of the frequency of initiation of recombination in crosses homozygous for cog^L by at least a factor of 4.3. We therefore conclude that a more accurate estimate is that recombination is initiated at cog^L in ~17% (3.84% × 4.3) of meioses. Assuming that distances between mutant sites greater than that separating K1201 and K874 would yield still greater numbers of His^+ progeny, even this estimate is conservative.

DISCUSSION

The data presented here (Figure 3) show that recombination events initiated by cog^L are distributed exponentially with respect to distance travelled. Thus we have shown that the exponential relationship between the distance separating two sites and the chance of co-conversion described at the *rosy* locus of Drosophila (Hilliker *et al.* 1994) and at *ade6* of *S. pombe* (Grimm *et al.* 1994) extends to the relationship between the *his-3* heteroallelic recombination frequency and the distance from *cog* in Neurospora. For co-conversion, the probability that a conversion tract will be at least a particular length x (in nucleotides) is ϕ^x, where ϕ is the probability at each nucleotide that a tract will continue (Hilliker *et al.* 1994). The formula presented here, $y = 963(0.99954)^x$, which describes the relationship between interallelic recombination frequency at *his-3* and the distance from the recombinator, is of a similar nature.

The formula derived for Drosophila describes the chance of co-conversion of a nucleotide site at a distance x from a site already showing conversion. When x is zero and the sites are adjacent, the chance of co-conversion combines ϕ (a value closely approximating unity) and the chance of conversion rather than restoration (a probability also close to one, as adjacent sites are likely to be covered by the same mismatch repair tract; Detloff and Petes 1992). So, the chance of co-conversion of adjacent sites is approximately equivalent to ϕ. In contrast, the data presented here do not reflect co-conversion but rather the relationship between interallelic recombination frequency at *his-3* and distance from the recombinator. Thus, in addition to the probability that a conversion tract will continue (ϕ^x), we must also consider the probability of initiation per spore (i) and the chance (k) that a tract, after passing K480, will result in a His^+ spore, resulting in an equation of the nature $y = k.i.(\phi^x)$, exactly the type of equation found to be a good fit for the data presented here. We suggest that both ϕ and the probability of initiation are constant at this locus in crosses homozygous for cog^L, but that k is variable and depends on the nature of each of the mutant sites and on the number of nucleotides separating the sites.

As yet, we cannot accurately estimate ϕ, as the effects of k and i cannot be separated from that of ϕ in this data set. However, co-conversion data collected previously during mapping of conversion tracts at *his-3* (Yeadon and Catcheside 1998) suggest that ϕ for this locus of Neurospora is greater than the 0.99717 estimated for the *rosy* locus of Drosophila (Hilliker *et al.* 1994). Of tracts in which *his-3* K26 was converted, only 2 of 15 extended less than another 270 bp, 11 extended more than 270 and less than 939 bp, and an additional 2 extended between 939 and 1387 bp past K26 (Yeadon and Catcheside 1998). If ϕ were as low as 0.99717, only 46% of the tracts should have extended >270 bp past K26, significantly less than the number observed ($\chi^2 = 9.8$; $P < 0.01$). Substituting 0.99954 (from the equation for y, shown above) for the Drosophila value of ϕ leads to the prediction that 87% of tracts would extend >270 bp past K26 and, in fact, 13 of 15 (87%) did so, suggesting that ϕ for this locus of Neurospora may be of this order.

However, when cog^L is >14 kb from *his-3*, the frequency of His^+ recombinants is predicted to be similar to that obtained when recombination is turned off by $rec\text{-}2^+$, suggesting that few events travel farther than 14 kb. The exponential relationship between distance from cog^L and the frequency of His^+ recombinants obtained from a cross indicates that most events will travel only a relatively short distance. Also, in crosses homozygous for cog^L, we estimate that recombination is initiated by cog^L in at least 17% of meioses although, since this estimate does not consider the chance that a conversion event may pass a mutant site without yielding a His^+ spore, the actual frequency of initiation is likely to be much greater.

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