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DOI: 10.1534/genetics.103.025080

Alleles of the Hotspot *cog* Are Codominant in Effect on Recombination in the *his-3* Region of Neurospora

P. Jane Yeadon, F. J. Bowring and D. E. A. Catcheside[1]

School of Biological Sciences, Flinders University, Adelaide, South Australia, 5001 Australia

Manuscript received November 26, 2003
Accepted for publication March 19, 2004

ABSTRACT

There are two naturally occurring functional alleles of the recombination hotspot *cog*, which is located 3.5 kb from the *his-3* locus of *Neurospora crassa*. The presence of the cog^+ allele in a cross significantly increases recombination in the *his-3* region compared to a cross homozygous for the *cog* allele. Data obtained shortly after discovery of cog^+ suggested that it was fully dominant to *cog*. However, a dominant cog^+ conflicts with observations of hotspots in *Saccharomyces cerevisiae* and *Schizosaccharomyces pombe*, in which recombination is initiated independently of homolog interactions, and suggests recombination mechanisms may differ in Neurospora and yeast. We present evidence that *cog* alleles are codominant in effect on both allelic recombination in *his-3* and crossing over between loci flanking *his-3*. In addition, we show that genetic background variation has at least a twofold effect on allelic recombination. We speculate that variation in genetic background, together with the complexities of recombination in crosses bearing close mutant alleles, accounts for the previous conclusion that cog^+ is dominant to *cog*.

MEIOTIC recombination is the process that shuffles genetic information during sexual reproduction. In conjunction with segregation of homologous chromosomes, recombination generates progeny with gene combinations that differ from those of either parent, thus increasing the variation upon which selection can act. Outcomes of recombination include crossing over (Morgan and Cattell 1912), an apparent breakage and rejoining of chromosomes in which the copy number of alleles is unchanged, and gene conversion (Lindegren 1953), where one parental allele increases in number at the expense of the other (Mitchell 1955; Fogel and Hurst 1967). Conversion and crossover events can be distinguished with certainty only by analysis of all eight meiotic products, as is possible in four- and eight-spored fungi in which the products of each meiosis are held within a single ascus. However, it has been shown that prototrophic progeny from heteroallelic crosses are usually generated by gene conversion events and that most exchanges between distant genetic markers result from crossovers (Mitchell 1955; Stadler 1959; Murray 1960; P. J. Yeadon, F. J. Bowring, D. R. Stadler and D. E. A. Catcheside, unpublished results).

Crossovers are not randomly distributed along chromosomes but tend to be clustered (Lichten and Goldman 1995; Baudat and Nicolas 1997; Jeffreys *et al.* 1998) in regions termed hotspots (Holliday 1968). Tetrad analyses indicate that crossovers are more likely close to a locus that has experienced conversion (Olive 1959; Stadler 1959; Murray 1960; Lissouba *et al.* 1962). In addition, evidence of gene conversion has been found close to hotspots for crossing over (Borts and Haber 1989; Jeffreys *et al.* 2001; Guillon and de Massy 2002; Jeffreys and Neumann 2002). The association between conversion and crossing over suggests that the same mechanism is responsible for both outcomes and this has been a central feature of recombination models (Szostak *et al.* 1983; Sun *et al.* 1991; Nassif *et al.* 1994; Pâques and Haber 1999). Recent studies suggest that crossover and noncrossover products arise from different pathways and that the decision between the two outcomes is made after initiation but before production of a recombination intermediate (Allers and Lichten 2001; Hunter and Kleckner 2001). However, both conversion and crossing over are thought to be initiated by the same mechanism.

In *Saccharomyces cerevisiae*, initiation of recombination is by a double-strand break (DSB) in one homolog, probably generated by the Spo11 protein (Keeney *et al.* 1997). *SPO11* homologs have been found in all eukaryotes in which they have been sought, including flies (McKim and Hayashi-Hagihara 1998), worms (Dernburg *et al.* 1998), mammals (Romanienko and Camerini-Otero 1999), plants (Grelon *et al.* 2001), and the filamentous ascomycete *Neurospora crassa* (F. J. Bowring, P. J Yeadon, R. J. Stainer and D. E. A. Catcheside, unpublished results), suggesting conservation of the initiation mechanism.

The Neurospora recombination hotspot *cog*, located centromere-distal of the *his-3* locus (Bowring and Catcheside 1991; Yeadon and Catcheside 1995a, 1998), influ-

[1]*Corresponding author:* School of Biological Sciences, Flinders University, Box 2100, Adelaide, South Australia, 5001 Australia.
E-mail: david.catcheside@flinders.edu.au

ences allelic recombination within *his-3* and crossing over in the chromosomal segments surrounding the gene (Angel *et al.* 1970). Two *cog* phenotypes, high (cog^+) and low (*cog*) frequency recombination, have been described (Angel *et al.* 1970), with the chromosome that bears cog^+ almost exclusively experiencing conversion (Catcheside and Angel 1974; Yeadon and Catcheside 1998). Although there are multiple differences between the *cog* region (Yeadon and Catcheside 1995a) sequences of cog^{Ea}, cog^{LA}, and cog^{EA}, all of which are *cog*, and cog^{La}, the only naturally occurring cog^+ allele known (Yeadon and Catcheside 1995b, 1999), a 10-bp sequence including two single-nucleotide polymorphisms (SNPs) is required for the high-frequency recombination phenotype (Yeadon and Catcheside 1998). Recombination is known to be initiated >2.2 kb from the 3′ end of *his-3* (Yeadon *et al.* 2001) and a peak in conversion close to these SNPs (Yeadon and Catcheside 1998) suggests that initiation may occur at this location, which is ~3.4 kb from *his-3*.

The presence of cog^+ increases allelic recombination frequency ~6-fold and crossovers between *his-3* and the centromere-distal gene, *ad-3*, ~4-fold when compared to similar crosses in which *cog* is homozygous (Catcheside and Angel 1974). The *trans*-acting *rec-2* gene imposes an additional level of regulation of recombination in this region of LG I as the dominant allele, *rec-2*$^+$, has an epistatic effect (Smith 1968; Catcheside 1979). In the presence of *rec-2*$^+$, recombination between *his-3* alleles is reduced 30-fold in crosses containing cog^+ and 4-fold in crosses of homozygous *cog* to the same low level (Angel *et al.* 1970). In addition, recombination events that occur in the presence of *rec-2*$^+$ appear to be initiated at the 5′ end of *his-3* and not at *cog* (Catcheside and Angel 1974; Yeadon and Catcheside 1998). It seems likely that the allelic recombination frequency attributable to each *cog* allele reflects the frequency with which recombination is initiated there and that the *rec-2*$^+$ product prevents initiation at either *cog* allele (Catcheside and Angel 1974).

Study of haploid meiosis indicates that the timing, frequency, and distribution of DSBs are independent of interhomolog interaction in *S. cerevisiae* (De Massy *et al.* 1994; Gilbertson and Stahl 1994) and in *Schizosaccharomyces pombe* (Young *et al.* 2002), suggesting that recombination is initiated independently at each allele of a particular hotspot. If recombination is initiated by a DSB at *cog* in Neurospora and each initiation is independent of initiation at the other *cog* allele, the sixfold increase in allelic recombination in crosses heterozygous for cog^+/*cog* (Catcheside and Angel 1974) implies that DSBs occur 11 times more frequently at cog^+ than at *cog* and predicts that a heteroallelic cross homozygous for cog^+ would yield close to twice as many recombinants as one in which cog^+ is heterozygous. However, Angel *et al.* (1970) found that, in crosses heteroallelic for *his-3* K26/K874, there was little difference in the allelic recombination frequency between cog^+ homo- and heterozygotes, leading to the conclusion that cog^+ is fully dominant to *cog*. Although *rec-2*$^+$ reduces allelic recombination in *cog*/*cog* diploids fourfold, there is no apparent decrease in crossovers between *his-3* and *ad-3* in the same diploids, even though Catcheside and Angel (1974) estimated that it should have been detectable.

In yeasts, no naturally polymorphic recombination hotspots have yet been found. The *ade6*-M26 mutation in *S. pombe* increases conversion in *ade6* 10- to 15-fold when compared to the closely linked *ade6*-M375 mutation (Gutz 1971; Fox and Smith 1998). During recombination M26 is preferentially converted to wild type (Gutz 1971), so the M26 chromosome, like that carrying cog^+, is usually the recipient of information. The use of an opal suppressor mutation, *sup9*, allowed measurement of meiotic intragenic recombination with M26 heterozygous or homozygous (Ponticelli *et al.* 1988). Ponticelli *et al.* (1988) concluded that crosses homozygous for M26 yielded 10 times more recombinants than those lacking M26. Moreover, recombination frequency in M26 homozygotes was approximately the sum of the two heterozygous frequencies. However, since *ade6*-M26 *sup9* spores form colonies only 50% as efficiently as *ade6*$^+$ *sup9* spores, Ponticelli *et al.* (1988) doubled the numbers of recombinants in the homozygous assay to reach this conclusion, so codominance of M26 and wild-type *ade6* hotspot alleles is far from certain.

Like cog^+, M26 increases crossing over nearby. The substitution of M26 for M375 results in a 2.5-fold increase in intrachromosomal crossing over, from 0.3 to 0.8% (Schuchert and Kohli 1988). In contrast, the *ura4-aim*–*tps16* genetic interval flanking *ade6* is not strongly affected by the presence of M26. The genetic distance increases to 12.5 cM, compared to the 11.8 cM measured in the absence of M26 (Zahn-Zabal *et al.* 1995). Zahn-Zabal *et al.* (1995) found that M26 convertants experience exchange between *ura4-aim* and *tps16* at the same frequency as M375 convertants and concluded that the slight increase in crossing over is due to the higher frequency of conversion at M26 and the resultant increase in conversion-associated crossovers.

With the exception of *ade6*-M26, all other artificial hotspot polymorphisms have been generated by deletion of part of the promoter region of the gene in which recombination was studied. Deletion of the *ade6* promoter removes the hotspot activity of M26 only when the deletion is *in cis* to M26, with no effect of the deletion *in trans* (Zahn-Zabal *et al.* 1995). Strangely, the conversion frequency in *ade6* in the absence of M26 is unaffected by the same deletion, whether *in cis* or *in trans* to M375.

In *S. cerevisiae*, homozygous deletion of a poly(dA·dT) tract in the promoter region of *ARG4* (Δ9) reduced conversion of the *arg4-RV* mutation to 0.8% from the wild-type level of 7.4% (Nicolas *et al.* 1989). Unlike the *ade6* promoter deletions in *S. pombe* (Zahn-Zabal *et al.*

1995), diploids heterozygous for Δ9 yielded a similarly low frequency of *arg4-RV* conversion of ~1% (6 tetrads of 562; Nicolas *et al.* 1989). To our knowledge, there appear to be no data on the effect of *ARG4* Δ9 on crossing over.

Regions in which the crossover rate is elevated also exist in the human genome (Janson *et al.* 1991; Oudet *et al.* 1992; Hubert *et al.* 1994). Recombination hotspots have been identified at several human loci (Chakravarti *et al.* 1984; Oudet *et al.* 1992; Yip *et al.* 1999), including within the human major histocompatibility complex class II region (Cullen *et al.* 1995). At one of the six hotspots in this region (Jeffreys *et al.* 2001), *DNA2*, the FG11G/A polymorphism, appears to alter the crossover frequency in sperm (Jeffreys and Neumann 2002). Haplotype-specific PCR primers were used to amplify recombinant DNA molecules from sperm taken from men heterozygous for various SNPs within the 5.5-kb amplification region. Two FG11G/G homozygotes yielded recombinant molecule frequencies of 0.1×10^{-5} and 0.7×10^{-5} while three of five A/G heterozygotes gave frequencies of between 2×10^{-5} and 3×10^{-5} (with the most extreme values in the other two men at 0.9×10^{-5} and 10×10^{-5}), suggesting that FG11A significantly increases the activity of the *DNA2* hotspot (Jeffreys and Neumann 2002). In addition, in recombinant molecules from FG11A/G heterozygotes, the FG11G SNP is overrepresented, suggesting that, as in other hotspot allele heterozygotes (Gutz 1971; Catcheside and Angel 1974; Nicolas *et al.* 1989), the FG11A strand is usually the recipient of information (Jeffreys and Neumann 2002). Since the crossover frequency (2.6×10^{-5}) in the single A/A homozygote falls in the middle of the range for A/G heterozygotes, one might conclude that the FG11A hotspot allele is fully dominant to the FG11G allele. However, the wide range of frequencies in the heterozygotes shows that factors other than the FG11 SNP have a large effect on crossing over at *DNA2*, making conclusions drawn from a single homozygote highly unreliable.

In recent years, we have constructed Neurospora strains that carry cog^+ *in cis* to *his-3* mutant alleles K480, K504, and K1201, allowing analysis of additional allele pairs, which are farther apart than the 215 bp separating K26 and K874 (Yeadon and Catcheside 1999). K26/K874 diploids yield a low frequency of His^+ progeny compared to those with more distant pairs of mutant sites (Angel *et al.* 1970), increasing the chance that measured recombination frequencies will be confounded by random variation. Higher recombination frequencies yielded by distant allele pairs reduce the impact of random factors and are likely to aid differentiation between His^+ frequencies from cog^+ hetero- and homozygotes. We have also constructed both cog^+ and *cog* stocks that are mutant in the centromere-proximal gene *lys-4*, and cog^+, *cog*, and $rec\text{-}2^+$ strains that are mutant in the centromere-distal gene *ad-3*, allowing measurement of the effect of all possible *cog* and *rec-2* genotypes on exchange in the *his-3* region.

MATERIALS AND METHODS

Construction of strains: The genotypes of all strains used in this study are listed in Table 1. The *his-3* mutation K26 was generated in a Lindegren Y8743 strain (Angel *et al.* 1970) and thus all nonrecombinant K26 strains have the high-frequency (cog^+) recombinator allele, cog^{La}. K480, K874, and K1201 mutations were generated in Emerson *a* strains (Angel *et al.* 1970) and so all nonrecombinant strains bearing these alleles have the low-frequency (*cog*) recombinator allele, cog^{Ea}. The *cog* allele found in St. Lawrence 79*a*, cog^{S79a} (Yeadon and Catcheside, 1995a), has the same sequence as cog^{La} and is also cog^+ (Yeadon and Catcheside 1995b). Lindegren *A* carries an allele of *cog* with a sequence different from that of cog^{La} and cog^{Ea} (Yeadon and Catcheside, 1995b, 1999), but appears to have a low-frequency recombination phenotype identical to that of cog^{Ea}.

Recombinant cog^+ K874 strains T4395, T11110, T11113 (Yeadon and Catcheside 1995a), T11125, T11126, T11132, and T6275 all have *his-3* from Emerson *a* and cog^{La}, each generated by a crossover in the *his-3* to *cog* interval. The K480 cog^+ strains, T11760, T11761, T11762, T11763, and T11764 and the K1201 cog^+ strain T11281 were constructed in the same way. Similarly, T11153 is *his-3* K874 cog^+ and has *his-3* from Emerson *a* and cog^{S79a} (Yeadon and Catcheside 1995a). K26 *cog* strains T11284 and T11318 have *his-3* from Lindegren Y8743 and cog^{Ea}. T11805 (*his-3* K1201 cog^+) has a mosaic version of *cog*, phenotypically cog^+, from T11257 (Yeadon and Catcheside 1998).

T11317, T12010, T12011, T12012, and T12013 are descendants of T11281 and are therefore all K1201 cog^+. Likewise, T10997, T10998, and T9149 are descendants of T6275 and are K874 cog^+. T10997 carries the dominant $rec\text{-}2^+$, so crosses to this strain have substantially reduced recombination in the *his-3* region (Smith 1968; Angel *et al.* 1970). T12078–T12081 are His^+ progeny of T11805 and T10998. T11997, a K1201 descendant of T11317 and phenotypically *cog*, was generated by an unselected conversion event within *cog*, which was detected by sequencing. T11311 and T11313 (*his-3* K874 *cog*) each have *his-3* from Emerson *a* and cog^{LA}. F3300, from the collection of D. G. Catcheside, is $rec\text{-}2^+$ and supposedly cog^+, although SNP analysis suggests it is *cog*.

T11039, T11041, and T11043 [Fungal Genetics Stock Center (FGSC) nos. 6526, 6077, and 6098, respectively] were generated in St. Lawrence 74*A* (Overton *et al.* 1989) as was *cog* (Yeadon and Catcheside 1995a). T11058 and T11059 are progeny of T11039, T11061, and T11062, and T11063 of T11041, T11065, and T11066 from FGSC no. 6085 (*A, his-3* 1-226-0503, cog^{Ea} $rec\text{-}2^+$), also generated in St. Lawrence 74*A* (Overton *et al.* 1989), and T11067 of T11043. Thus, these strains carry a variety of mutant *his-3* alleles, but are all *cog*.

T11782 and T11789 were made by replacement transfection of the *his-3* K458 recipient strains T11644 and T11630 (Yeadon *et al.* 2001), respectively, with a PCR product including *his-3* K26.

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

Recombination assays: Ascospores were harvested from a

TABLE 1

Neurospora stocks

Stock no.	Genotype
T4395, T11110/13/25/26/32/53	*A his-3* K874 cog^{La}; *rec-2*
T10998	*A his-3* K874 cog^{La}; *ad-3; rec-2*
T6275	*a his-3* K874 cog^{La}; *rec-2*
T9149	*a his-3* K874 cog^{La}; *ad-3; am rec-2*
T10997	*A his-3* K874 cog^{La}; *ad-3; am* $rec\text{-}2^{+}$
T4396, T11092, T11127	*A his-3* K874 cog^{Ea}; *rec-2*
T11093/099/104/105/117	*A his-3* K874 cog^{Ea}; *am rec-2*
T11089	*A his-3* K874 cog^{Ea}; *ad-3; am rec-2*
T11311, T11313	*a his-3* K874 cog^{LA}; *am rec-2*
T11045	*a his-3* K874 cog^{Ea}; *ad-3; am rec-2*
T11281	*A his-3* K1201 cog^{La}; *ad-3; am rec-2*
T11317	*A his-3* K1201 cog^{La}; *am rec-2*
T11805	*a lys-4 his-3* K1201 cog^{La}; *am rec-2*
T12010/11/12/13	*a his-3* K1201 cog^{La}; *rec-2*
T9144	*A his-3* K1201 cog^{Ea}; *am rec-2*
T9194, T11997	*a his-3* K1201 cog^{Ea}; *am rec-2*
T11801, T11802	*a lys-4 his-3* K1201 cog^{Ea}; *am rec-2*
T10988	*A arg-1 his-3* K26 cog^{La}; *am rec-2*
T10989	*a arg-1 his-3* K26 cog^{La}; *am rec-2*
T11782	*A his-3* K26 cog^{La}; *rec-2*
T11789	*a his-3* K26 cog^{La}; *rec-2*
T11286	*A arg-1 his-3* K26 cog^{La}; *ad-3; am rec-2*
T11284	*A arg-1 his-3* K26 cog^{Ea}; *ad-3; am rec-2*
T11318	*a his-3* K26, cog^{Ea}; *am rec-2*
F3300	*A arg-1 his-3* K26 cog^{Ea}; $rec\text{-}2^{+}$
T11760/764	*a his-3* K480 cog^{La}; *am rec-2*
T11681	*a lys-4 his-3* K480 cog^{Ea} *ad-3*; *am rec-2*
T12078/79	*a lys-4* cog^{La}; *ad-3; am rec-2*
T12080/81	*A lys-4* cog^{La}; *ad-3; am rec-2*
T11081	*A* cog^{La} *ad-3; am rec-2*
T4400	*a* cog^{Ea} *ad-3; rec-2*
T11039 (FGSC no. 6526)	*A his-3* 1-306-0218 cog^{Ea}; $rec\text{-}2^{+}$
T11041 (FGSC no. 6077)	*A his-3* 1-226-0408 cog^{Ea}; $rec\text{-}2^{+}$
T11043 (FGSC no. 6098)	*A his-3* 1-234-0567 cog^{Ea}; $rec\text{-}2^{+}$
T11051	*a his-3* 1-306-0127 cog^{Ea}; $rec\text{-}2^{+}$
T11057	*a his-3* 1-306-0127 cog^{Ea}; *am rec-2*
T11058	*a his-3* 1-306-0218 cog^{Ea}; *am rec-2*
T11059	*A his-3* 1-306-0218 cog^{Ea}; *am rec-2*
T11061/62/63	*A his-3* 1-226-0408 cog^{Ea}; *am rec-2*
T11065/66	*A his-3* 1-226-0503 cog^{Ea}; *am rec-2*
T11067	*A his-3* 1-234-0567 cog^{Ea}; *am rec-2*

The stock numbers for strains with the same genotype may be abbreviated. For example, T11110/13 indicates that T11110 and T11113 have the same genotype. The *am* allele is K314, *arg-1* is K166, *lys-4* is STL4, and *ad-3* is K118. All strains except T11039, T11041, T11043, and T11058 include the colonial temperature-sensitive mutation *cot-1* C102t. In the absence of $rec\text{-}2^{+}$, cog^{La} has the high-frequency recombination phenotype cog^{+}; both cog^{Ea} and cog^{LA} have the low-frequency recombination phenotype *cog*.

single crossing tube in distilled water. After estimation of the number of spores by hemocytometer, an appropriate volume was added to 20-ml layer agar (0.8% Difco agar, 2% sucrose, 2% Vogel's N medium) kept at 60°. Following serial dilution in layer agar and incubation at 60° for 45–70 min, 3-ml aliquots of the highest and lowest dilutions were plated onto selective and nonselective medium, respectively. Plates were incubated overnight at 20° and then moved to 34° for 24–48 hr to express *cot-1* and to restrict colony size. The dilution factor between selective and nonselective plates varied from 1/1600 to 1/100 for allelic recombination assays and from 1/10 to 1/100 for intergenic assays, depending on the recombination frequency.

Statistical analysis of recombination data: For *his-3* K1201/K874 heterozygotes (Figure 1), *his-3* K26/K874 heterozygotes (Figure 6), *lys-4 ad-3* (Figure 4), and *his-3 ad-3 trans*-heterozygotes (Figure 5), data from crosses homozygous for cog^{+} were compared to those from crosses heterozygous for cog^{+}/cog. The significance of any difference between frequency distributions was determined by a two-tailed *t*-test. Since the data are expressed as frequencies, each frequency was transformed ($P \rightarrow \sin^{-1}\sqrt{P}$) before comparison. Each comparison of frequency distributions was also subjected to an *f*-test to assess the level of variance in each distribution and thus to determine whether to perform a *t*-test for equal or unequal variances.

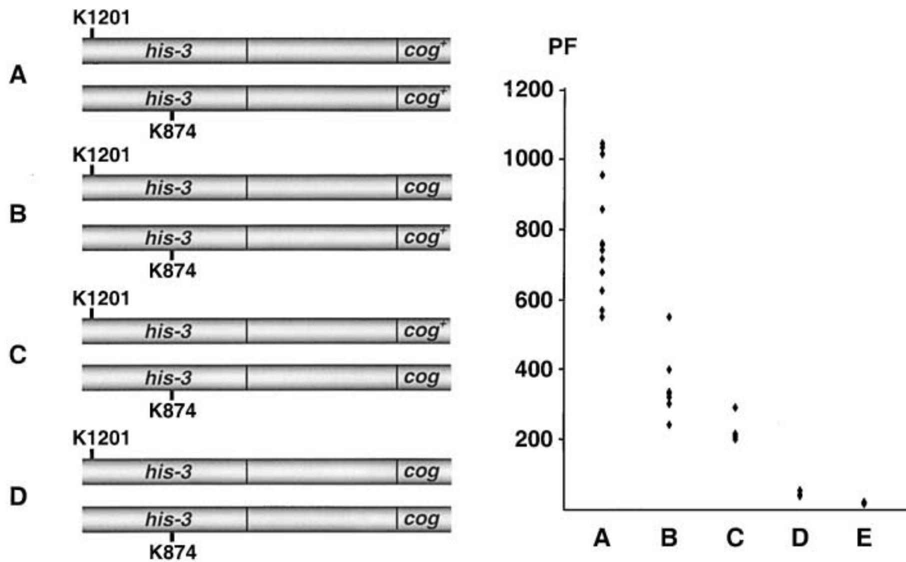


Figure 1.—Allelic recombination in diploids heteroallelic for K1201/K874. The centromere is at the left of the figure. The mutant sites are separated by 1605 bp (Yeadon and Catcheside 1999). PF is the frequency of His$^+$ progeny yielded by each cross, multiplied by 10^5. His$^+$ frequencies were obtained from *cog*$^+$ homozygotes (A: T12010 × T4395, T12011 × T4395, T12012 × T4395, T12013 × T4395, T12010 × T11126, T12010 × T11153, T12011 × T11113, T12011 × T11126, T12011 × T11132, T12011 × T11153, T11805 × T4395, T11805 × T10998, T11317 × T6275, and T11317 × T9149), *cog/cog*$^+$ (B: T11997 × T4395, T11997 × T11110, T11997 × T11113, T11997 × T11125, T11997 × T11132, T11997 × T11153, and T11801 × T10998), *cog*$^+$/*cog* heterozygotes (C: T11805 × T11089, T11317 × T11045, T11281 × T11311, and T11281 × T11313), *cog* homozygotes (D: T11801 × T11089, T9144 × T11311, T9144 × T11313, and T9144 × T11045), and a *rec-2*$^+$ heterozygote (E: T11805 × T10997).

To determine heterogeneity of recombination frequencies from repeats of a single cross or within a single genotype, a χ^2 test was used to assess the probability that the colony counts could differ by chance. Where repeat crosses gave homogeneous counts and the data set was large (Figures 1 and 2), repeat counts were combined to give a single recombination frequency.

Determination of flanking markers in His$^+$ progeny: Approximately 100 His$^+$ progeny were extracted from *his-3* K1201/K874 heterozygotes that were also heterozygous for *lys-4* and *ad-3* (Figure 4), homozygous for *cog*$^+$ (type A, Figure 1), heterozygous for *cog*$^+$/*cog* (Figure 1, type B), homozygous for *cog* (Figure 1, type D) and heterozygous *rec-2*/*rec-2*$^+$ (type E, Figure 1). Each strain was tested for a requirement for lysine or adenosine.

RESULTS

Allelic recombination frequency is elevated in *cog*$^+$ homozygotes: While it was thought that *cog*$^+$ is dominant to *cog* (Catcheside and Angel 1974), we find that the His$^+$ frequency is higher when *cog*$^+$ is homozygous than when it is heterozygous (Figures 1–3), with the exception of crosses heteroallelic for K26/K874 (Figure 6), discussed below. For crosses heteroallelic for K1201/K874 (Figure 1), the His$^+$ frequency from crosses homozygous for *cog*$^+$ (Figure 1A; $786/10^5$ viable spores) is greater ($P = 4 \times 10^{-9}$) than that ($343/10^5$) when *cog*$^+$ and K874 are *in cis* (Figure 1B). When *cog*$^+$ and K1201 are *in cis* (Figure 1C), the recombination frequency is somewhat lower ($218/10^5$), but still higher than that from crosses homozygous for *cog* ($32/10^5$; Figure 1D). Similarly, for diploids heteroallelic for K874/K480, the recombination frequencies are $257/10^5$, $171/10^5$, $160/10^5$, and $16/10^5$ (Figure 2, A–D, respectively). For diploids heteroallelic for K1201/K26, the recombination frequencies are $253/10^5$, $140/10^5$, $112/10^5$, and $41/10^5$ (Figure 3, A–D, respectively). Therefore, it appears that *cog*$^+$ is not dominant to *cog* but that the hotspot alleles operate codominantly to influence the frequency of allelic recombination at *his-3*.

Crossing over is elevated in *cog*$^+$ homozygotes: *cog*$^+$ copy number also has a substantial effect on crossing over in chromosomal intervals flanking *his-3*. Crosses homozygous for *cog*$^+$ yield on average 10.8% Lys$^+$ Ad$^+$ (Figure 4A) and 7.5% His$^+$ Ad$^+$ (Figure 5A) progeny, which is double the frequencies of 4.7% Lys$^+$ Ad$^+$ (Figure 4B) and 3.7% His$^+$ Ad$^+$ (Figure 5B) from crosses heterozygous for *cog*$^+$. The increase is significant, with $P = 3 \times 10^{-4}$ (Figure 4) and $P = 6 \times 10^{-5}$ (Figure 5). We thus conclude that *cog*$^+$ and *cog* operate codominantly to influence the frequency of exchange in the *his-3* region.

Heterogeneity of assay data and the effect of genetic background on recombination: Repeat assays of the same cross almost invariably yield consistent recombination frequencies. For example, three assays of T11997 × T11110 gave His$^+$ frequencies of $327/10^5$, $330/10^5$, and $346/10^5$ (χ^2 heterogeneity test gives $P = 0.88$). Four repeat Lys$^+$ Ad$^+$ assays of T11805 × T10998 also gave homogeneous crossover frequencies ($P = 0.07$), and similar results were obtained for 13 other repeat assays (P values range from 0.02 to 0.77). These data show that technical variations, sampling error, and other random factors have little effect on the recombination frequencies measured in this study. (Recombination assay data are available in Tables A1 and A2 in an electronic appendix at http://www.genetics.org/supplemental/.)

In contrast, recombination frequencies from crosses of strains with the same *cog*, *his-3*, and *rec-2* alleles can be variable. Since recombination frequencies do not vary between repeats of the same cross, other factors must be affecting recombination in these crosses. For crosses of the *his-3* K1201 *cog* strain T11997 to T11110, T11125,

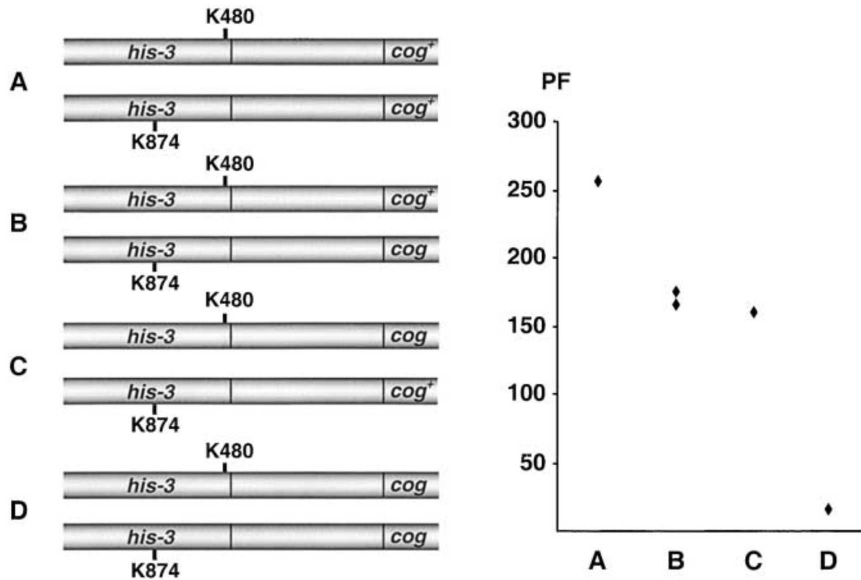


Figure 2.—Allelic recombination in diploids heteroallelic for K874/K480. The centromere is to the left of the figure. The mutant sites are separated by 888 bp (Yeadon and Catcheside 1999; Yeadon *et al.* 2002). PF is the frequency of His$^+$ progeny yielded by each cross, multiplied by 10^5. His$^+$ frequencies were obtained from a *cog*$^+$ homozygote (A: T11125 × T11760), two *cog*/*cog*$^+$ heterozygotes (B: T4396 × T11760 and T4396 × T11764), a *cog*$^+$/*cog* heterozygote (C: T11125 × T11681), and a *cog* homozygote (D: T4396 × T11681).

and T11132 (Figure 1C), three *his-3* K874 *cog*$^+$ strains extracted from a single cross, recombination frequencies are homogeneous (χ^2 heterogeneity test gives P = 0.32). A similar result is obtained for crosses of T10989 (*his-3* K26 *cog*$^+$) to the same three strains (Figure 6A; P = 0.10). However, if we include crosses of T11997 and T10989 to T11113 (Figures 1B and 6A, respectively), which has the same parents as T11110, T11125, and T11132, in each case the data become less homogeneous (P = 0.0002 and 0.02, respectively). When T10989 is crossed to T11092, T11093, T11099, T11104, T11105, T11117, and T11127 (Figure 6C), which are *his-3* K874 *cog* strains with the same parents as T11110, T11113, T11125, and T11132, the recombination frequencies are substantially heterogeneous ($P = 1.6 \times 10^{-8}$). In addition, crosses of the *his-3* K1201 *cog*$^+$ strain T12011 to *his-3* K874 *cog*$^+$ strains T11113, T11126, T11132, and T11153 (Figure 1A) yield homogeneous recombination frequencies (P = 0.22), but data from T12011 × T4395 (a *his-3* K874 *cog*$^+$ strain made >40 years ago; Angel *et al.* 1970; Figure 1A) are substantially heterogeneous ($P = 1 \times 10^{-17}$). These data suggest the existence of more than one gene, each with a small effect on recombination, and that the parents of the K1201 and K874 strains described above carried different alleles of these genes.

Crossover frequency also varies within crosses of a single known genotype (Figures 4 and 5). T12078, T12079, T12080, and T12081 are *his-3*$^+$ *cog*$^+$ *ad-3* progeny of a cross between T11805 and T10998. The frequency of His$^+$ Ad$^+$ progeny from crosses of these strains to T11782 and T11789 (*his-3* K26 *cog*$^+$) falls into two distinct groups (χ^2 yields $P = 4 \times 10^{-9}$), with crosses to T12078 and T12080 yielding frequencies of 8.8% (P = 0.99) and those to T12079 and T12081 yielding lower frequencies of 6.6 and 6.0%, respectively (P = 0.29; Figure 5). A likely explanation is that alleles of a gene that affects the frequency of crossing over in the *his-3* region are segregating in the progeny of T11805 and T10998.

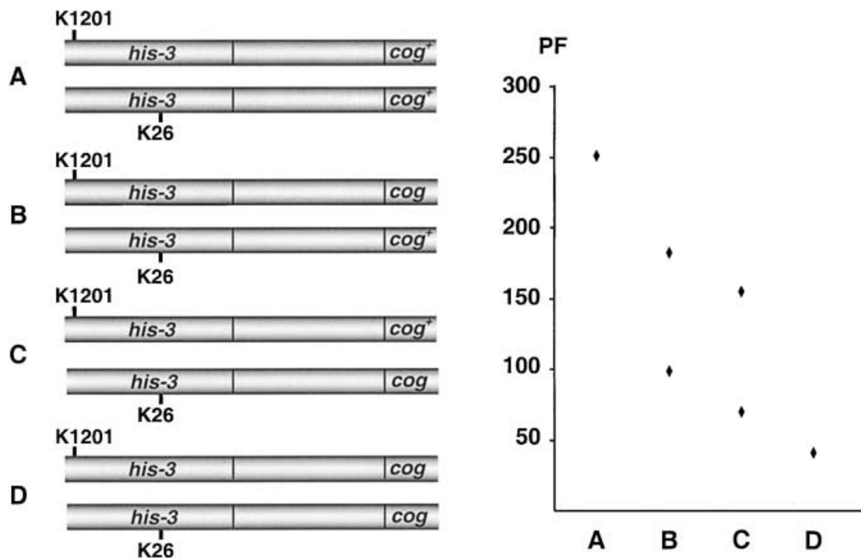
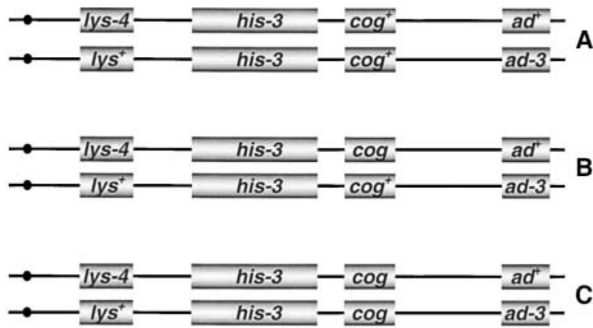


Figure 3.—Allelic recombination in diploids heteroallelic for K1201/K26. The centromere is to the left of the figure. The mutant sites are separated by 1390 bp (Yeadon and Catcheside 1999). PF is the frequency of His$^+$ progeny yielded by each cross, multiplied by 10^5. His$^+$ frequencies were obtained from *cog*$^+$ homozygotes (A: T11317 × T10989 and T11281 × T10989), *cog*/*cog*$^+$ (B: T9194 × T10988 and T9194 × T11286), *cog*$^+$/*cog* heterozygotes (C: T11317 × T11318 and T11281 × T11318), and a *cog* homozygote (D: T9194 × T11284).



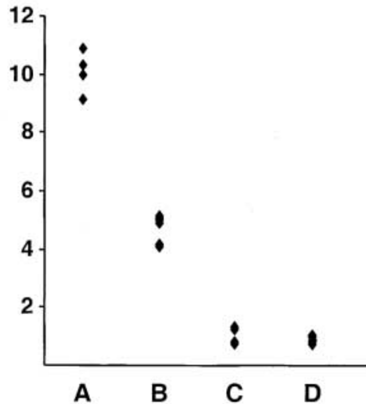
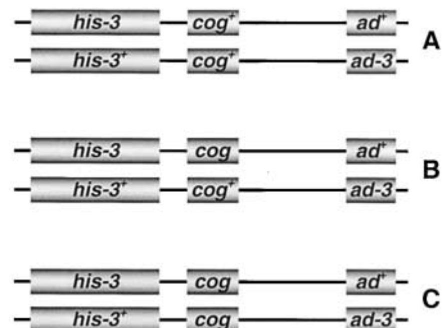


FIGURE 4.—Crossing over between *lys-4* and *ad-3*. The centromere, to the left of the figure, is represented by a solid disc. The figure is not to scale. The frequency of Lys$^+$ Ad$^+$ progeny is given as a fraction of the total number of viable spores. Lys$^+$ Ad$^+$ frequencies were obtained from four replicate crosses of a single *cog*$^+$ homozygote (A: T11805 × T10998), three *cog*$^+$/*cog* heterozygotes (B: T11089 × T11805, T10998 × T11801, and T10998 × T11802), two *cog* homozygotes (C: T11089 × T11801 and T11089 × T11802), and three *rec-2*$^+$ heterozygotes (D: T10997 × T11801, T10997 × T11802, and T10997 × T11805).



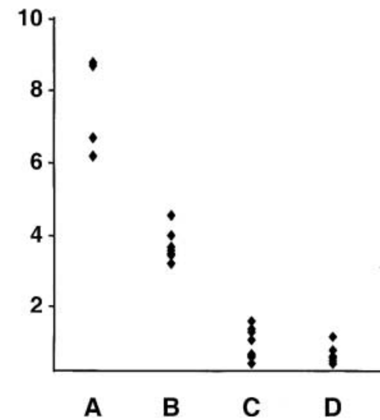


FIGURE 5.—Crossing over between *his-3* and *ad-3*. The centromere is on the left. The figure is not to scale. The frequency of His$^+$ Ad$^+$ progeny is given as a fraction of the total number of viable spores. His$^+$ Ad$^+$ frequencies were obtained from *cog*$^+$ homozygotes (A: T12080 × T11789, T12078 × T11782, T12081 × T11789, and T12079 × T11782), *cog*$^+$/*cog* heterozygotes (B: T11081 × T11058, T4400 × T4395, T11081 × T11089, T11081 × T11057, T11668 × T11782, T11667 × T11789, and T4400 × T11113), *cog* homozygotes (C: T4400 × T4396, T4400 × T11067, T4400 × T4396, T4400 × T11066, T4400 × T11059, T4400 × T11063, T4400 × T11062, T4400 × T11065, T4400 × T11061, and T4400 × T11058), and *rec-2*$^+$ heterozygotes (D: T4400 × T11039, T4400 × T11043, and T4400 × T11041).

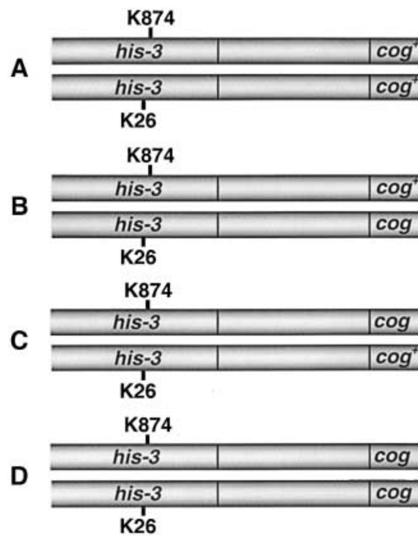
T12078 and T12080 received the higher-frequency allele, and T12079 and T12081 the lower-frequency allele.

Allelic recombination in K874/K26 heterozygotes: Crosses homozygous for *cog* and heteroallelic for K26/K874 yield a very low frequency of His$^+$ progeny ($3/10^5$; Figure 6D), so we expect the contribution of initiation at *cog* to have little effect on recombination in *cog*/*cog*$^+$ heterozygotes. When *cog*$^+$ and K874 are *in cis* (Figure 6B), the His$^+$ frequency is $29/10^5$-$44/10^5$ and when *cog*$^+$ and K26 are *in cis* (Figure 6C), it is $14/10^5$–$30/10^5$. From crosses homozygous for *cog*$^+$ (Figure 6A), the His$^+$ frequency is $21/10^5$–$31/10^5$ viable spores. Thus, there is no apparent increase in His$^+$ frequency when *cog*$^+$ is homozygous (for A and B, a *t*-test yields $P = 0.03$; A and C, $P = 0.39$; B and C, $P = 0.01$). These data confirm the previous results (ANGEL *et al.* 1970; CATCHESIDE and ANGEL 1974) and show how analysis of a few K874/K26 heterozygotes led to the conclusion that *cog*$^+$ is fully dominant to *cog*.

However, His$^+$ frequencies in K26 by K874 crosses are heterogeneous for all genotypes in which *cog*$^+$ is present (Figure 6, A–C; χ^2 heterogeneity tests yield $P = 0.02$, $P = 0.001$, and $P = 1.6 \times 10^{-8}$, respectively), suggesting the influence of factors other than *cog*.

Flanking marker exchange in progeny experiencing allelic recombination: Forty-five percent (57/128) of His$^+$ progeny of a K1201/K874 diploid, homozygous for *cog*$^+$, were recombinant for flanking markers *lys-4* and *ad-3*. Similar frequencies ($P = 0.92$) were obtained from K1201/K874 diploids heterozygous for *cog*$^+$/*cog* (44% or 55/126), homozygous for *cog* (43% or 51/119), or heterozygous for *rec-2*$^+$ (40% or 50/124).

The effect of *rec-2*$^+$ on allelic recombination and on crossing over: As seen in crosses heteroallelic for K1201/K874 (Figure 1), the presence of *rec-2*$^+$ significantly reduces allelic recombination compared to that measured in crosses homozygous for *cog* ($P = 8 \times 10^{-4}$).



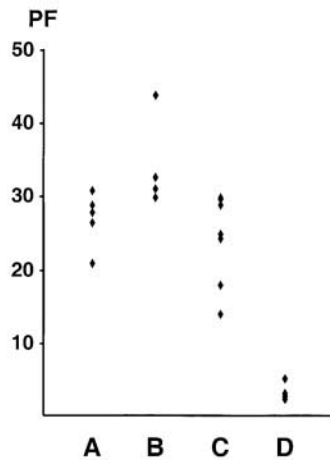


FIGURE 6.—Allelic recombination in crosses heteroallelic for K874/K26. The centromere is at the left of the figure. The mutant sites are separated by 215 bp (YEADON and CATCHESIDE 1999). PF is the frequency of His$^+$ progeny yielded by each cross, multiplied by 10^5. His$^+$ frequencies were obtained from *cog*$^+$ homozygotes (A: T10989 × T11113, T10989 × T11110, T10989 × T11125, T10989 × T11126, and T10989 × T11132), *cog/cog*$^+$ (B: T11318 × T4395, T11318 × T11113, T11318 × T11125, and T11318 × T10998), *cog*$^+$/*cog* heterozygotes (C: T10989 × T11092, T10989 × T11093, T10989 × T11099, T10989 × T11104, T10989 × T11105, T10989 × T11117, and T10989 × T11127), and *cog* homozygotes (D: T11318 × T4396, T11318 × T11093, T11318 × T11099, T11318 × T11104, T11318 × T11105, and T11318 × T11127).

In contrast, *rec-2*$^+$ does not significantly reduce the frequencies of either Lys$^+$ Ad$^+$ or His$^+$ Ad$^+$ spores from those seen in crosses homozygous for *cog* (Figures 4 and 5) with two-tailed *t*-tests for equal variances yielding values of $P = 0.4$ and 0.3, respectively.

DISCUSSION

***cog* alleles are codominant:** Two copies of *cog*$^+$ yield twice as many local crossovers as a single copy. The *lys-4–ad-3* interval is 20 cM in crosses homozygous for *cog*$^+$, 9.4 cM in those heterozygous for *cog*$^+$/*cog* and 2 cM in those homozygous for *cog* (Figure 4). Similarly, the *his-3–ad-3* interval is 15.5 cM in crosses homozygous for *cog*$^+$, 7.5 cM in crosses heterozygous *cog*$^+$/*cog*, and 1.4 cM in those homozygous for *cog* (Figure 5). Alleles of *cog* are therefore codominant in effect on local crossing over.

In addition, in heteroallelic crosses, providing that the mutant alleles are distant (Figures 1–3), two copies of *cog*$^+$ result in approximately twice the average frequency of allelic recombination as that of a single copy. Thus we conclude that *cog*$^+$ is not dominant to *cog*, but rather that the two alleles operate independently of one another to attract recombination events.

The relative frequency of recombination initiation at *cog* and *cog*$^+$: In any comparison of crosses carrying the same pair of mutant *his-3* alleles, the His$^+$ frequency should be directly related to the rate of initiation at the *cog* hotspot. This rate, in the absence of *rec-2*$^+$, is dependent on the *cog* alleles in the cross.

For crosses heteroallelic for K1201/K874, the average His$^+$ frequency (786/10^5 viable spores) from crosses homozygous for *cog*$^+$ is 25 times higher than that (32/10^5) from crosses homozygous for *cog*. For crosses heteroallelic for K874/K480, the ratio is 16 (257/16) and for those heteroallelic for K1201/K26, only 6 (253/41). The previous estimate of an 11-fold increase in DSBs at *cog*$^+$ relative to *cog*, based on data from crosses heteroallelic for K874/K26 (ANGEL *et al.* 1970), is consistent with our estimate using the same allele pair (9-fold, or 27/3) and falls in the middle of the range of our estimates. It is possible therefore that recombination is initiated as much as 25 times more frequently at *cog*$^+$ than at *cog*.

Genetic background variation alters recombination frequency: Although recombination frequency in the *his-3* region depends upon which alleles of *cog* and *rec-2* are present, it seems that these are not the only factors involved. His$^+$ frequency, for a single pair of mutant *his-3* alleles, varies over a twofold range in crosses with identical *cog* and *rec-2* genotypes (Figures 1–3 and 6). Crossover frequency varies in a similar way (Figures 4 and 5). Analysis of recombination frequencies from crosses between strains with the same or similar genetic backgrounds suggests that genes with small effects on recombination segregate in our laboratory strains. Such an effect has been detected previously, where allelic recombination at the *nit-2* locus was found to vary with parental provenance (CATCHESIDE 1970).

The effect of *rec-2*$^+$ on crossing over in the *his-3* region: In a *cog* homozygote, recombination in *his-3* is reduced fourfold when *rec-2*$^+$ is present (Figure 1 and CATCHESIDE and ANGEL 1974) and that which persists appears to be initiated from the *rec-2*$^+$-independent hotspot at the 5′ end of *his-3* (CATCHESIDE and ANGEL 1974). Therefore, if crossovers resulting from initiation at this or other *rec-2*$^+$-independent hotspots in the *lys-4–ad-3* interval occur autonomously, unaffected by those generated by initiation at *cog*, the absence of *rec-2*$^+$ in a cross should increase crossing over in this region. However, between *lys-4* and *ad-3*, crossovers occur at an average frequency of 2.0% in *cog rec-2* homozygotes and 1.7% in crosses including *rec-2*$^+$ (Figure 4, C and D). In the *his-3–ad-3* interval, the equivalent average frequencies are 1.4 and 1.0%, respectively (Figure 5, C and D). The crossover frequency for each interval in *cog* homozygotes

is thus unaffected by the presence of *rec-2*$^+$ (P = 0.4 and 0.3, respectively). How can this be?

Perhaps we simply failed to detect the increase in crossing over due to the absence of *rec-2*$^+$. We consider this unlikely, as we detected a difference between 0.8 and 0.3% in mean His$^+$ frequency for crosses heteroallelic for K1201/K874 (Figure 1, A compared to B; $P = 2 \times 10^{-6}$), despite highly heterogeneous data (for A, $P = 4 \times 10^{-19}$; for B, $P = 3 \times 10^{-250}$). To obtain the crossover data, a similar number of colonies were counted but spore suspensions experienced fewer dilutions than in estimations of the yield of His$^+$ progeny from K1201/K874 crosses, thus decreasing sampling error and increasing our chance of differentiating between the frequencies. In addition, the His$^+$ Ad$^+$ frequencies (Figure 5, C and D) are no more heterogeneous (for C, $P = 1 \times 10^{-88}$, and for D, $P = 1 \times 10^{-9}$) than the K1201/K874 His$^+$ frequencies, so it seems improbable that a real difference in frequency due to the presence of *rec-2*$^+$ has been confounded by variation in genetic background.

If events initiated at *cog* were more likely than those initiated at *cog*$^+$ to proceed by synthesis-dependent strand annealing (Nassif *et al.* 1994; Pâques and Haber 1999), the absence of *rec-2*$^+$ would stimulate conversion but not crossing over in a *cog* homozygote. If this were the case, His$^+$ progeny of *cog* homozygotes would experience fewer crossovers than His$^+$ progeny extracted from crosses where *cog*$^+$ is present. However, the frequency of flanking marker exchange is the same in His$^+$ progeny from all crosses heteroallelic for K1201/K874 (P = 0.92).

We must therefore conclude that our original assumption, that *rec-2*$^+$-independent and *cog*-associated crossovers occur autonomously, is incorrect and that reduction of the latter yields an increase in the former type of crossover. It may be that events are initiated autonomously but that, as *cog*-associated crossovers decrease, events initiated elsewhere have an increased chance of yielding crossovers, the phenomenon of crossover interference (Muller 1916). Alternatively, since competitive interaction between two nearby hotspots in *S. cerevisiae* has been observed to reduce the activity of both (Xu and Kleckner 1995; Fan *et al.* 1997), the lack of DSBs at *cog* when *rec-2*$^+$ is present may increase the frequency of initiation at other locations in the *his-3* region.

***cog*$^+$ appears dominant in K26/K874 heterozygotes:** In crosses heteroallelic for distant *his-3* alleles, a *cog*$^+$ homozygote gives a His$^+$ frequency close to the sum of the heterozygotes, suggesting that the His$^+$ frequency is determined by the frequency of recombination initiation. In contrast, in crosses heteroallelic for K26 and K874 (Figure 6A), which are 215 bp apart (Yeadon and Catcheside 1999), *cog*$^+$ homozygotes yield a His$^+$ frequency lower than that of one of the heterozygotes (P = 0.03), despite a presumed doubling in initiation frequency. Alleles in close proximity experience co-conversion more often than widely separated sites (Hilliker *et al.* 1994; Yeadon *et al.* 2002). Therefore, a recombination event initiated at *cog* (Yeadon *et al.* 2001) on the chromosome bearing K874 is less likely to terminate between K874 and K26 to yield a His$^+$ spore than is a more distant allele pair. In addition, when nearby alleles are co-converted, both mismatches may be included in a single repair tract (Modrich and Lahue 1996). Thus, the probability that a conversion event initiated on the K26 chromosome and covering K874 and K26 will result in a His$^+$ spore is reduced compared to a similar event involving remote alleles. However, despite this effect, a *cog*$^+$ homozygote should yield His$^+$ progeny at the sum of the two heterozygous frequencies, whether the mutant alleles are close or distant.

Our His$^+$ frequencies are in most cases heterogeneous within a single known genotype, suggesting segregation of genes, unlinked to *cog*, that affect recombination. K26/K874 heterozygotes are no exception, with the frequencies yielded by K874 *cog*/K26 *cog*$^+$ crosses especially heterogeneous ($P = 1.6 \times 10^{-8}$). We therefore suggest that variation in genetic background is responsible for the apparent lack of additivity of our K26/K874 recombination frequencies. Since our strains are descendants of those of D. G. Catcheside, genetic background variation is also a likely explanation for the previous nonadditive data (Angel *et al.* 1970; Catcheside and Angel 1974). It is clear that recombination involving K874 and K26, the only allele pair available to Catcheside and Angel (1974) to test the dominance relationship of *cog* and *cog*$^+$, is a special case, and that this relationship is more easily investigated by analysis of crosses heteroallelic for more distantly separated alleles.

We have shown that naturally occurring alleles of the recombination hotspot *cog* are codominant. Since initiation of recombination is thought to be independent of interaction between homologs (De Massy *et al.* 1994; Gilbertson and Stahl 1994; Young *et al.* 2002), codominance of hotspot alleles was predicted, but not demonstrated prior to this study. This work supports the conclusion that the frequency of conversion in crosses homozygous for *ade6* M26 in *S. pombe* is equal to the sum of the two heterozygous frequencies (Ponticelli *et al.* 1988), making it likely that all hotspot alleles operate codominantly to influence recombination nearby.

We also present evidence that genes that influence both conversion and crossing over are polymorphic in laboratory strains of *N. crassa*. Recombination at the naturally polymorphic human *DNA2* hotspot has yielded data (Jeffreys and Neumann 2002) that suggest that genetic background may have a similar effect on recombination in humans. Identification and investigation of the polymorphic genes involved in the genetic background effect in Neurospora may assist with identification of similar polymorphisms present in humans.

This work was supported by grants from the Australian Research Council and the Flinders University Research Budget.

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Communicating editor: A. Nicolas