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High density analysis of randomly selected Neurospora octads reveals conversion associated with crossovers located between *cog* and *his-3*

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ABSTRACT

We analysed 148 octads from a Neurospora cross maximised for sequence heterology in the *his-3* region and detected non-Mendelian segregation at *his-3*, *cot-1* and *lys-4* loci. This was in all cases 6:2 or 2:6, with no evidence of post-meiotic segregation (PMS) in these genes. High density snp analysis was used to place crossovers between *his-3* and the centromere-distal marker *ad-3*, and sequencing to refine the location of crossovers between *his-3* and the recombination hotspot *cog*. Crossovers appeared to have a non-random distribution, falling close to *his-3* or more than 40 kb distal, and all those in which the location was determined were flanked by sequences showing gene conversion and/or PMS amongst the polymorphisms. This octad study confirms the validity of assumptions made during random spore analyses and suggests that recombination hotspots at *cot-1* and *lys-4* may, unlike the relatively cold recombination initiator at the *am* locus, be high frequency recombinators similar to *cog*.

1. Introduction

Arguably the most attractive feature of ascomycetes to the student of meiotic recombination is the ordered packaging by these fungi of all products from a single meiosis into the ascus, forming a tetrad or octad of haploid spores. Study of tetrads or octads has yielded insights into meiotic recombination unavailable where this process must be probed using individual spores taken at random. Despite this, plundering the treasures of the ascus is not without cost, as a huge effort is usually required to isolate the contents of the asci and large numbers of asci are essential if a meaningful analysis is to be carried out.

In a Herculean effort, Mitchell (1955) isolated and tested thousands of Neurospora octads to find 32 *pdx*$^+$ recombinants from a cross heterozygous for two different *pdx* mutants. Although these *pdx* prototrophs could have arisen by crossing over between the mutant alleles, the absence of corresponding *pdx* double mutants in the ascus from whence each came provided the first unambiguous demonstration of gene conversion. Stadler (1959) subsequently demonstrated that unlike crossovers, which suppress additional crossovers in nearby intervals (interference), gene conversion events do not.

In the yeast *Saccharomyces cerevisiae*, where recombination frequency is usually relatively high and informative tetrads are correspondingly more frequent, the tetrad is used almost routinely. In a seminal study, Hurst et al. (1972) reported that for the *arg4*, *thr3*, *his-1* and *SUP6* loci, where the flanking markers used were in each case $\leqslant$20 centiMorgans (cM) apart, half of the tetrads showing gene conversion also had a crossover between the flanking markers. This observation provided a foundation for the majority of recombination models that followed, with each positing that the prior existence of a recombination intermediate is signaled by gene conversion and that a crossover results when this intermediate is resolved in a certain way.

The power of the ascus as a tool for dissecting the mechanism of meiotic recombination increased substantially with the advent of molecular techniques. However, with the notable exception of Ascobulus where spore colour difference can be used to identify exceptional octads (e.g. Maloisel and Rossignol, 1998), there is a paucity of organisms in which meiotic recombination has been subjected to this level of scrutiny, as a huge effort is required for significant reward from such studies in non-yeast organisms. In contrast, there is a large and convincing dataset provided by the elegant studies that have used yeast.

For example, Borts and Haber (1989) used *S. cerevisiae* diploids with a duplication of the mating type genes, separated by *URA3* and *leu2* loci and sequences from the plasmid pBR322, to investigate the relationship between conversion and crossing over at the molecular level. Dissection of a total of 2273 tetrads allowed the conclusion that when URA3 was converted, 57% of the conversion events had also experienced an exchange of flanking markers,

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while conversion of at least one molecular marker was detected in 61% of crossover tetrads. Conversion tract lengths were also estimated, concluding that there was no difference between those identified by a crossover or by conversion. Although most recombination events were simple, consisting of a conversion tract terminated by a crossover, both discontinuous conversion tracts and crossovers separated from a conversion tract by an unconverted marker were observed, at a frequency of about 2%, in the RHB633 cross from which over 900 tetrads were analysed.

However, the studies described above (Borts and Haber, 1987, 1989) highlighted a shortcoming of yeast, as it was found that more than six sequence heterologies within the 9 kb region under analysis distorted the recombination events being measured (Borts and Haber, 1987). Thus, although it is theoretically possible for the resolution of a molecular analysis of recombination to be at the level of a single base pair, the sensitivity of recombination to sequence heterology severely limits resolving power in yeast, and it has become increasingly obvious that a complete understanding of meiotic recombination requires a synthesis of evidence from more than one model organism (e.g. Cromie and Smith, 2007).

Our standard *Neurospora crassa* laboratory strains display high levels of polymorphism (Germann et al., 1988; Yeadon and Catcheside, 1998, 1999) and the recent sequencing of the Neurospora genome (Galagan et al., 2003) provides an economical way to identify such polymorphism, potentially yielding a rich set of molecular markers that can be used to probe recombination at high resolution in this organism. Neurospora also has a well-characterised system of local regulation of recombination, consisting of *trans*-acting *rec* genes of which the dominant allele of each decreases recombination in specific target regions (reviewed in Catcheside (1986)). *rec-2*, on Linkage Group V (Smith, 1968) regulates recombination in the *his-3* region, probably by interaction with the recombination hotspot *cog*, centromere-distal of the *his-3* coding sequence (Bowring and Catcheside, 1991; Yeadon and Catcheside, 1995a, 1998; Yeadon et al., 2001). *cog* has two co-dominant alleles (Yeadon et al., 2004), of which cog^+ (Angel et al., 1970) is a more efficient recombination hotspot than *cog* (Angel et al., 1970; Yeadon and Catcheside, 1995a). In the absence of $rec\text{-}2^+$, cog^+ increases both recombination within *his-3* and crossing over between *his-3* and the distal locus *ad-3* (Angel et al., 1970; Catcheside, 1979).

Thus, by choosing strains containing the high frequency recombinator cog^+ and lacking $rec\text{-}2^+$, octads exhibiting recombination events nearby can be recovered at higher frequency than elsewhere in the genome (e.g. *pdx*, see above), so it is possible to obtain a meaningful dataset from a moderate number of octads. In view of this attractive combination of features, we have carried out the first high resolution analysis of meiotic recombination in Neurospora using octads.

Here we report the outcome of a detailed analysis of 148 octads largely selected at random. We found gene conversion at three loci, *his-3*, *cot-1* and *lys-4*, with a frequency at the latter two loci comparable to that at *his-3*. Using single nucleotide polymorphisms (snps) we found that crossovers were distributed non-randomly over the 51 kb interval bounded by the most distant snps used in our analysis, and that where a crossover in this interval was examined at high resolution it was without exception close to a region in which gene-conversion or post-meiotic segregation had occurred.

2. Materials and methods

2.1. Neurospora strains

The genotypes of strains used in this study are listed in Table 1. The parents of the heterozygote used for octad analysis, T12105 and T12282, are both *rec-2* and contain cog^+ and *cog* respectively, to ensure recombination initiated at *cog* occurs at high frequency. In T12105, LGI sequence is of Lindegren origin, and in T12282 it is of Emerson/St. Lawrence/Mauriceville origin, strains that are polymorphic in the *his-3* region (Yeadon and Catcheside, 1995a, 1998, 1999). As a result, the strains differ at multiple snps on Linkage Group I, which we have utilised for analysis of recombination events (Fig. 1). Henceforth, T12105 will be termed L, for Lindegren, and T12282 termed E, for Emerson.

Table 1
Neurospora strains. The *am* allele is K314, *lys-4* is STL4, *cot-1* (colonial temperature-sensitive mutation) is C102t and *ad-3* is K118.

Stock number	Genotype
T2326	*fl a*
T2327	*fl A*
T9135	Lindegren 25*a* wild-type
T12092	*A*, *lys-4*, *his-3*, cog^+, *ad-3*; *cot-1*; *rec-2*
T11588	*a his-3* K480 *cog ad-3*; *cot-1*; *am*, *rec-2*
T12275/FGSC 5436	*A*, *lys-4*
T12105 (L)	*a*, cog^+; *cot-1*; *rec-2*
T12282 (E)	*A*, *lys-4*, *his-3* K480 *cog ad-3*; *am*, *rec-2*

The L strain was extracted from a cross between Lindegren 25*a* wild-type T9135 and T12092. Since *rec-2* alleles are distinguished by an insertion–deletion (Bowring, unpublished), the presence of *rec-2* was determined by the size of a PCR product. The E strain was extracted from a cross between T11588, a strain of Emerson origin that carries mutations in *his-3* and *ad-3*, and FGSC 5436, a strain of St. Lawrence/Mauriceville origin that bears a mutation in *lys-4*. Thus mating type, *lys-4*, *his-3*, and *ad-3* are all available for use as genetic markers on LGI. E also has a mutation in *am*, and L has a mutation in the colonial temperature-sensitive locus *cot-1*, providing markers on LGV and LGIV respectively to assist in ordering octads. The chosen *his-3* mutation is K480, at the 3′ end of the *his-3* gene (Yeadon et al., 2002), the closest available mutant site to *cog*, which would therefore be expected (Yeadon et al., 2002) to experience conversion more frequently than more distant markers.

In addition, this particular pair of strains was chosen because it shows wild-type reproductive behaviour: L is a good female parent in a plate cross, the cross is very fertile with high spore viability, bubble asci are rare and nearly all mature asci have eight black spores.

2.2. Culture methods and media

As described by Bowring and Catcheside (1996), except where described below. Vegetative cultures were supplemented with 200 μg/ml l-histidine, 500 μg/ml l-alanine, 400 μg/ml adenosine and 400 μg/ml l-lysine. Mating type tester cultures were produced by inoculation of either T2326 or T2327 onto the centre of a petri dish of solid medium (1× SC, 2% sucrose, 2% agar), and incubation at 25 °C for 7 days in the dark. Mating type tester strains carry the *fl* mutation and are aconidiate (Perkins et al., 1989).

2.3. Crosses

Crosses were performed in glass petri dishes on 50 ml solid crossing medium (1× SC, 2% agar) with Whatman filter paper (Cat No. 1001 090) as carbon source. A conidial suspension of the female parent (L) was spread onto the filter paper and incubated at 25 °C. The male parent (E) was similarly inoculated onto the paper after 4–7 days, and the cross incubated at 25 °C. Spores were collected 10–12 days after fertilisation.

2.4. Spore collection

Octads were collected by allowing spores to shoot from the cross plate onto water agar (1.5% agar) in a petri dish suspended