Blocked recombination along the mating-type chromosomes of \textit{Neurospora tetrasperma} involves both structural heterozygosity and autosomal genes

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Running title:
Mating-type chromosomes of *Neurospora tetrasperma*

Keywords:
*Neurospora*, mating-type chromosome, recombination block, sex chromosome

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ABSTRACT

The *Neurospora tetrasperma* mating-type chromosomes have been shown to be structurally heterozygous by reciprocal introgression of these chromosomes between *N. tetrasperma* and *N. crassa*. This structural heterozygosity correlates both with a previously described recombination block and with cytologically visible unpaired chromosomes at pachytene. Genes on the autosomes are also implicated in blocking recombination.
The filamentous fungus *Neurospora tetrasperma* is self-fertile with a vegetative thallus (mycelium) that is heterokaryotic for mating type (pseudohomothallic). Sexual development in *N. tetrasperma* has been genetically and developmentally reprogrammed so that two haploid nuclei of opposite mating type (*mat A* and *mat a*) are delivered into each of the four ascospores (DODGE 1927; RAJU and PERKINS 1994). To accomplish this nuclear packaging, crossing over is suppressed in the mating-type bivalent (but not in the autosomes) ensuring that *mat A* and *mat a* will segregate at the first division of meiosis. Recombination is blocked for over 120 map units, which is about 90% of this chromosome (Linkage Group I) and ~12% of the total genome (GALLEGOS et al. 2000). The two homologs of the mating-type bivalent are seen to remain unpaired at pachytene over most of its length. Chiasmata near the ends hold the homologs together and ensure proper disjunction at anaphase I. Spindles at the second division are precisely repositioned pair-wise so as to ensure that two nuclei of opposite mating type are enclosed in each ascospore, rendering it self-fertile. Pseudohomothallism, as exemplified in *N. tetrasperma*, leads to sustained but not absolute inbreeding, with profound consequences for population structure and evolution (POWELL et al. 2001).

Because recombination is blocked on the mating-type chromosomes of *N. tetrasperma*, the regions linked to *mat A* and *mat a* have undergone substantial sequence divergence (MERINO et al. 1996). Sequences in the region of suppressed crossing over exhibit heteroallelism (heterozygosity), even when sibling nuclei from the same wild type isolate are compared. In contrast, the autosomes recombine freely and sibling nuclei of opposite mating type from each wild-type isolate exhibit nearly complete
homoallelism (homozygosity) along the autosomes. *N. tetrasperma* mating-type chromosomes resemble the sex chromosomes in animals, in failing to recombine over most of their length. The structure, function, and evolution of fungal mating-type loci and chromosomes is an area of current interest (e.g., Fraser et al. 2004; Hood et al. 2004).

Three questions arise from these observations: 1) Why has the crossover block come to be extended over most of the chromosome? A recombination block in the short region between mat and centromere would suffice to provide the mechanism for pseudohomothallism by maintaining first division segregation of mating type and the subsequent packaging of opposite mating-type nuclei into individual ascospores. 2) What are the consequences of an extended recombination block for a haploid organism in the context of free recombination in the autosomes? The mating-type chromosome should be subject to Muller’s ratchet, resulting in accumulation of alleles of reduced fitness as expected for a nonrecombining chromosome in an otherwise recombining genome (Muller 1964). 3) What is the cellular basis of blocked recombination? This study concerns the third question.

A likely explanation for the cellular basis of the recombination block would be structural heterozygosity between the *mat a* and *mat A* mating-type chromosomes of *N. tetrasperma*, involving rearrangements such as inversions. However, blocked recombination might be under some other genetically controlled mechanism, based on particular gene(s), on the mating-type chromosome and/or on the autosomes. To test these alternatives, the mating-type chromosomes were introgressed reciprocally between *N. tetrasperma* and *N. crassa*. The interval introgressed (ro-10 to un-18)
encompasses most of the linkage group (Figure 2A and B) (PERKINS et al. 2001). Classical genetic markers (mutations) were followed in the crosses reported here, and were present both within the recombination block (leu-3, mep, mat, the centromere, cyh-1, and al-2) and outside it (ro-10 and un-18) (GALLEGOS et al. 2000).

Nearly complete sterility between *N. crassa* and *N. tetrasperma* required the use of a hybrid strain, C4,T4 (FGSC 1778), in the initial bridging cross (METZENBERG and AHLGREN 1969). The *N. tetrasperma* strains used to construct the C4,T4 hybrid were different than those used in the present study. The cross DJ1307, therefore, has been designated the first hybrid cross of the present introgression series (Figure 1). This cross had very low fertility and fecundity. The programmed recombination block of *N. tetrasperma* broke down upon initial hybridization with *N. crassa*. Recombination along the mating-type chromosomes was evident even in the small number of viable progeny available (Figure 2C). Four subsequent series of introgressions were necessary for a completely reciprocal design. Pedigrees and selection strategies are shown in Figure 1. Mating types from *N. tetrasperma* and *N. crassa* are designated as mat A$^T$ or mat a$^T$ and mat A$^C$ or mat a$^C$, respectively. Recombination rates were measured among progeny of the sixth backcross (Figure 2C).

**Recombination in introgression series:** Series 1 – the mat a$^T$ chromosome introgressed into *N. crassa*: No recombination block was evident after six generations (Figure 2C, Series 1). Recombination frequencies along the mating-type chromosomes in this introgression were very similar to those of wild type *N. crassa* (compare Figure 2C with 2A). Progeny viability was normal and allele ratios were near 1:1.
Series 2 – the mat \( A^T \) chromosome introgressed into N. crassa: In contrast, crossing over remained blocked between \( \text{mat} \) and \( \text{al-2} \) in this opposite orientation (Figure 2C, Series 2). The <1\% recombination in this interval was reminiscent of wild type \( N. \) \( \text{tetrasperma} \). However, even after six generations, crosses of \( \text{mat} \ A^T \times \text{mat} \ a^C \) showed a high proportion of aborted, unpigmented ascospores. Viability was low among black ascospores, and allele ratios were highly skewed among those that germinated, for example, 14 \( \text{mat} \ A \) to 85 \( \text{mat} \ a \) progeny. These factors indicated general developmental problems in the crosses, which perhaps confounded measurements of recombination.

Series 3 and 4 – the \( N. \) crassa mating-type chromosomes introgressed into \( N. \) \( \text{tetrasperma} \): By the fourth backcross into \( N. \) \( \text{tetrasperma} \), the crosses in both series produced normal four-spored asci (Figure 2C, Series 3 and 4). Genes centromere-distal to crossovers undergo second-division segregation in one-half of crossover asci. Because of ascus programming in \( N. \) \( \text{tetrasperma} \), such genes become homoallelic in ascospores, while genes proximal to the crossover remain heteroallelic (RAJU and PERKINS 1994). Therefore, recombination was scored as the number of homoallelic recessive progeny over the total progeny. This proportion of homoallelic ascospores, as a percentage, is the same as the distance in map units between the centromere and the marker locus (HOWE 1963).

Series 3 – the \( \text{mat} \ a^C \) chromosome introgressed into \( N. \) \( \text{tetrasperma} \): Recombination was blocked between \( \text{leu-3} \) and \( \text{cyh-1} \) (Figure 2C, Series 3). However, 14\% recombination (3/21 progeny) occurred in the interval between \( \text{nit-2} \) and \( \text{leu-3} \), an area normally within the recombination block of \( N. \) \( \text{tetrasperma} \) (Figure 2B). The leftmost marked interval in
Series 3, therefore, was more comparable to the normal 17% recombination in *N. crassa* (Figure 2A).

*Series 4 – the mat a<sup>c</sup> chromosome introgressed into N. tetrasperma:* In the opposite orientation, the recombination block was also reestablished from *mat* to *al-2* (Figure 2C, Series 4). Recombination between the distal markers (inside and outside the recombination block) on both chromosome arms was lower than expected compared to normal *N. tetrasperma* (Figure 2B). Cytological observation of pachytene chromosomes in the sixth backcross of *mat A<sup>c</sup>* into *N. tetrasperma* showed the typically long unpaired central chromosome segment and the short paired terminal segments (N. B. Raju, pers. comm.), similar to Figure 4 in (GALLEGOS et al. 2000).

**Mating-type chromosome structure:** In the *N. crassa* background, recombination is normal between the *mat a<sup>T</sup>* and *mat A<sup>c</sup>* chromosomes (Figure 2C, Series 1). This demonstrates that these two chromosomes are colinear. Normal recombination is defined in *N. crassa* by *mat a<sup>c</sup> × mat A<sup>c</sup>*; therefore, it follows that *mat a<sup>T</sup>* must also be colinear with *mat a<sup>c</sup>* (although, obviously, recombination cannot be tested by directly crossing *mat a<sup>T</sup> × mat a<sup>c</sup>*, strains of the same mating type).

In the *N. crassa* background, recombination is blocked in the opposite orientation, between the *mat A<sup>T</sup>* and *mat a<sup>c</sup>* chromosomes (Figure 2C, Series 2). This strongly suggests that *mat A<sup>T</sup>* is structurally different from both *mat A<sup>c</sup>* and *mat a<sup>c</sup>* chromosomes. This was not seen in the earlier backcrosses because of low fertility and fecundity (data not shown).
In the *N. tetrasperma* background, recombination is blocked from *leu-3* to *cyh-1* in *mat a<sup>c</sup> × mat A<sup>T</sup>, suggesting that these chromosomes are structurally different (Figure 2C, Series 3). Recombination was blocked between *mat* and *al-2* even in the first hybrid cross (DJ1725, 0/27 recombinants). However, the *mat a<sup>c</sup>* and *mat A<sup>T</sup>* chromosomes did recombine in the *nit-2-leu-3* interval, a region still within the *N. tetrasperma* recombination block, contrary to expectations if structural differences extend left of mating type.

**Other factors controlling the recombination block in *N. tetrasperma*:**

Introgression Series 4 further indicates that a structurally unique *mat A<sup>T</sup>* chromosome cannot be the only explanation for the recombination block in *N. tetrasperma*. If blocked recombination was due solely to differences in chromosome structure, the expectation would be that the colinear mating-type chromosomes would recombine freely in the *N. tetrasperma* background, as was demonstrated for *mat A<sup>c</sup>* and *mat a<sup>T</sup>* in the *N. crassa* background (Series 1). The original hybrid cross of *mat A<sup>c</sup> × mat a<sup>T</sup>* (DJ1307) did show recombination (Figure 2C, f<sub>1</sub> hybrid progeny), but during the six backcrosses of *mat A<sup>c</sup>* to *N. tetrasperma* the recombination block was reestablished despite the absence of a *mat A<sup>T</sup>* chromosome (Figure 2C, Series 4).

These results show that the mating-type chromosomes of *N. tetrasperma* are structurally different from each other. Structural heterozygosity could, of itself, cause the recombination block between those chromosomes. However, complete genetic control of the recombination block is likely more complicated than simple differences in
chromosome structure. Blocked recombination was reestablished during backcrossing to *N. tetrasperma*, even when the mating-type chromosomes were colinear (*mat A^C × mat a^T*). Genetic background must, therefore, have a critical role in this qualitative regulation of recombination. The data also suggest that genes affecting recombination are present both on the mating-type chromosomes and on the autosomes.

Involvement of autosomal genes raises the possibility that differences in mating-type chromosome structure in *N. tetrasperma* may have occurred concomitantly with blocked recombination, rather than causing it. Determining the evolutionary history of the mating-type chromosome structures would address issues of cause and effect. When did the structure of *mat A^T* and *mat a^T* diverge? Was it coincident with the origin of pseudohomothallism or subsequent to it? The genetic data reported here leave these and other questions unanswered. An assembled genome sequence of *N. tetrasperma*, that includes both mating types, will likely provide the definitive answers by enabling both *mat A^T* and *mat a^T* chromosome structures to be compared with each other and with those of *N. crassa*. This might also explain why the recombination block is much larger than necessary for pseudohomothallism. Continued genetic work will then be needed to identify the genes that affect recombination in the *N. tetrasperma* background.

I deeply thank David D. Perkins for helping me recognize the significance of these data. Thanks also to Donald O. Natvig for providing unpublished linkage data for the right arm of the mating-type chromosome. The work was supported by National Science Foundation grants MCB-9713015 to DJJ and MCB-9728675, MCB-0235698, and MCB-0417282 to D. D. Perkins.
LITERATURE CITED


Figure 1. Flow chart of crosses and selection strategy for reciprocal introgression of mating-type chromosomes between *N. crassa* and *N. tetrasperma*. Classical genetic markers along the mating-type chromosome (mutations and mating-type alleles) were followed in the crosses (see Perkins et al. 2001 for explanation of markers). For crosses, haploid parental genotypes are indicated above and below a bold line, this also indicates the zygote genotype. *N. crassa* or *N. tetrasperma* origin of the *mat* allele is indicated by superscript (C or T, respectively); mutant alleles for morphological and auxotrophic markers are shown in italics with the corresponding wild-type alleles indicated by (+); resistance or susceptibility is indicated by superscript (R or S, respectively) for the methylpurine resistance or cycloheximide resistance-1 loci. Cross number, where appropriate, is indicated by DJxxxx. Strain numbers are given in parentheses to the right of genotypes, FGSC = Fungal Genetics Stock Center, DJ = strain derived from a Jacobson cross. DJ1243-2 is a methylpurine resistant strain of *N. tetrasperma* (see Gallegos et al. 2000) and DJ 1687-82 is a strain of *N. crassa* with a novel marker combination developed for this study. The bridging cross (DJ1292) initiated all series. Even though strain C4,T4 is itself a hybrid between *N. crassa* and *N. tetrasperma*, the progeny from cross DJ1307 were considered the first hybrid strains (f1) for Series 1, 2, and 4. Series 3 required the opposite mating type of *N. crassa* (*mat a*) and different markers than were available from DJ1307. These were introduced in crosses DJ1700 and DJ1725, which again employed progeny from the Bridging Cross (DJ1258). Progeny from DJ1725 were considered the first hybrid strains (f1) for Series 3. Progeny from the sixth backcross generation of each series were analyzed for recombination of the markers indicated (Figure 2).
Figure 2. Crossing over between *mat A* and *mat a* mating-type chromosomes (Linkage Group I) in conspecific crosses of *N. crassa* (A.) and *N. tetrasperma* (B.), and after reciprocal introgression from one species into the other (C.).

A. *N. crassa*. Numbers along the linkage group are distances between *N. crassa* markers, expressed as map units (≅ percent recombination). Parentheses around *ro-10* and *cyt-21* indicate equivocal gene order. This map is based on PERKINS et al. (2001) and Jacobson (unpublished).

B. *N. tetrasperma*. Recombination rates expressed as the ratio of number of recombinants over total progeny tested. Heavy lines and ratios in bold are data from cross P556 *mat A* × P581 *mat a*, except those marked with (‡), which are results of a selfing cross (P556 *mat A* × P556 *mat a*) (GALLEGOS et al. 2000). The recombination block extends between the markers *nit-2* on the left and *arg-13* on the right. Tetrad analysis confirmed that 100% of meioses underwent crossing over in the interval between *nit-2* and *cyt-21* (marked with *) and 86% underwent crossing over between *arg-13* and *un-18* (marked with †). Other ratios are from previous studies of *N. tetrasperma* (data from D. D. Perkins, unpublished, and G. S. Saenz and D. O. Natvig, unpublished).

C. Reciprocal introgression crosses between *N. crassa* and *N. tetrasperma*. Crosses are described in Table 1. First Hybrid Cross. The fractions indicate the number of crossovers scored over the total *f1* hybrid progeny. Series 1 and 2. The fractions indicate the number of crossovers scored over the total number of progeny in the sixth backcross. Series 3 and 4. The fractions indicate the number of homoallelic recessive progeny over the total progeny scored in the sixth
backcross. The percent homoallelics is the same as the distance in conventional map units (HOWE 1963) (see text).
**BRIDGING CROSS – DJ1292:**

\[
\begin{align*}
\text{ro-10 mep}^S \text{ mat A} & \text{C al-2 un-18} \\
+ \text{ mep}^R \text{ mat a}^T & + + \\
\text{(FGSC 3789)} & \\
\text{C4, T4 (FGSC 1788)} &
\end{align*}
\]

**FIRST HYBRID CROSS – DJ1307:**

\[
\begin{align*}
\text{ro-10 mep}^S \text{ mat A} & \text{C al-2 un-18} \\
+ \text{ mep}^R \text{ mat a}^T & + + \\
\text{(DJ1292-58)} & \\
\text{N. tetrasperma (DJ1243-2)} &
\end{align*}
\]

**SERIES 1 N. tetrasperma mat a^T INTO N. crassa:**

First backcross:

\[
\begin{align*}
+ \text{ mep}^R \text{ mat a}^T & + + \\
\text{ro-10 mep}^S \text{ mat AC al-2 un-18} & \\
\text{f}_1 \text{ (DJ1307-2)} & \\
\text{N. crassa (FGSC 3789)} &
\end{align*}
\]

Five additional backcrosses:

selecting + mep^R mat a^T + + progeny

**SERIES 2 N. tetrasperma mat A^T into N. crassa:**

Cross to switch mating type – DJ1540:

\[
\begin{align*}
+ \text{ mep}^R \text{ mat a}^T & + + \\
\text{ro-10 mep}^S \text{ mat AC al-2 un-18} & \\
\text{f}_1 \text{ (DJ1307-2)} & \\
\text{N. tetrasperma (FGSC 2508)} &
\end{align*}
\]

First backcross:

\[
\begin{align*}
+ \text{ mep}^R \text{ mat a}^T & + + \\
\text{ro-10 mep}^S \text{ mat AC al-2 un-18} & \\
\text{f}_1 \text{ (DJ1540-2)} & \\
\text{N. crassa (FGSC 3790)} &
\end{align*}
\]

Five additional backcrosses:

selecting + mep^R mat a^T + + progeny

**SERIES 3 N. crassa mat a^C INTO N. tetrasperma:**

Cross to switch mating type and markers – DJ1700:

\[
\begin{align*}
\text{ro-10} & + + + + \text{ mat A}^C \text{ cyh-15 al-2 un-18} \\
+ \text{ nit-2 leu-3 un-3 mat a}^C \text{ cyh-1R} & + + \\
\text{N. crassa (DJ1687-82)} &
\end{align*}
\]

First hybrid cross – DJ1725:

\[
\begin{align*}
\text{nit-2 leu-3 un-3 mat a}^C \text{ cyh-1R} & + + \\
+ + + + \text{ mat A}^T \text{ cyh-1S} & \\
\text{N. tetrasperma (FGSC 2508)} &
\end{align*}
\]

Six backcrosses:

begining with nit-2 leu-3 un-3 mat a^C cyh-1R f_1 progeny (DJ1725-1) and using N. tetrasperma (FGSC 2508) as the recurrent parent

**SERIES 4 N. crassa mat A^C INTO N. tetrasperma:**

First backcross:

\[
\begin{align*}
\text{ro-10 mep}^S \text{ mat AC al-2 un-18} & f_1 \text{ (DJ1307-14)} \\
+ \text{ mep}^R \text{ mat a}^T & + + \\
\text{N. tetrasperma (DJ1243-2)} &
\end{align*}
\]

Five additional backcrosses:

selecting ro-10 mep^S mat AC al-2 un-18 progeny

**Figure 1.**
Figure 2.

A. *N. crassa* ($mat^A C \times mat^A C$) No recombination block

B. *N. tetrasperma* ($mat^A T \times mat^A T$) Recombination blocked between *nit-2* and *arg-13*

C. Introgressions of mating-type chromosomes between *N. crassa* and *N. tetrasperma*

First Hybrid Cross (DJ1307): $mat^A C \times mat^A T$
hybrid progeny (Recombination reduced but not blocked)

Series 1: $mat^A T \times mat^A C$
After 6 generations in *N. crassa* background (No recombination block)

Series 2: $mat^A T \times mat^A C$
After 6 generations in *N. crassa* background (Recombination blocked except for *nit-2-leu-3*)

Series 3: $mat^A C \times mat^A T$
After 6 generations in *N. tetrasperma* background (Recombination blocked)

Series 4: $mat^A C \times mat^A T$
After 6 generations in *N. tetrasperma* background (Recombination blocked)