**Why are Neurospora crassa crosses that are homozygous for a large duplication barren?**

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Crosses homozygous for the duplication *Dp(AR17)* are barren regardless of RIP. *Sad-1*, a semi-dominant suppressor of meiotic silencing, suppresses the barrenness of duplication-heterozygous but not of duplication-homozygous crosses. Could it be that in the context of the homozygous cross the *sad-1+* allele is not detected as being unpaired, and consequently, *Sad-1* fails to suppress meiotic silencing?

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In *Neurospora crassa*, crosses between some translocations and normal sequence strains can yield segregants that are now duplicated for the translocated segment (Perkins 1997). When the duplication strains are themselves crossed with normal sequence strains they impart a characteristic barren phenotype, that is, the crosses produce normal-looking perithecia but yield only very few progeny ascospores. The barrenness is caused by a process called meiotic silencing which degrades mRNA from any gene that is unpaired in meiosis, and consequently also silences all homologous genes, including those that may themselves be paired (Lee *et al.* 2004). Since duplications can include genes required for meiosis and ascus development, and one copy of each such gene remains unpaired in the meiosis of a duplication-heterozygous cross, their silencing renders the cross barren. The *Sad-1* gene which encodes an RNA-dependent RNA polymerase homologue is required for meiotic silencing. Semi-dominant *Sad-1* mutations can suppress the barren phenotype of duplication-heterozygous crosses (Shiu *et al.* 2001). Crosses between duplication and normal sequence strains also show a significant increase in the frequency of mutations in duplication-borne genes (Bhat and Kasbekar 2001; Perkins *et al.* 1997). The mutations are caused by the pre-meiotic genome defense mechanism called RIP (repeat-induced point mutation) which induces multiple G:C to A:T mutations in DNA sequences that are duplicated in the otherwise haploid genome (Selker 1990). The *rid-1* gene, which encodes a DNA cytosine methyltransferase homologue, is required for RIP and RIP does not occur in crosses that are homozygous mutant for *rid-1* (Freitag *et al.* 2002). We have reported previously that crosses homozygous for the duplication *Dp(AR17)* were as unproductive as the *Dp(AR17)* heterozygous cross (Bhat and Kasbekar 2001). If we assume that duplication-borne genes are not unpaired in the homozygous cross, then we would not expect them to be subjected to meiotic silencing. It has been suggested that the low productivity of duplication-homozygous crosses might be due to the occurrence of RIP in both the parental nuclei (Shiu *et al.* 2001). However, this suggestion was not consistent with our proposal that even heterozygosity for a large duplication was capable of titrating out the RIP machinery (Bhat and Kasbekar 2001; Bhat *et al.* 2003). In which case the homozygous cross should not experience more RIP than the heterozygous cross. Here we ask whether the barren phenotype of *Dp(AR17)* homozygous crosses can be accounted for by RIP or meiotic silencing.

We constructed *mat A* and *mat a* strains of the genotypes *Dp(AR17), dow+/dow, rid-1; Dp(AR17), dow+/ dow,* and *Sad-1; Dp(AR17), dow+/dow* (Bhat 2004) and used them to set up crosses that were homozygous for *Dp(AR17)* as well as (1) homozygous for *rid-1*, (2) heterozygous for *rid-1* or (3) heterozygous for *Sad-1*. These strains were also crossed with the wild type to obtain crosses that were (4) heterozygous for *Dp(AR17)* and (5) heterozygous for both *Sad-1* and *Dp(AR17)*. Multiple crosses were examined for each type of cross. All crosses were coded and scored "blind" as barren, intermediate or fertile (by A. B., Meenal Vyas, Ranjan Tamuli and Prakash Arumugam) and the results are summarized in Table 1. Crosses of type 1, 2, 3 and 4 were all about equally barren and only type 5 crosses were non-barren.

That crosses of type 1 (homozygous for *rid-1* and *Dp(AR17)*) and type 2 (heterozygous for *rid-1* but homozygous for *Dp(AR17)*) were indistinguishable ruled out the possibility that the barren phenotype of the duplication-homozygous cross was due to RIP. The barrenness of type 4 (heterozygous for *Dp(AR17)*) and the non-barrenness of type 5 (heterozygous for *Sad-1* as well as for *Dp(AR17)*) re-confirmed earlier studies which demonstrated suppression of the barrenness of *Dp(AR17)* heterozygous crosses by the semi-dominant *Sad-1* mutant (Bhat *et al.* 2003). The barrenness of type 3 crosses (heterozygous for *Sad-1* but homozygous for *Dp(AR17)*) was a novel and unexpected result for which we do not have any simple explanation. That a semi-dominant *Sad-1* mutation failed to confer fertility to duplication-homozygous crosses was also noted by Shiu *et al.* (Shiu *et al.* 2001).

We suggest the following model to account for the barrenness of the type 3 crosses. The duplication-borne genes may frequently switch "partners" in this cross and they may be transiently unpaired during these switches. Pairing and unpairing of multiple genes might reflexively impair detection of the absence of pairing at *Sad-1*. The *sad-1+* allele must be detected as being unpaired for meiotic silencing to become suppressed (Shiu and Metzenberg 2002). If the *Sad-1* mutant allele is rendered recessive in this way, the triggering of meiotic silencing during the transient unpairing would make type 3 crosses as

unproductive as those of type 4 (heterozygous for *Dp(AR17)*) and type 2 (effectively only homozygous for *Dp(AR17)*). We thank Prakash Arumugam, Meenal Vyas and Ranjan Tamuli for help in scoring the crosses.

Table 1: Productivity of *Dp(AR17)* homozygous and heterozygous crosses, in the presence and absence RIP.

| Type of cross | Productivity of the cross |
|---|---|
| 1 rid homozygous; Dp homozygous | (B, I, B) (B, B, B) (I, I, I) (I, F, F) (B, I, I) (B, I, I) (B, I, B) (B, I, I) |
| 2 rid heterozygous; Dp homozygous | (B, I, B) (B, I, B) (B, B, B) (I, I, I) |
| 3 Sad-1 heterozygous; Dp homozygous | (I, F, I) (I, I, F) (B, I, I) (I, F, I) (B, B, B) |
| 4 rid-1 heterozygous; Dp heterozygous | (I, I, B) (I, F, I) (B, B, B) (B, I, I) (B, I, B) |
| 5 Sad-1 heterozygous; Dp heterozygous | (F, F, F) (F, F, F) (F, F, F) |

Dp= Dp(AR17), B= barren, F= fertile and I= intermediate fertility Each set of three scores, within parentheses, represents "blind" scoring of an individual cross by AB, MV and RT, in that order.

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