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Wild-isolated Neurospora crassa strains that increase fertility of crosses with segmental aneuploids used to establish that a large duplication suppresses RIP in a smaller duplication

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Wild-isolated Neurospora crassa strains that increase fertility of crosses with segmental aneuploids used to establish that a large duplication suppresses RIP in a smaller duplication

Abstract

Crosses involving strains bearing large segmental duplications are characteristically barren and produce only very few viable ascospores. We report here that the productivity of duplication x euploid crosses can be influenced by the euploid parent. The yield of ascospores was exceptionally low in crosses with the wild-isolated strains Golikro (FGSC# 4830) and Costa Rica (FGSC# 852) and exceptionally high in crosses with Lahore-1 (FGSC #1824), Dagguluru-1 (FGSC #3360), Okeechobee (FGSC # 3968), and Tiassale (FGSC # 4825). The four strains that increased productivity were used in crosses with strains bearing the duplication *Dp(IBj5)* together with a small (1.3 kb) duplication of the *erg-3* gene. This made it possible to obtain sufficient numbers of progeny to establish that presence of *Dp(IBj5)* suppresses RIP in *erg-3*.

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Wild-isolated *Neurospora crassa* strains that increase fertility of crosses with segmental aneuploids used to establish that a large duplication suppresses RIP in a smaller duplication

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Crosses involving strains bearing large segmental duplications are characteristically barren and produce only very few viable ascospores. We report here that the productivity of duplication x euploid crosses can be influenced by the euploid parent. The yield of ascospores was exceptionally low in crosses with the wild-isolated strains Golikro (FGSC# 4830) and Costa Rica (FGSC# 852) and exceptionally high in crosses with Lahore-1 (FGSC #1824), Dagguluru-1 (FGSC #3360), Okeechobee (FGSC # 3968), and Tiassale (FGSC # 4825). The four strains that increased productivity were used in crosses with strains bearing the duplication *Dp(IBj5)* together with a small (1.3 kb) duplication of the *erg-3* gene. This made it possible to obtain sufficient numbers of progeny to establish that presence of *Dp(IBj5)* suppresses RIP in *erg-3* .

Neurospora strains bearing large (e.g., >100 kb) chromosomal segment duplications (segmental aneuploids) can be obtained as segregants from crosses heterozygous for some insertional or quasiterminal translocations (reviewed in Perkins 1997 Adv. Genet. **36:** 239-398). Sexual crosses made with segmental aneuploid strains display a characteristic "barren" phenotype; they form perithecia but only a few viable ascospores. Although the barren phenotype is useful in signalling the presence of segmental aneuploidy, it makes subsequent genetic analyses very tedious because of the difficulty in obtaining sufficient numbers of progeny. Therefore a need was felt to identify euploid strains that might increase ascospore yields in crosses with segmental aneuploid strains.

A recent study involving crosses between *Dp(AR17)* duplication strains and eight wild-isolated strains revealed that crosses with two wild strains, Golikro (FGSC# 4830) and Costa Rica (FGSC# 852), produced significantly (approx 100x) fewer ascospores (Noubissi *et al*. 2000 Fungal Genet. Biol. **31:** 91-97). Results summarized in Table 1 show this is true even in crosses with two other duplication strains, *Dp(OY329)* and *Dp(S1229)* and suggested that the productivity of barren crosses can be influenced by the euploid parent. This motivated us to examine wild-isolated strains for any that *increased* ascospore production in otherwise barren crosses.

We examined 71 of the 74 wild-isolated *N. crassa* strains listed as *mat A* in the FGSC strain catalogue (7th edition, 1998). The three strains not tested were Lankala Koderu-1, FGSC 3358, Roanoke-1m, FGSC 2227; and Libreville, FGSC 4823. Each wild strain was crossed with *Dp(AR17), Dp(OY329)* and *Dp(S1229)* strains. The *Dp(S1229)* strains FGSC #264 and FGSC #265 were obtained from the FGSC. The *Dp(AR17)* strains *A30* and *A40* , and the *Dp(OY329)* strain *C25-3* are described by Bhat and Kasbekar (2001 Genetics **157**: 1581-1590). Another *Dp(OY329)* strain, *C23-13,* was constructed by a similar approach to that used for constructing *C25-3*. The crosses were made on synthetic crossing medium in petri dishes and after 31 days the ascospore yields were estimated by inspecting the lids in a dissection microsope. Discernibly more ascospores were produced in the crosses with the wild-isolates Lahore-1 (FGSC #1824), Dagguluru-1 (FGSC #3360), Okeechobee (FGSC # 3968) and Tiassale (FGSC # 4825). *Dp(AR17), Dp(OY329)* and *Dp(S1229)* have previously been shown to suppress RIP in a smaller gene sized duplication, presumably by titrating out the RIP machinery (Bhat and Kasbekar 2001 Genetics **157**: 1581-1590). We verified that the Lahore-1, Dagguluru-1, Okeechobee and Tiassale strains did not interfere with the ability of *Dp(AR17)* and *Dp(OY329)* to effect such suppression (data not shown).

Dp(IBj5) suppresses RIP in *Dp1.3^{ec} hph*:

We wanted to test whether *Dp(IBj5)* also suppresses RIP in a small duplication. *Dp(IBj5)* is an insertional duplication on IR of a VIL segment (from *cpc-1* through *ylo-1*) and strains bearing this duplication can be obtained as segregants from crosses of the translocation strain *T(VIL > IR) IBj5 cpc-1 mat A* (FGSC #443) with normal sequence strains. The small duplication was represented by *Dp 1.3 ^{ec}hph*, which is comprised of a 1.3 kb fragment of *erg-3* that is marked with the *hph* gene for hygromycin-resistance, and targets RIP to the *erg-3* locus on LGIIIR (Prakash *et al*., 1999 Microbiology **145**: 1443-1451). Colonies generated from *erg-3* mutant ascospores have a characteristic morphology on Vogel's-sorbose agar medium and are easily scorable under a dissection microscope (Noubissi *et al*. 2000 Fungal Genet. Biol. **31:** 91-97). The IBj5 translocation strain was crossed with a *Dp 1.3^{ec} hph* strain to generate *Dp 1.3^{ec} hph* ; *Dp(IBj5)* segregants. Crosses of these segregants with the standard laboratory wild type strains *74-OR23-1 mat A* or *OR8-1 mat a* were so severely barren that despite repeated attempts we could not obtain sufficient numbers of progeny to test whether *Dp(IBj5)* suppresses RIP in *Dp 1.3^{ec} hph*. Therefore we resorted to crossing a *Dp 1.3^{ec} hph* ; *Dp(IBj5) mat a* segregant with the Lahore-1, Dagguluru-1, Okeechobee and Tiassale strains. In a single attempt we recovered, respectively, 182, >2500, 81 and 34 progeny from these crosses. None of these progeny were mutant in *erg-3*. Combining the results for the crosses with the Lahore-1, Okeechobee and Tiassale strains, the *erg-3* mutation frequency is estimated to be less than 1/297 (i.e., < 0.3%). We examined 1003 progeny from the cross with Dagguluru-1, thus the *erg-3* mutation frequency in this cross was less than 1/1003 (i.e., < 0.1%). These values are much lower than the *erg-3* mutation frequencies in crosses of these four wild-isolates with a *Dp 1.3^{ec} hph mat a* strain (Noubissi *et al*. 2000 Fungal Genet. Biol. **31:** 91-97) and therefore allow us to conclude that the IBj5 duplication, like the AR17, OY329 and S1229 duplications, suppresses RIP in the smaller duplication *Dp 1.3^{ec} hph*.

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Figure 1.: Relative map positions in the *Dp 1.3^{ec} hph* ; *Dp(IBj5) mat a* strain.

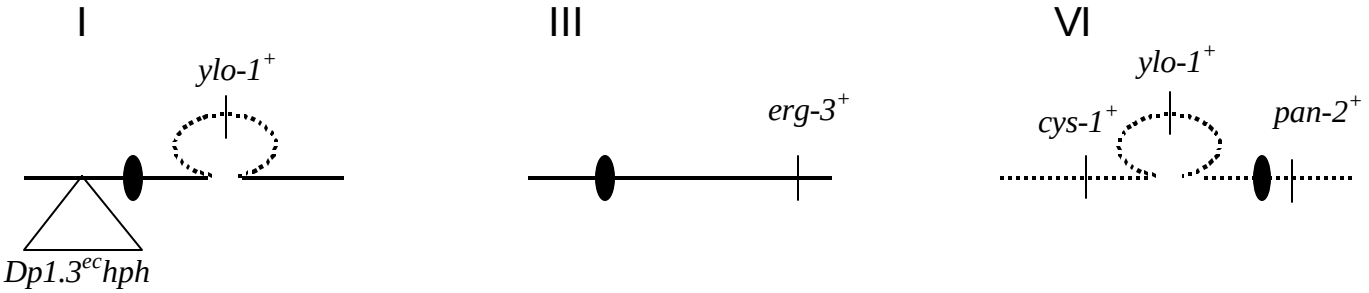

Table 1 : Yield of ascospores from crosses of *mat a* segmental aneuploid strains *Dp(S1229*) and *Dp(OY329*) with selected wild isolates

Segmental Aneuploid strains	"Low-RIP" strains			"High-RIP" strains			
	Dacca (FGSC 4704)	Carrefour Dufort (FGSC 4760)	Adiopodoume (FGSC 430)	Golikro (FGSC 4830)	Ravenswood-1 (FGSC 3212)	Agudas Rd-1 (FGSC 6203)	Costa Rica (FGSC 852)	Merger (FGSC 4713)
Dp(S1229)	$6.6x10^4$	$5x10^5$	$8.3x10^4$	19	10^5	$1.5x10^5$	20	$1.5x10^5$
Dp(OY329)	$5x10^4$	$5x10^4$	82	19	$2.5x10^4$	20	78	$2.5x10^4$

Estimation of ascospore numbers as is in Noubissi *et al*, 2000, Fungal Genet. Biol. **31** : 91-97.