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Repeat-Induced Point Mutation (RIP) in Crosses with Wild-Isolated Strains of *Neurospora crassa:* Evidence for Dominant Reduction of RIP

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Noubissi, F. K., McCluskey, K., and Kasbekar, D. P. 2000. Repeat-induced point mutation (RIP) in crosses with wild-isolated strains of *Neurospora crassa:* Evidence for dominant reduction of RIP. *Fungal Genetics and Biology* 31, 91–97. Seventy-one wild-isolated strains of *Neurospora crassa* were examined for their ability to support repeat-induced point mutation (RIP) in the *erg-3* locus. RIP was exceptionally inefficient but detectable in crosses with the strain FGSC 430 from Adiopodoume, Ivory Coast. We could find no consistent differences in ascospore yields when wild isolates identified as "low-RIP" or "high-RIP" strains were crossed with strains bearing the segmental duplication *Dp(IIIR > [I; II])AR17*. This suggested that RIP may not be responsible for the barren phenotype of crosses involving segmental duplication strains. © 2001 Academic Press

Index Descriptors: RIP; dominant suppression; segmental aneuploidy; barren phenotype; *erg-3; dow*.

A unique mutational process called repeat-induced point mutation (RIP) that causes multiple G:C to A:T transition mutations and methylation of cytosine residues in DNA sequences that are duplicated in an otherwise haploid nucleus occurs during crosses in *Neurospora crassa*, in the dikaryotic stage between fertilization and karyogamy (for reviews see Selker, 1990; Irelan and Selker, 1996). Although RIP has been known for several years, no mutants have thus far been reported with a "RIP-less" phenotype. *N. crassa* is a heterothallic species, that is the two nuclei of the dikaryon are contributed by different parental strains. Consequently, in the absence of suitable balancer chromosomes, it is difficult to obtain homozygosity for recessive mutations that affect the diplophase (but see Leslie and Raju, 1985). However, even dominant, single-gene, mutations affecting RIP have not been reported thus far. Recent studies in our laboratory showed that a chromosome segment duplication, *Dp(IIIR → [I;II])AR17*, can dominantly suppress RIP in a smaller gene-sized duplication, possibly by titrating out the RIP machinery from the dikaryotic cell (A. Bhat and D. P. Kasbekar, unpublished observations). Since *N. crassa* is a highly polymorphic outbreeder (Perkins *et al.*, 1976), it was conceivable that additional classes of dominant RIP suppressors might be present in natural populations. The primary objective of this work was to screen wild-isolated strains of *N. crassa* for the ability to dominantly suppress RIP.

The *erg-3* gene provides an excellent target for scoring the occurrence of RIP. It is located in linkage group (LG) IIIR and encodes the *erg*osterol biosynthetic enzyme sterol C-14 reductase (Perkins *et al.*, 1982; Papavinasasundaram and Kasbekar, 1994). Prakash *et al.* (1999) constructed strains in which a tagged duplicate copy of a 1.3-kb fragment of *erg-3*, designated *Dp 1.3^{ec} hph*, was introduced ectopically to target RIP to *erg-3*. RIP-induced *erg-3* mutants are viable and have altered sensitivities to isoflavonoids and to the steroidal glycoside α-tomatine (Sengupta *et al.*, 1995). Whereas the wild type is resistant to isoflavonoids and sensitive to tomatine, *erg-3* mutants are resistant to tomatine and sensitive to isoflavonoids.

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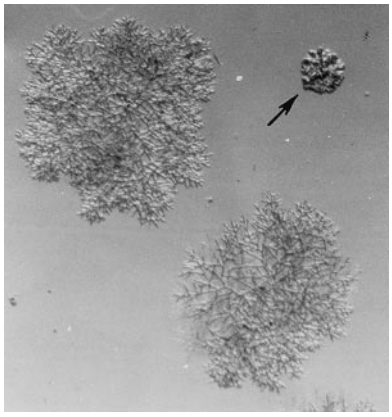

FIG. 1. Colonies from two wild-type and one *erg-3* mutant (arrow) ascospores on Vogel's–sorbose medium.

Most importantly for our present objective, the colonies generated from *erg-3* mutant ascospores exhibit a characteristic growth morphology on Vogel's–sorbose agar medium that allows them to be unambiguously identified by merely inspecting the plates under a dissection microscope (Fig. 1). In this study, *Dp 1.3^{ec} hph* strains were crossed with the wild-isolated strains and the frequency of *erg-3* mutants among the progeny was scored. Our results suggest that a wild-isolate from Adiopodoume, Ivory Coast can exert a dominant suppression of RIP in *erg-3.* To our knowledge this is the first investigation of RIP in wild-isolated strains of Neurospora.

It has been suggested that RIP may be responsible for the "barren" phenotype of crosses involving strains bearing large chromosome segment duplications (Perkins *et al.*, 1997). Barren crosses produce perithecia but few or no asci or ascospores. It was conceivable that mutation and methylation of large stretches of the genome by RIP in some way interfered with perithecial development and thus caused barrenness. As a preliminary investigation of this hypothesis we examined whether the barren phenotype was suppressed (i.e., more ascospores were produced) in crosses between *Dp(IIIR → [I;II])AR17* segmental aneuploid strains and four wild isolates that were identified as "low-RIP" strains.

MATERIALS AND METHODS

Strains

Hundreds of Neurospora strains have been isolated from various "wild" populations from all over the world. These are maintained by the Fungal Genetics Stock Center (FGSC), Department of Microbiology, University of Kansas Medical Center, Kansas City, Kansas. The strains are maintained in suspended animation on silica gels that were prepared shortly following their isolation from the wild (Perkins *et al.*, 1976). Many of the wild-isolated strains have been crossed with standard reference strains representing each of the nonhomothallic species in this genus and this has resulted in the ascertainment of species and mating type of a large subset of wild-isolated strains. We set out to screen the *N. crassa* wild isolates of the *mat A* mating type for any that may exert dominant effects on RIP. The FGSC strain catalogue (6th edition, 1996) lists 74 such strains. Three of them (Groveland-1c, FGSC 1945; Esterillo Este Rd-3, FGSC 6206; and Tucamanduba, FGSC 7556) behaved in our hands as *mat a.* One strain (Lankala Koderu-1, FGSC 3358) is a thiamine auxotroph and was not tested and two strains (Roanoke-1m, FGSC 2227; and Libreville, FGSC 4823) did not grow following their transit from the United States to India. Thus, this study reports on 71 wild-isolated strains.

The 74-OR23-1 *mat A* (FGSC 987) and OR8-1 *mat a* (FGSC 988) strains represent the standard Oak Ridge wild-type background and were obtained from the FGSC.

The construction of the *Dp 1.3^{ec} hph mat a* and *Dp 1.3^{ec} hph mat A* strains has been described by Prakash *et al.* (1999). These strains contain a transgene on LG I, designated *Dp 1.3^{ec} hph*, that is marked by the bacterial *hph* gene for resistance to the antibiotic hygromycin B. Additionally, the transgene contains a 1.3-kb fragment of *erg-3* which serves to target RIP to the *erg-3* locus.

The details of the construction of the *Dp(IIIR → [I;II])AR17 mat a* strains (A17, A40, A52, and B23) will be described elsewhere. Briefly, the A17, A40, and A52 strains were obtained as progeny from a cross between *Dp 1.3^{ec} hph mat A* and translocation strain *T(IIIR → [I;II])AR17, mat a* (Perkins lab stock No. xx-366 = FGSC 1463). The translocation strain was provided by David D. Perkins (Stanford University) and is described by Perkins (1997). Roughly one-third of the viable progeny from this cross inherit LG's I and II from the translocation parent and LG III from the euploid parent and consequently become duplicated for the translocated segment of LG III. B23 is a *Dp(IIIR → [I;II])AR17* segregant that was obtained from a cross between the wild type and a *Dp(IIIR → [I;II])AR17* sibling of the A17, A40, and A52 strains. The duplicated segment includes a wild-type allele at the LG III locus *dow;* and the identification of *dow* mutants among the progeny from crosses of A17, A40, A52, and B23 with the wild type allowed us to verify that

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they were indeed *Dp(IIIR → [I;II])AR17* strains. The *dow* mutants were presumably generated by RIP in the segmental aneuploid parent.

Growth Conditions

Crossing and maintenance of the Neurospora strains were essentially as described by Davis and De Serres (1970). Antibiotic resistance was scored by streaking conidia onto 1.5% agar plates containing Vogel's N medium plus FGS (0.05% fructose, 0.05% glucose, and 2% sorbose) and supplemented with the antibiotic. The antibiotics tested were α-tomatine (Sigma) at 90 μg/ml made from a 25 mg/ml stock solution in dimethylformamide and hygromycin B (Sigma) at 200 μg/ml made from a 100 mg/ml aqueous stock solution. After an overnight incubation at 30°C on tomatine-supplemented medium, growth of only the *erg-3* mutant strains can be observed (Sengupta *et al.*, 1995). Only strains expressing the *hph* gene could grow on hygromycin medium.

Ascospore Collection

Crosses were performed by confrontation between mycelia inoculated as plugs on synthetic crossing medium in petri dishes. Generally, ascospores began to be shot within 14–16 days. They were harvested by washing the lids with ~1 ml water. A first harvest was made 31 days after the crosses were set up; then the petri dish lids were replaced and a second harvest was made after an additional 14 days.

Scoring RIP Efficiencies

Ascospores bearing RIP-induced *erg-3* mutants exhibit a characteristic slow-growth morphology on Vogel's–FGS agar medium that enables them to be scored merely by inspection (Fig. 1). Reliability of identifying the *erg-3* mutant phenotype in this way was established by confirming the ability of the conidia to germinate and grow on tomatine-medium.

Double-Blind Experiment to Identify the Adiopodoume Strain

Five cultures of the Adiopodoume strain (FGSC 430) and 14 other wild-isolated strains were handed over by F.K.N. to her colleague A. Bhat (A.B.), who relabeled three Adiopodoume cultures and seven other wild isolates with the numerals 1,2, . . . 10 and handed them to another colleague, A. Prakash (A.P.), who rerelabeled the cultures A, B, C, . . . J and returned them to F.K.N. Thus, there were two keys, one with A.B. indicating which cultures were numbered 1,2,3, . . . 10 and the other with A.P. indicating which of these was labeled A, B, C, . . . J. F.K.N. knew only that at least 1 of the 10 cultures was Adiopodoume. Only A.B. was aware that there were in fact 3 Adiopodoume cultures. The challenge was to predict which of the individual cultures A, B, C, . . . J were Adiopodoume and which were non-Adiopodoume on the basis of RIP efficiencies in crosses with *Dp 1.3^{ec} hph mat a* and to compare the predictions by reference to the keys. The cultures coded B, C, and E were correctly identified as Adiopodoume and A, D, F, G, H, I, and J were correctly identified as the non-Adiopodoume strains (see Results). The non-Adiopodoume strains used in the crosses were A, Maripasoula (FGSC 6240); D, Old Man Bay-2 (FGSC 8182); F, Georgetown-4 (FGSC 4723); G, Carrefour Dufort (FGSC 4710); H, Pescail (FGSC 4714); I, Arena Reser (FGSC 7547); and J, Ravenswood-1 (FGSC 3212).

Estimating the Yield of Ascospores from the Barren Crosses

The crosses between the wild-isolated strains and the *Dp(IIIR → [I;II])AR17* segmental aneuploid strains were barren and yielded very few ascospores. On the 31st day following the cross, the ascospores were harvested from the petri dish lids in 1 ml water, pelleted by spinning, and resuspended in 100 μl water. The concentration of the resuspended ascospores was determined using a hemocytometer. From this value we obtained an estimate of the total number of harvested ascospores.

RESULTS

erg-3 Mutation Frequencies in Crosses Involving the Wild-Isolated Strains

The crosses between the *Dp 1.3^{ec} hph* strains and the wild isolates were all quite productive. Table 1 summarizes the RIP frequencies among ascospores harvested at 31 and 45 days following the setting up of the cross. None of the crosses showed an absolute inability to RIP. Thus, none of the 71 wild isolates tested contains a completely penetrant dominant RIP suppressor. However, there was a relatively wide range in RIP efficiencies. At 31 days the *erg-3* mutation frequency was >10% in 5 crosses (involv-

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TABLE 1

erg-3 Mutation Frequencies in Crosses of Wild-Isolated *N. crassa* Strains with *Dp 1.3*^{ec} *hph mat a*

Strain designation		FGSC Nos.	Number of segregants examined		Frequency of *erg-3* mutants	
			31 days	45 days	31 days	45 days
Bangladesh	Dacca	4704	967	283	0.1	0.8
British West Indies	Old Man Bay-2	8182	136	418	5.1	19.1
Congo	Madingo	4822	493	502	2.6	3.6
Continental United States	Bayou Chicot-5	3227	258	1162	3.1	3.7
	Coon-4	3199	323	205	4.6	8.7
	Elizabeth-4	3223	590	619	4.2	17.6
	Everglades	3972	360	1232	5.0	15.2
	Florida City	3973	192	196	1.6	9.2
	Franklin	7833	341	466	2.6	3.0
	Groveland-1c	1945*	232	192	25.0	24.47
	Homestead-2	3970	360	315	1.1	5.1
	Houma-1n	2220	276	631	5.4	23.8
	Iowa-1	2222	240	803	4.6	8.7
	Mauriceville-1c	2225	500	512	3.8	8.2
	Northside Planting	7838	365	348	4.6	10.1
	Okeechobee	3968	383	371	3.1	8.1
	Ravenswood-1	3212	157	316	17.5	21.5
	Roanoke-1m	2227	ND	ND	ND	ND
	Saratoga-1l	3226	274	337	5.1	10.4
	Scott A	3885	210	354	1.9	9.0
	Spurger-3	3201	319	318	1.6	9.4
	Sugartown-1	3210	923	352	0.9	8.2
	Welsh-1e	2229	157	290	2.6	10.3
	Yeehaw Junction	3969	411	199	8.3	18.1
Costa Rica	Agudas Rd-1	6203	337	182	11.9	18.7
	Costa Rica	851	235	268	6.8	16.8
	Costa Rica	852	188	330	10.6	13.0
	Covolar	6212	141	414	2.1	5.8
	Esterillo Este	6208	272	277	3.2	5.1
	Esterillo Este Rd-3	6206*	328	321	5.5	8.0
	Jaco-1	6202	99	337	4.0	20.8
	Jaco-2	6211	767	782	3.9	6.0
Gabon	Libreville	4823	ND	ND	ND	ND
Haiti	Berard	4708	451	297	0.9	3.4
	Carrefour Dufort	4710	670	540	0.4	2.0
	Carrefour Mme.Gras	4824	214	150	9.8	15.3
	Haut Diquini	4711	208	569	4.3	14.4
	Merger	4713	478	331	12.6	15.7
	Pescail	4714	223	448	6.3	11.8
	Puilboreau Mt	4716	407	939	3.4	3.7
India	Aarey-1e	2499	500	249	5.6	6.0
	Dagguluru-1	3360	210	167	0.9	5.4
	Lankala Koderu-1	3358	ND	ND	ND	ND
	Madurai	4717	318	195	2.5	5.1
	Vallancheri	4720	378	284	1.1	3.2
	Vickramam	6688	471	284	2.5	3.2
Ivory Coast	Adiopodoume	430	308	397	<0.3	0.3
	Golikro	4830	383	321	0.3	2.8
	Ibogue	4833	218	358	5.5	9.5
	Issia	4834	464	901	1.9	4.8
	N'Douci	4835	250	387	1.6	2.3
	Sakota	4837	650	445	4.8	8.1
	Tiassale	4825	397	478	4.5	16.1

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TABLE 1—*Continued*

Strain designation		FGSC Nos.	Number of segregants examined		Frequency of *erg-3* mutants	
			31 days	45 days	31 days	45 days
Liberia	Liberia	961	738	158	3.3	9.5
Malaya	Georgetown-4	4723	105	225	4.8	3.6
	Georgetown-5	4725	414	239	3.5	8.8
	Georgetown-6	4726	224	201	8.0	14.4
Mexico	Chemax	6634	527	250	1.3	1.6
	Kabah	6638	184	318	2.7	7.9
Pakistan	Lahore-1	1824	241	360	0.8	1.7
Panama	Panama CZ30.6	1131	329	444	1.8	13.3
Puerto Rico	Colonia Paraiso	3693	167	328	7.2	10.4
South America	Arena Reser	7547	159	459	5.0	15.5
	Digitima Creek-1	5910	117	512	2.6	5.7
	Maripasoula	6240	582	373	5.0	18.8
	Orinoco Delta-2	7552	604	388	6.3	18.3
	Puerto Ayachucho	4730	111	232	4.5	4.7
	Rondon	4705	1462	736	1.56	2.7
	Torani Canal	5914	448	379	2.5	3.9
	Tucamanduba	7556*	280	371	2.9	3.77
	Tucamanduba-2	7851	308	884	4.9	3.4
Thailand	Khao Eto	6490	231	148	2.9	3.4
	Klong Rangsit-57	6488	219	965	2.7	2.1
Trinidad	Caroni Swamp	8147	226	520	6.1	12.1
Standard reference strain	74-OR23-1A	987	635	306	8.7	29.4

Note. ND, not determined.
* These strains behaved as *mat a.*

ing the wild isolates Agudas Rd.-1, Costa Rica (FGSC 852), Merger, Ravenswood-1, and Groveland-1c) and these wild isolates were identified as "high-RIP" strains. The frequency was <0.5% in four other crosses (involving Adiopodoume, Dacca, Carrefour Dufort, and Golikro) and the corresponding wild isolates were identified as "low-RIP" strains. In the remaining 62 crosses (87%) the *erg-3* mutation frequencies were in the 0.5–10% range.

At 45 days only the Adiopodoume strain continued to show an *erg-3* mutation frequency <0.5%. In 25 crosses the frequency had increased to >10% and in the remaining crosses it was in the 1.6–10% range. These results are consistent with the observation of Singer *et al.* (1994) that the frequency of RIP increases with the "age" of the cross; "late" harvests show greater RIP frequencies than "early" harvests. However, in 3 crosses (with the strains Georgetown-4, Tucamanduba-2, and Klong Rangsit-57) the *erg-3* mutation frequency at 45 days was, in fact, slightly lower than that at 31 days. It is possible that in these crosses the efficiency of RIP may have "plateaued" by 31 days.

To confirm that the low frequency of *erg-3* mutants in the cross involving the Adiopodoume (FGSC 430) strain was not merely a sampling artifact we performed this cross two more times (independently by F.K.N. and A. Bhat) and again the *erg-3* mutation frequencies were very low (Table 2). In fact, the frequencies in these crosses were comparable with those observed in crosses of *Dp 1.3^{ec} hph*

TABLE 2
erg-3 Mutation Frequencies in Crosses Involving *Dp 1.3^{ec} hph* Strains

Cross	1st Harvest (%)	2nd Harvest (%)
1. *Dp 1.3^{ec} hph mat a* × Adiopodoume	<0.12 (787)	<0.18 (570)
2. *Dp 1.3^{ec} hph mat a* × Adiopodoume	0.05 (1772)	Not determined
3. *Dp 1.3^{ec} hph mat a* × 74-OR23-1 *matA*	8.9 (158)	14.7 (471)
4. *Dp 1.3^{ec} hph mat a* × *Dp 1.3^{ec} hph matA*	10.4 (96)	8.6 (499)
5. *Dp 1.3^{ec} hph matA* × OR8-1 *mat a*	12.8 (39)	12.6 (278)

Note. Number of segregants examined is indicated in parentheses. 1st harvest at 33 days (for cross 2, 31 days); 2nd harvest at 45 days.

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TABLE 3
Yield of Ascospores from Crosses of *Dp(IIIR → [I;II])AR17 mat a* Segmental Aneuploid Strains with Selected Wild Isolates

	Segmental aneuploid strains (*mat a*)			
	A17	A40	A52	B23
Dacca (FGSC 4704)	2.1×10^4	3.2×10^4	2×10^3	2.6×10^4
Carrefour Dufort (FGSC 4760)	9.3×10^3	17.5×10^3	1.5×10^3	6×10^3
Adiopodoume (FGSC 430)	3.5×10^3	4.3×10^3	67	1.5×10^3
Golikro (FGSC 4830)	75	10^3	32	0
Ravenswood-1 (FGSC 3212)	3.0×10^4	5.5×10^3	6.5×10^3	1.8×10^4
Agudas Rd-1 (FGSC 6203)	2.7×10^4	2.1×10^4	4.5×10^3	3.5×10^3
Costa Rica (FGSC 852)	0.5×10^3	66	62	28
Merger (FGSC 4713)	1.3×10^4	2.2×10^4	10^3	1.2×10^4

Note. The first four are low-RIP strains and the bottom four are high-RIP strains.

mat a with *Dp(AR17) mat A* segmental aneuploid strains (A. Bhat and D. P. Kasbekar, unpublished observations). This suggested that the Adiopodoume strain exerts a dominant suppression of RIP in *erg-3*.

Identifying the Adiopodoume Strain Based on Its Suppression of RIP in erg-3

We tested whether the suppression of RIP can be used as a defining character to identify the Adiopodoume strain from among a collection of wild-isolated strains in a "double-blind" experiment. Crosses were set up between *Dp 1.3^{ec} hph mat a* and 10 cultures of wild-isolated strains coded A, B, C, . . . J. (The details of the coding and the wild strains used are described under Materials and Methods.) Ascospores were harvested after 31 days, and the following *erg-3* RIP efficiencies were obtained (%): A, 1.1; B, <0.06; C, 0.03; D, 3.2; E, 0.03; F, 1.9; G, 1.7; H, 2.4; I, 1.3; and J, 5.3. Based on these results the strains coded B, C, and E were correctly identified as Adiopodoume and A, D, F, G, H, I, and J were correctly identified as the non-Adiopodoume strains. These results provide conclusive support to our claim that the Adiopodoume strain differs from all the other wild-isolated strains tested by virtue of its ability to dominantly reduce RIP in *erg-3*.

Is RIP Responsible for the Barren Phenotype Caused by Segmental Aneuploidy?

If RIP was responsible for the barren phenotype of crosses involving segmental aneuploid strains, we might expect crosses between the *Dp(IIIR → [I;II])AR17 mat a* segmental aneuploid strains A17, A40, A52, and B23 and the 8 *mat A* wild isolates identified as "low-" or "high-RIP" strains (see above) to show differences in their barren phenotypes. Ascospores were harvested from the petri dish lids and their numbers were estimated as described under Materials and Methods. The results are summarized in Table 3. As can be seen in Table 3 most of the crosses (with the possible exception of those involving the strains Golikro and Costa Rica) produced between 10^3 and 3.2×10^4 ascospores and therefore were about equally barren. Significantly, the crosses with the "low-RIP" strain Adiopodoume produced fewer ascospores than the crosses with the "high-RIP" strains Agudas Rd.-1, Ravenswood, and Merger. Thus, these results do not support the hypothesis that the barrenness of crosses involving segmental aneuploid strains is because of RIP.

The duplicated segment in *Dp(IIIR → [I;II])AR17* includes a wild-type allele at the LG III locus *downy* (*dow*). Earlier studies in our laboratory had revealed that crosses between the strains A17, A40, A52, and B23 and the wild type can generate *dow* mutants, presumably via RIP in the *Dp(IIIR → [I;II])AR17* nucleus, at frequencies that were comparable with those reported by Perkins *et al.* (1997) (A. Bhat and D. P. Kasbekar, unpublished observations). No *dow* mutants were obtained among the 112 segregants that were examined from the cross between A40 and the Adiopodoume strain (RIP frequency < 0.89%). However, *dow* mutants were produced in crosses of A40 with Ravenswood-1 and Merger, at frequencies of 7 of 147 (4.76%) and 2 of 200 (1%), respectively. Although the number of segregants compared in these experiments is small, these results are consistent with the hypothesis that the dominant reduction of RIP by Adiopodoume might extend even to large segmental duplications.

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DISCUSSION

The most important finding in this work was that the Adiopodoume strain exerted a dominant reduction of RIP in *erg-3.* A second finding was that despite this reduction, crosses between Adiopodoume and strains bearing the large chromosomal segment duplication *Dp(AR17)* were not more productive. This suggests either that the reduction of RIP in *erg-3* might not be generalizable to other loci, particularly those covered by *Dp(AR17),* or that RIP might not be a primary cause for the barren phenotype of segmental duplication strains. Our evidence that the reduction of RIP extends to *dow* tends to favor the latter possibility.

The ability of Adiopodoume to support RIP has been investigated previously by Kinsey *et al.* (1994). They found, as did we, that RIP can indeed occur in crosses that are heterozygous for the Adiopodoume genetic background, but they did not compare RIP efficiencies in crosses involving Adiopodoume and non-Adiopodoume strains. It is possible that we detected the reduction because our study compared Adipodoume with 70 other wild-isolated strains. Another reason could be that we monitored RIP events in the *erg-3* gene, whereas they used the *am* gene. It is possible that different genes and genomic regions differ in susceptibility to RIP and to its reduction. One way to make a valid comparison between our results and those of Kinsey *et al.* (1994) would be to segregate their ectopically duplicated *am* gene into a *Dp 1.3*^{ec} *hph mat a* strain and compare the mutation frequencies in *erg-3* and *am* among the progeny from crosses of these segregants with Adiopodoume and 74-OR23-1 *mat A.*

It is interesting that translocations also have been reported to be unusually frequent in crosses involving the Adiopodoume strain (reported by Kinsey and Helber, 1989, as a personal communication from David Perkins). Insertional and quasiterminal translocations have the potential to generate segmental aneuploid segregants via the segregation of the translocation chromosome(s) with normal chromosomes in meiosis (Perkins, 1997). Recent results from our laboratory have suggested that the presence of segmental duplication can protect a smaller duplication from destruction by RIP (A. Bhat and D. P. Kasbekar, unpublished observations). Selker (1990) has suggested that RIP might serve to protect the genome against the proliferation of transposable elements. In this context it is noteworthy that Adiopodoume is the only Neurospora strain known to harbor active copies of a LINE-like transposable element named *Tad.* All other Neurospora strains examined contain only RIP-altered relics of *Tad* (Kinsey *et al.,* 1994). Given the possibility of both dominant RIP suppressors and translocations, the survival of *Tad* in Adiopodoume is perhaps not quite as surprising after all.

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