

Table 2. Allelic frequencies at six loci in French and Porto-Alegre populations.
(N=sample size; *mean allelic frequencies obtained with 15 populations;
for Pgm, see Singh et al. 1982).

		ACPH			ADH			EST-C				
Populations		N	F	S	N	F	S	N	F	S	rare	
French*		2688	0.99	0.01	2668	0.96	0.04	2625	0.88	0.09	0.03	
Porto- Alegre	Sept.	100	0.98	0.02	100	0.63	0.37	100	0.94	0.05	0.01	
	Nov.	145	0.88	0.12	143	0.59	0.41	123	0.96	0.04	-	
		EST-6			α -GPDH			PGM				
Populations		N	F	S	rare	N	F	S	N	F	S	rare
French*		2640	0.27	0.71	0.02	2543	0.53	0.47	-	0.98	0.02	-
Porto- Alegre	Sept.	89	0.37	0.58	0.05	100	0.81	0.19	100	0.86	0.09	0.05
	Nov.	144	0.25	0.75	-	143	0.90	0.10	145	0.84	0.04	0.12

Despite seasonal changes in gene frequency at Porto-Alegre, allelic frequencies in the French populations and in the Brazilian population are very similar for three loci (Acph, Est-C, and Est-6). Of course, migrations of *Drosophila melanogaster* between France and Brasil are not possible and therefore cannot explain the homogeneity in allele frequencies observed at the Acph and esterase loci. Moreover how explain in this case the differentiation at the three other loci (Adh, α -Gpdh, Pgm)? Consequently, our results are not in agreement with neutralist theory. Another conclusion can be drawn: differentiation in allozyme frequencies are shown only for enzymes of energetic metabolism (especially Adh and α -Gpdh) which probably play in the adaptation a more important role than non-specific enzymes (Cavener & Clegg 1978).

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Chaudhuri, G.K. and A.S. Mukherjee.
University of Calcutta, India. Effect of α -Methyl-DL-Methionine on the replication of polytene chromosomes in *Drosophila melanogaster*.

The nucleoproteins of the eukaryotic cell contain acetyl, phosphoryl and methyl side chain groups which are metabolically active. It has been suggested that enzymatic control of side chain metabolism may be involved in the specific control of gene expression and in a more general way in such biochemical pro-

cesses as may be involved in gene activity and cell division (Goodman & Benjamin 1972; Felsenfeld & McGhee 1982). In the present investigation possible effect of methylation using a methyl donor on DNA replication has been examined in *Drosophila* polytene chromosome.

In these experiments salivary glands from late third instar larvae of *Drosophila melanogaster* (Oregon R⁺) were dissected out in buffered *Drosophila* Ringer (pH 6.8) and incubated for 20 mins in α -Methyl-DL-Methionine (obtained from Sigma Chemical Co., USA) at a concentration of 10^{-3} M. The control sets were incubated for the same period in Ringer. The glands were then incubated for 20 minutes in 3 H-thymidine (conc. 400 μ Ci/ml, sp. activity 12,700 mCi/m mole, obtained from Bhabha Atomic Research Centre, Trombay, Bombay, India). The squash preparations of chromosome were processed for autoradiography using Kodak AR 10 stripping film, exposure time being 24 days.

Table 1. Data on the frequency of labelled chromosomes in control and α -Methyl-DL-Methionine treated salivary glands of male and female *Drosophila melanogaster*.

	$^3\text{H-TdR}$ Pulse time(min)	No. of nuclei	Number of labelled nuclei			Number of unlabelled nuclei
			Early patterns (DD-1C)	Mid patterns (2C-3C)	Terminal patterns (3C-3D-CL)	
CONTROL σ	20	649	16 (2.46%)	115 (17.72%)	402 (61.93%)	116 (17.87%)
α -Methyl-DL- Methionine Treated σ	20	894	3 (0.33%)	45 (4.99%)	554 (70.13%)	292 (22.26%)
Control φ	20	518	29 (5.49%)	87 (16.48%)	275 (52.08%)	127 (22.16%)
α -Methyl-DL- Methionine Treated φ	20	1065	2 (0.19%)	24 (2.27%)	735 (70.2%)	304 (28.81%)

Results of the two sets of experiments are presented in Table 1. Data revealed that the frequency of initial and mid labelling patterns (termed DD-1C and 2C-3C) is drastically decreased. In contrast, the frequency of terminal patterns (marked by discontinuous labelling on dark bands) is increased in both sexes. These results suggest that methylation does take place in this system and inhibits the initiation of replication, since it has been suggested earlier that both DD-1C and 2C-3C labelling patterns include successive stages of initiation in *Drosophila* polytene chromosomes (Mukherjee 1982; Mukherjee & Chatterjee 1983).

Interestingly the frequency of labelling of the 20 sites on the autosome 2R is remarkably similar in the two sexes for both control and α -Methyl-DL-Methionine treated preparations, whereas, for the 45 replicating sites in the X-chromosome, the frequencies of labelling are distinctly different in the two sexes. They are much less in the male than in the female. The sites 3C, and 7E, 11A and 12DE are exceptions. When the mean silver grain numbers on each X chromosome segments (1A-12DE) and the autosomal segment 56AB to 60F are compared in the control and treated sets and the ratios of grains X/A are analyzed, it becomes evident that the inhibition is more drastic in the male X chromosome than in the female X (Table 2).

Table 2. Summary of grain count data in control and α -Methyl-DL-Methionine treated glands.

Samples (No. of nuclei)	Mean grain no. on the segment 1A-12DE of X (\pm SE)	Mean grain no. on segment 56AB to 60F of 2R (\pm SE)	X/A ratio (\pm SE)
Control σ (32)	204.5 \pm 4.72*	123 \pm 1.24	1.66 \pm 0.06*
Treated σ (28)	124.14 \pm 6.84	113.2 \pm 2.33	1.09 \pm 0.03
Control φ (24)	215.3 \pm 6.57*	121 \pm 0.77	1.77 \pm 0.1 *
Treated φ (30)	140.4 \pm 6.61	105.5 \pm 5.33	1.33 \pm 0.043

* P < 0.05

These results suggest firstly that methylation - demethylation may indeed be involved in initiation of DNA replication and secondly, X chromosomal organization in male and female may determine a differential methylation in the two sexes. Further works are in progress to find out the amount and distribution of methylating sites on the X and autosomes.

References: Goodman, R.M. & W.B. Benjamin 1972, Chromosoma; Felsenfeld, G. & J. McGhee 1982, Nature 296:602; Mukherjee, A.S. 1982, Current Science; Mukherjee, A.S. & C. Chatterjee 1983, J. of Cell Science.