The In(3L)P, D^3 combination used in the above synthesis was derived in a somewhat similar manner; h was inserted into In(3L)P by a rare double crossover, and In(3L)P, Me h D^3 was constructed from these chromosomes and In(3L)P, Me.

Large paracentrics exist for all major autosomal arms--In(2L)DTD27 (21B; 40), $In(2R)bw^{VDe1}$ (41; 59), In(3L)C90, and In(3R)P110 (81F; 99). Stocks of $In(2L)NS^{L}DTD27^{R}/In(2L)DTD27^{L}NS^{R}$ and $In(2L)Cy^{L}DTD27^{R}/In(2L)DTD27^{L}Cy^{R}$ have been constructed in addition to the $In(3L)C90^{L}P^{R}/In(3L)P^{L}C90^{R}$ complex. These stocks were derived by applying the methods which I have described for deriving crossover products of pericentric inversions (Genetics 99:75-77, 1981).

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Differential puffing activity in two E chromosomal arrangements of D.subobscura.

A comparison of the E chromosome puffing patterns of the different gene arrangements were carried out in order to investigate the possible effect of inversions on gene expression. Two strains of Drosophila subobscura were used: H271 which is homozygous for E_{st} arrangement and Ra121 which is homozygous for $E_{1+2+9+12}$ arrangement. The puffing patterns of late third instar larvae and different aged prepupae were analyzed. The prepupal samples were taken at 0, 4, 10 and 18 hrs after the eversion of the anterior spiracles. 20 individuals were analyzed per developmental stage and strain. Five nuclei were observed from each of the individuals analyzed. For the average degree of puffing activity two criteria were taken into account: (a) size of puffs, and (b) frequency of appearance of each puff at every stage analyzed. The puffs and breakpoints of $E_{1+2+9+12}$ inversion were located using the standard salivary gland chromosome map of Kunze-Mühl and MUller (1958). The breakpoints of $E_{1+2+9+12}$ arrangement are the following: E_1 58D/59A-62D/63A, E_2 58D/62D-64B/64C,

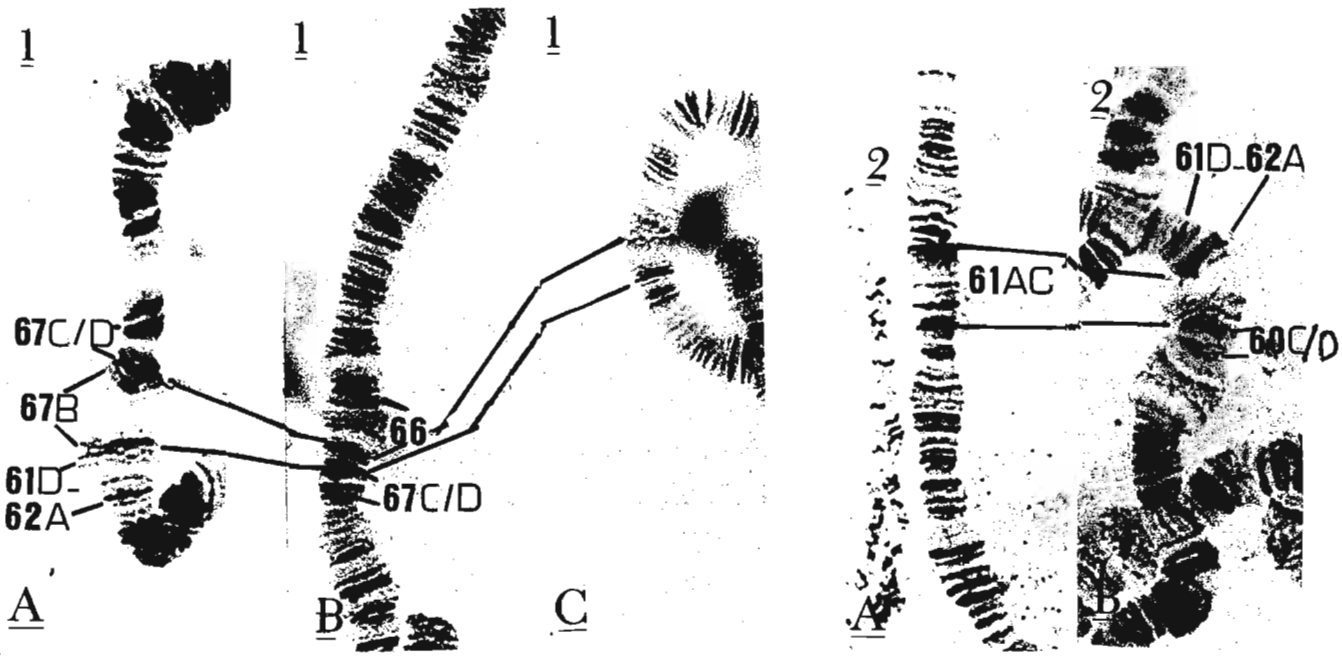

Figure. E chromosomes of D.subobscura: (1A) $E_{1+2+9+12}$ arrangement of Ra121 strain (18h prepupa). (1B) E_{st} arrangement of H271 strain (4h prepupa). (1C) E_{st} arrangement of H271 strain (0h prepupa). (2A) $E_{1+2+9+12}$ arrangement of Ra121 strain (0h prepupa). (2B) E_{st} arrangement of H271 strain (18h prepupa).

E_9 58D/64D-68B/68C, E_{12} 61C/61D-67A/67B. All individuals were dissected in Ringer Drosophila solution (pH 7.2). Salivary glands were fixed in ethyl alcohol: acetic acid (3:1), and were stained in lacto-aceto orcein (acetic orcein 80%, lactic acid 20%). All experiments, cultures and cytological preparations were carried out in a thermoregulated room at 19±1°C.

Several puffs show a similar pattern of activity in E_{st} and $E_{1+2+9+12}$ arrangements, for instance 68DE,69B, 70A and 70BC. Other puffs show quantitative differences or differences in the timing of activity. Finally, a few puffs display strong differences. Among this group can be included the large puffs 67B and 61AC. In the $E_{1+2+9+12}$ arrangement 67B is active in third larvae, regresses by 0h prepupae and after puparium formation increases its activity throughout the prepupal period (Figure 1A). In spite of this, 67B is an occasional puff in the E_{st} chromosome. It only appears actively in the third instar and at the beginning of prepupation and always at low frequency (Figures 1B and 1C).

Another striking difference is the size of 67B in both chromosomes. In $E_{1+2+9+12}$ arrangement 67B is a large puff as can be seen in Figure 1A. The size of this puff varies little thoughout its time of activity. In spite of this, 67B is a small puff in E_{st} chromosome. Its maximum size can be observed on Figure 1C. 61AC shows complementary behaviour (Fig. 2A and 2B). It is a large puff that maintains its activity throughout the prepupal period in E_{st}, and a small occasional puff in $E_{1+2+9+12}$ chromosome. It is important to emphasize the location of these puffs on the E chromosome. Both puffs are located at the boundaries of E_{12} inversion. In the E_{st} chromosome (Fig. 1B and 2B), 67B is located between the active locus 66 and the occasional puff 67C/D, and 61AC is located between the occasional loci 60C/D and 61D-62A. In the $E_{1+2+9+12}$ chromosome 67B is located between 67CD and 61D-62A. It is interesting to note the behaviour of this last site. 61D-62A shows activity both in E_{st} and in $E_{1+2+9+12}$, whereas in E_{st} it is close to 61AC and is never very large (Fig. 2B), in $E_{1+2+9+12}$ it is close to 67B and is always very large (Fig. 1A). It is obvious that 67B and 61AC show the greatest differences in puffing activity between E_{st} and $E_{1+2+9+12}$ arrangements. Both are located at the boundaries of the E_{12} inversion, and not only do they change their position in the chromosome but also the sites close to both are different in the two arrangements. It is possible that these differences in gene activity at puff level can be due to position effect.

References: Kunze-MUhl, E. & E.MUller 1958, Weiter Untersuchungen Uber die chromosomale Strukturtypen bei Drosophila subobscura, Coll.Z.indukt.Abstamm.-Vererb.Lehrer 87:65-84.

Di Pasquale Paladino, A. and P.Cavolina.
University of Palermo, Italy. Caffeine effect on tumor manifestation in the tu-pb stock of D.melanogaster.

Table 1. Complete development in medium containing various concentrations of caffeine.

	% ♀♀ tu	N.♀♀	% ♂♂ tu	N.♂♂
Control	32.82	1301	7.22	1149
Caffeine 500 ug/ml	29.13	1253	5.73	1238
Caffeine 1000 ug/ml	17.61*	1221	3.68*	1060
Caffeine 0.01 M	7.23*	166	1.45*	207

* $P < 0.05$ (compared to control)

We have now investigated the effect of caffeine added to the nutrient medium of developing tu-pb larvae. Statistical analysis of results obtained after complete development of larvae on medium containing various concentrations of caffeine (Table 1) demonstrates that a dose of 500 ug/ml does not exert any influence upon tumor incidence, while concentrations equal to 1000 ug/ml and 0.01 M (corresponding to 1984 ug/ml) exert a significant inhibitory effect upon tumor appearance in adult insects. 0.01M concentration was chosen for experiments involving exposition of larvae to caffeine medium for a limited period of time. Results are shown in Table 2.

Experiments involving egg deposition onto normal medium, followed by transfer onto caffeine medium: tumor incidence is significantly decreased, if compared to control, only in the 48h group. This finding suggests that caffeine is active only during the first stages of development.

Experiments involving egg deposition onto caffeine medium followed by transfer onto normal medium: tumor incidence was found to be lower in groups of larvae transferred from