using Ca^{++} from both female (figure 2, a-e) and male (figure 2, f). These fibres form globules of 300 Å (figure 2, a and b) which are interconnected by thin (~ 30 Å) filaments (figure 2, c). Each globule consists of about 8–10 nucleosomes (80 Å) disposed in a circular way at the globule periphery (figure 2, d). We also observed that these globules are arranged in larger coils along the fibres (figure 2, a, b and e).

The findings in this and also in our previous work[9] indicate that the condensed chromatin fibre is organized in globules composed by the assembly of nucleosomes. We think that, although these globules might correspond to the knobs or supernucleosomes reported by Hozier et al.[10], the arrangement of the nucleosomes in the globules suggests that each globule could be an uncoiled turn of the superhelix described by Finch and Klug[8]. The agglomerates of globules observed suggest also that the supercoils form major coils along the fibre.

The presence of smooth and fine threads on the NaCl preparations suggests they could be products of changes in the protein-DNA complex[12]. Alternatively, they could belong to the category of free DNA as found in 10% of the genome[13]. As to the 30 Å interglobule fibrils, these may correspond to the internucleosomal filaments observed by others[6]. They presumably contain DNA in its B-structure configuration of 3.4 Å/base pair. We do not know whether such connecting fibril is a structural component or is an artefact resulting from stresses during the preparation.

1 This work was supported by grants from Brazilian CNPq, FAPESP and FEDIB.
2 We thank Dr A. Brunner Jr for the permission to use the electron microscope.
3 A.L. Olins and D.E. Olins, J. Cell Biol. *59*, 252a (1973).
4 R.D. Kornberg and J.O. Thomas, Science *184*, 865 (1974).
5 D.R. Hewish and L.A. Burgoyne, Biochem. biophys. Res. Commun. *52*, 504 (1973).
6 P. Oudet, M. Gross-Bellard and P. Chambon, Cell *4*, 281 (1975).
7 H. Ris and D.F. Kubai, A. Rev. Genet. *4*, 263 (1970).
8 J.T. Finch and A. Klug, Proc. natl Acad. Sci. USA *73*, 1897 (1976).
9 M.L. Beçak, K. Fukuda and S.M. Carneiro, Experientia *33*, 1314 (1977).
10 J. Hozier, M. Renz and P. Nehls, Chromosoma (Berl.) *62*, 301 (1977).
11 O.L. Miller Jr and B.R. Beatty, Science *164*, 955 (1969).
12 T. Tanaka and T. Oda, Exp. Cell Res. *103*, 143 (1976).
13 C.B. Chae, Biochemistry *13*, 1110 (1974).

A duplication involving the esterase 6 locus in a wild population of *Drosophila melanogaster*

R. Costa, G.A. Danieli and E. Morbini

Institute of Animal Biology, University of Padua, Via Loredan 10, I-35100 Padova (Italy), 16 May 1978

Summary. A possible duplication of the esterase 6 locus in *D. melanogaster* is reported. Recombination between the duplicated loci is about 30%.

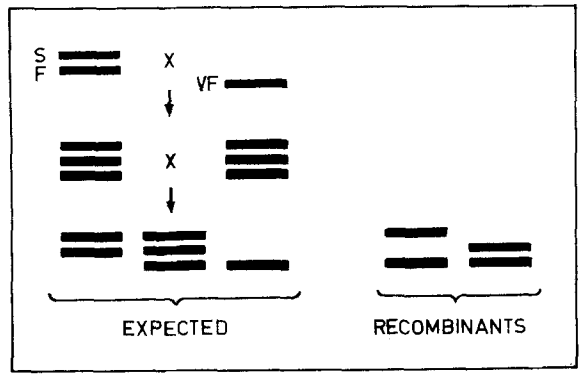
In *Drosophila melanogaster,* the esterase 6 locus *(Est-6)* maps on the 3rd chromosome at position 36.8; 6 different alleles have been detected so far by acrylamide gel electrophoresis[1]. Isoelectrophoretic alleles with reduced enzymic activity or with thermal lability have also been described[2,3]. The electrophoretic variants show codominance, without hybrid bands in the heterozygotes[4].

In the course of an extensive study on the dynamics of the *Est-6* polymorphism in wild populations[5], in a sample of 1327 males collected near Vicenza (Italy) 9 individuals with an unusual 3-band phenotype were observed[6].

From stocks of the original population reared in the laboratory, males were singly crossed with virgin females homozygous for the allele $Est\text{-}6^{VF}$. This allele is quite rare in the wild and shows the greatest electrophoretic mobility (1.21). By scoring the F_1, in the progeny of some crosses, individuals with a 3-band phenotype were observed. These invariably showed a pattern corresponding to the simultaneous presence of the allelic variants S, F, and VF with mobilities 1.0, 1.07, and 1.21, respectively. Since the allelic variant VF was contributed by the parental female, homozygous for $Est\text{-}6^{VF}$, it was clear that the S and F variants were transmitted together by the male parent. By inbreeding the F_1, it was possible to obtain an homozygous stock for the 2-banded paternal pattern. A detailed analysis, performed with the use of different substrates revealed that the 2 bands show differential affinity: in a mixture of α- and β-naphthyl acetate, one of the 2 bands (mobility 1.07) shows α-specificity, while the slow band (mobility 1.00) shows the usual β-specificity peculiar for the *Est-6* variants. The α-specific band is generally more heavily stained in females than in males.

The analysis of the F_2 progeny showed the presence of some recombinants between the α- and the β-specific esterase loci (figure). A further experiment was set up in order to estimate the frequency of recombination between these loci. The results are reported in the table. The recombination frequency calculated according to Fisher's formula resulted in about 28.7%. On the basis of these results, the hypothesis of the existence of 2 different cistrons appears unquestionable. However, the transposition of a large chromosome segment appears unlikely, since in salivary gland chromosomes preparations no evident morphological changes were noted.

The location of the presumed duplication was attempted by crossing individuals homozygotes for the duplication, with those of the marker stock 'sepia'. Unfortunately, in most of the F_2 individuals the α-specific band was extremely faint, so that a nonambiguous classification of the electrophoretic



Parental, F_1 and F_2 esterase phenotypes observed in a cross S, F × VF with the presumed parental genotypes $Est\text{-}6^{S}$, $Est\text{-}6^{F}/Est\text{-}6^{S}$, $Est\text{-}6^{F} \times Est\text{-}6^{VF}/Est\text{-}6^{VF}$.

phenotypes was impossible. This phenomenon might be explained by supposing that the continuous inbreeding of the lines used in the experiment have made homozygous unknown regulatory genes, controlling the expression of the *Est-6* locus.

Beardmore and Ahmad[7] found in *D. simulans* that 3 esterase loci (one of which is tightly linked to esterase 6) influence the appearance of bands at positions corresponding to those characteristic of the *Est-6* alleles; active alleles at these loci are present in wild populations at very low frequencies. It is possible that a similar situation exists also in *D. melanogaster,* a species very closely related to *D. simulans,* even if in our case the additional cistron does not map close to the *Est-6* locus, and shows a different substrate specificity. Beardmore and Ahmad suggest that the appearance of unusual esterases may be in relation to the exposure of the wild populations of *D. simulans* to insecticides. In fact Tsakas and Krimbas[8] in *Dacus oleae,* reported evidence in favour of selection for the new enzymes. On the other hand, it must be noticed that *Est-6* is probably not involved in the physiology of the nervous system, as indirectly indicated by the data of Wright[4] and of Korochkin et al.[9]. The fact that in our case the frequency of the duplication in the original population of *D. melanogaster* is quite low and that no apparent variation in frequency has been observed in the population samples maintained in the laboratory for several generations, leads us to assume that there probably is no selective pressure acting on this duplication.

Segregation of esterase phenotypes S (slow), F (fast), and VF (very fast) in the F_2 from inter se crosses of F_1 flies from the cross S, F × VF

Phenotypes in F_2	S, F, VF	S, F	VF	S, VF	F, VF
No. of individuals	191	63	69	65	53

1 K.A. Danieli, R. Costa and E. Rodinò, Accad. naz. Lincei. *58,* 441 (1975).
2 G. Trippa, G.A. Danieli, R. Scozzari and R. Costa, Atti Ass. genet. ital. *22,* 13 (1977).
3 B.J. Cochrane, Nature *263,* 131 (1976).
4 T.R.F. Wright, Genetics *48,* 787 (1963).
5 G.A. Danieli and R. Costa, Genetica *47,* 37 (1977).
6 R. Costa, G.A. Danieli and E. Morbini, Drosoph. Inf. Serv. *52,* 31 (1977).
7 J.A. Beardmore and M. Ahmad, Genetica *46,* 257 (1976).
8 S. Tsakas and C. Krimbas, Evolution *24,* 807 (1970).
9 L.I. Korochkin, A.A. Aronshtam and N.M. Matveeva, Biochem. Genet. *12,* 9 (1974).

A pathogenic hypothesis of malformation caused by amniocentesis[1]

G.G. Garzetti, U. Bellati, F.G. Serri, V.E. Pettorossi and R. Mancinelli

Istituto di Clinica Ostetrica e Ginecologica, Università Cattolica S. Cuore, via della Pineta Sacchetti 644, I-00100 Roma (Italy), and Istituto di Fisiologia Umana, Università Cattolica S. Cuore, Roma (Italy), 19 May 1978

Summary. The role of amniocentesis in producing fetal malformations has been investigated in the rat. Relevant uterine contractions were observed after amniocentesis. The pathogenetic mechanism of fetal malformations is discussed on the basis of the present results.

According to literature[2,3] in rodents, malformations occur in high percentage when amniocentesis is carried out during the organogenetic stage of gestation. These malformations seem to be related to histologically observed haemorrhagic processes occurring in rat limbs after about 120 min from amniocentesis. Throughout the period of survival, no fetal haemorrhages take place when amniocentesis is performed within the amniotic sac outside the uterus[3].

On the other hand, vasopressine administration in pregnant rats provokes fetal malformations[2]. These observations support the hypothesis that alterations are due to an increase of intrauterine pressure. Furthermore it has been demonstrated experimentally[4] that needle puncture alone in amniotic sac does not give rise to pathological events; therefore, as far as fetal malformations are concerned, all the data suggest a pathogenic role of uterine volume reduction which could provoke contractions greater than those occurring during pregnancy, even when pathologic. Thus we decided to control directly in the rat the intraamniotic pressure after amniocentesis, during the same period of gestation in which, in previous experiments on the same species[4], teratogenetic effects were caused.

The experiments were carried out on 20 Wistar rats (270 g) at 15th day of gestation. The day of fecundation was established by the presence of spermatozoa in the vaginal smear. The animals were anaesthetized with ether. After laparotomy, the uterine horns were exposed and protected with warm mineral oil (37 °C). The measure of endouterine pressure was performed by means of a needle type Butterfly No. 21, introduced in the amniotic sac and connected through a pressure transducer (Sanborn Mod. 268) to a preamplifier (Sanborn 350-3000). The signals were recorded by a Tektronix oscilloscope and a Grass recording camera. The same needle was connected to a syringe by a T catheter for fluid withdrawal or introduction (0.04 ml).

During the control period, intrauterine pressure inside various sacs showed a mean value of 4 mm Hg and wave amplitude was about 3 mm Hg. The frequency of contraction was about 3/min (figure 1, A).

The pattern of wave progression shown in figure 1, A, during the control period corresponds to the variation

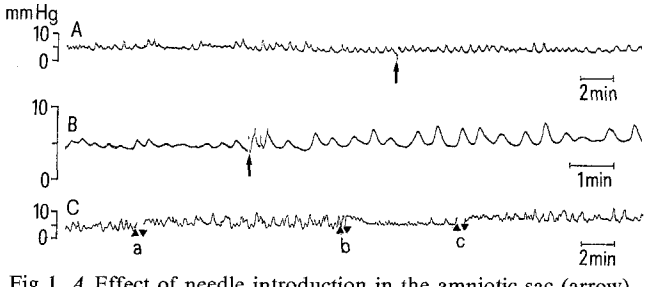


Fig. 1. *A* Effect of needle introduction in the amniotic sac (arrow) without fluid withdrawal. *B* Effect on the amniotic pressure of amniocentesis (arrow). *C* Effect of amniocentesis and of reintroduction of fluid on endouterine pressure; before a: control, a: amniocentesis (0.04 ml); b: reintroduction of the fluid (0.04 ml) withdrawn in a; c: amniocentesis. Continuous tracing. All the trials have been carried out on Wistar rats at 15th day of gestation.