Roca, A. and J.Rubio. Universidad de Oviedo, Espana. Breakage of polytene chromosomes of D.virilis under genetic control.

Some individuals from a stock of D.virilis kept in our laboratory since 1975, were found to experience abundant and variable breakage of their salivary gland polytene chromosomes when treated in the usual preparatory way for observation, that is dissection in aceto-lactic acid, followed by staining the glands in a drop of 2% aceto-lactic orcein on a slide for 30 min, and squashing the glands covered by a cover-slide (by observation under a stereomicroscope we can control, in the squash, the degree of extension of the chromosomal arms).

The breakage takes place even at normal squashing pressure apparently as a result of the squashing since a direct relationship between the pressure applied and the amount of chromosome fragmentation is observed. The chromocenter region is always the first to break up; then, the oligotenic points of the chromosomes (α-heterochromatin) are broken with increasing pressure, indicating a higher frailty than the rest of the chromosome. A typical view after a minimal pressure upon the stained glands would be to have all 5 chromosomes disjointed from each other at the chromocentric region and their arms broken at the following points: chromosome 1 at point P-Q; chromosome 2 at Mc and chromosome 4 at F-G (notation as in HSU maps, Patterson & Stone 1952); the chromosomes 3 and 5 are usually found without breakage when squashed lightly. When the squashing pressure increases to normal (as necessary for a good extension of the chromosomes), more breakages occur at various sites, so that eventually many chromosome fragments of variable size appear and the breakage points seem to be random (Figure 1).

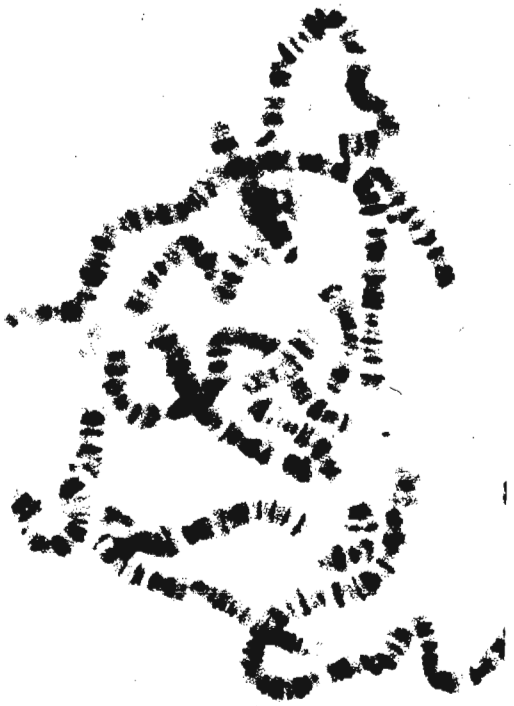

Figure 1. Frail chromosomes.

Taking off components from the preparatory solution (orcein or acetic or lactic acid) does not improve the stability of the polytene chromosomes to squashing pressure, nor change the pattern of breakage. It was not possible to make preparations without aceitc and lactic acids (only in distilled water) nor in saline solution because no nuclear membrane disruption is obtained under these conditions, probably due to the lack of elasticity that the acetic acid (Lefevre 1976) and the lactic acid (unpubl.) confer to the membrane and the chromosomes. The use of detergenets did not show any results, because they do not break the nuclear membranes.

All these results move us to consider that although the squash is the main cause of chromosomal breakage, the preparatory solutions can not be considered as related to the breakage phenomenon.

A true-breeding stock for such polytene chromosome frailty was established, where the adults are otherwise phenotypically normal and without any signs of their viability or fertility being affected. Reciprocal crosses to a normal stock of D.virilis from different origins yield progenies segregating as expected for a recessive sex-linked allele with 100% penetrance (Table 1).

We called "fra" to the allele responsible for the chromosome frailty.

Closer examination of the banding in heterozygous individuals under the optic microscope does not reveal any difference between the two homologous endocopy fasces. Even though it is indubitable that the structure of the polytene chromosomes of individuals with chromosome frailty is different than that of the normal individuals in the species. How the chromosome fine structure might differ remains to be seen.

Table 1. Progenies of the reciprocal crosses.

PARENTS:	♀♀ frail chrom.;	♂♂ normal chrom.
1st GENERATION:	♀♀ all normal;	♂♂ all frail
2nd GENERATION:	♀♀ normal (22);	♀♀ frail (27)
	♂♂ normal (14);	♂♂ frail (17)
PARENTS:	♀♀ normal chrom.;	♂♂ frail chrom.
1st GENERATION:	♀♀ all normal;	♂♂ all normal
2nd GENERATION:	♀♀ normal (45);	♀♀ frail (0)
	♂♂ normal (15);	♂♂ frail (19)

References: Lefevre,Jr, G. 1976, Genetics and Biology of Drosophila, V1a, Academic Press Inc., London; Patterson,J.T. & W.S.Stone 1952, Evolution in the genus Drosophila, Macmillan Co., New York.

Sampsell, B.[1] and B.Latham.[2] 1-Roswell Park Memorial Institute, Buffalo, New York USNA. 2-Chicago State University, Illinois USNA. Survival of ADH thermostability variants on naturally-occurring alcohols.

Wild Drosophila melanogaster probably feed and breed on a variety of fermenting fruits and other substances derived from human activities. Examination of the alcohol content of several rotting fruits showed that ethanol was the most common alcohol present with concentrations ranging up to 4%, while 1-propanol and 2-propanol occurred in concentrations of 1% or usually less (McKechnie & Morgan 1982). Ethanol concentrations as high as 12% were observed in certain portions of a pile of grape residues outside a winery (McKenzie & McKechnie 1979). We have measured the ability of flies with various Adh genotypes to survive on several alcohols presented singly and in combination over a range of concentrations.

Alcohol tolerance was measured using a modification of the method of Starmer et al. (1977). Newly-emerging adults were collected from uncrowded breeding vials and transferred to fresh food vials to age for 7 days. The flies were then lightly etherized and groups of 20 males or females selected. They were returned to an empty vial for 4 hours to recover from the ether. They were then transferred to a 35 ml food vial, empty except for a 1-inch square of filter paper on which 0.1 ml of an alcohol solution of the indicated concentration (v/v) had been absorbed. From this point on in the experiment, this alcohol solution served as the sole source of nourishment and moisture. It should also be noted that flies were exposed to the alcohol by inhalation as well as ingestion. The vials were covered with parafilm, and observations were made at regular intervals to count the number of dead flies. After more than 50% of the flies in a given vial had succumbed, the number of hours to 50% mortality was determined by interpolation between observed points. Each point in the graphs represents the average of 6 vials (3 of males and 3 of females). Females generally lived somewhat longer than males; a fact that is probably the result of their larger body size and hence greater store of food. Three strains of flies, each homozygous for one of the three common alleles, were tested. The strains were Sf 1 (Adh-Fr), Ore (Adh-Fm), and SSS (Adh-Sm). Information about these strains may be found in Sampsell & Steward (1983). All tests were conducted at room temperature.

As previously reported, certain alcohols can serve as nutrients for the flies prolonging their life beyond that possible on plain water. In the case of ethanol, survival peaks occurred at 4-8%; however, survival time decreased sharply at concentrations above this level. On 1-propanol, the survival peak was seen at much lower concentrations of only 0.5 to 1%. Either this alcohol offers less nutritive benefit than ethanol or higher concentrations are more toxic. Survival on a combination of the two alcohols suggested that the latter explanation is probably correct. When ethanol and 1-propanol were both present in a 10:1 ratio, survival times peaked in the 2-4% ethanol range. 2-propanol was apparently even more toxic, since on this alcohol alone, flies did not survive longer than on plain water. Presented in combination with ethanol, 2-propanol's toxic effect was again apparent from the fact that peak survival occurred at around 4% ethanol. Finally when all three alcohols were present (with ethanol concentrations 10X that of either of the other two alcohols) a peak in hours to 50% mortality occurred at 2% for all three strains. The most interesting thing about these findings is the fact that the alcohol concentrations at which longest survival times were observed are very similar to those observed in natural food sources.

Comparisons among the strains for differential survival times were hampered by the small sample sizes, but several conclusions can be drawn. Based on measurements of ADH activity levels in which Ore > Sf 1 > SSS, we might have expected survival times to parallel these differences. In fact, SSS (Adh-Sm) flies generally had shorter survival times when there were any significant differences among the strains. Sf 1 (Adh-Fr) survival times, however, were generally equal to or longer than those of Ore (Adh-Fm). The Adh-Fr allele occurs in most natural populations, but only at low frequencies (1-16%). Flies with this allele, however, do not seem to have impaired alcohol tolerance compared to individuals with