

total progeny;  $k_2$  for segregation of third chromosomes =  $st^+$  individuals/total progeny). A further count of  $k_1$  and  $k_2$  in the progeny of  $F_2$  males makes it possible to evaluate the degree of distorted segregation of chromosomes 2 and 3 and their reciprocal effects on segregation.

A study on two wild populations collected in October 1978 in northern Italy (Mareno, Veneto) and in southern Italy (Nardò, Puglia) has led to the recovery of a third chromosome,  $III^{Nr}$  ( $III^{Nardò}$ ) which alters the segregation of second chromosomes (Table 1). This chromosome normally segregates in  $III^{Nr}/st$  heterozygous males ( $n = 41$ ;  $k = 0.53$ ). As things stand at present, two general hypotheses can be put forth to interpret the results obtained: (1)

Table 1. Effect of the  $III^{Nr}$  chromosome on the segregation of second chromosomes.

| Cross | $\sigma \frac{+}{bw-5} \frac{st-5}{st-5} \times \text{♀ } bw-5; st-5$ |            |                 | $\sigma \frac{+}{bw-5} \frac{+}{st-5} \times \text{♀ } bw-5; st-5$ |            |                 |
|-------|---|------------|-----------------|--|------------|-----------------|
|       | n   | tot. prog. | $k \pm S.E.$    | n  | tot. prog. | $k \pm S.E.$    |
| #13   | 13  | 1100       | $0.51 \pm 0.06$ | 41   | 3885       | $0.96 \pm 0.05$ |

there may be an Sd-like factor acting like other Sd factors so far detected on the second chromosome but located on the third chromosome; (2) there may be on chromosome 3 an Sd modifier (enhancer?) acting on the  $II^+$  Nardò chromosome (which is actually an SD chromosome, despite the fact that in the  $+/bw-5; st-5/st-5$  heterozygous males  $k = 0.51$ ) thus causing segregation distortion of second chromosomes. The frequency of genotypes with the SD trait which is a consequence of the interaction of wild chromosomes II and III is in the Mareno population 0.00% (0/137 chromosomes) and in the Nardò population about 0.03% (3/108 chromosomes). In the Nardò population, besides #13, there are two examples of SD with  $k$  value equal to 0.67 in  $+/bw-5; st-5/st-5$  males and 1.00 in  $+/bw-5; +/st-5$  males. These data indicate that there is a significant difference ( $P \gg 0.001$ ) in the  $k$  values of the two genotypes and that this difference depends on the presence of wild third chromosomes which enhance distortion by the wild second chromosome.

It is interesting to note that while the Nardò (South Italy) population shows an SD frequency of about 3% (a very similar value to those observed in wild populations from many parts of the world), the Mareno population (North Italy) shows no cases of SD. This is the second example of a wild population with no cases of SD, after that extensively examined in Austin, Texas (see Hartle and Hiraizumi 1976). If it is true that one of the mechanisms contrasting the spread of SD in populations is the appearance and increase of normal chromosomes resistant to Sd, it would be particularly stimulating to test the degree of sensitivity to distortion by Sd of the second chromosomes of these two populations.

References: Hartl and Hiraizumi 1976, in: Genetics and Biology of Drosophila (Ashburner and Novitski, eds.) vol. 1b; Trippa et al. 1972, DIS 49:81; Trippa and Loverre 1975, Genet. Res. 26:113.

Tsakas, S.C. Agricultural College of Athens, Athens, Greece. Chromosomal breaks and alteration in staining observed in vitro after ultrasonication of salivary glands of *D. subobscura* species.

It is known that many chemical agents and physical factors produce chromosomal breaks and aberrations. The purpose of this work was to discover if ultrasonics also have an effect of this kind in vitro.

Salivary glands of the "Küsnacht" strain, first pupal stage, were used. This strain has a standard/standard chromosomal structure for the five long chromosomes, X, O, U, E, and J. Tap water was used as dissecting solution; its chemical analysis is as follows: pH = 7.2;  $SO_4^{--} = 30$  mg/l;  $NO_3^- = 2$  mg/l;  $Cl^- = 34$  mg/l;  $HCO_3^- = 150$  mg/l;  $Ca^{++} = 60.1$  mg/l;  $Fe^{++} = 0.2$  mg/l;  $Mg^{++} = 29.4$  mg/l;  $Cl_2$  free = 0; hardness:  $CaCO_3 = 150$  mg/l, the remaining = 32 mg/l; total = 182 mg/l. The staining solution was composed of 2 g of synthetic orcein (Edward Gurr, Ltd., London) dissolved into 50 ml hot glacial acetic acid, plus 50 ml of 85% lactic acid after removing from heat (Strickberger 1962).

Immediately after dissection of eight pairs of salivary glands, four of these pairs were placed on one slide and four on another. Each slide contained one drop of dissecting solution. One slide was kept as a control and the other was placed under the sonicator's microphone. A TECH Ultrasonicator (company, Japan) was used, with a crystallic twiter microphone.

Conditions were: (a) distance between the slide and the microphone, 6.0 cm; (b) sine waves; (c) frequency, 40,000 cps, first harmonic waves at 120,000 cps; (d) power,  $N = 0.3$  watts; (3) time, 30 min; (f) temperature, 19°C.

After sonication each pair of salivary glands was placed on a slide containing a drop of staining solution. So, there were four slides produced from the control slide and four from the treated. After a 10 min. interval for staining, a cover slip was placed without applying pressure on top of each slide. The first observation then took place using a magnification of 100X. After this, the cover slide was pressed slightly until the point enabling magnification of 700X and another observation was made.

The above procedure was performed six times, so the total number of slides observed was 24 control and 24 treated. In every slide of treated glands a significant number of chromosomal breaks appeared as well as a significant alteration in the effectiveness of staining by orcein, whereas the control slides showed chromosomes without breaks and clear and sharp staining. Figs. 1 and 2 are presented as an example of these findings. The only difference between the two figures is that ultrasonication was applied to the glands in Fig. 2; all other conditions from dissection through the development of the photograph were the same.



Fig. 1. Partial view of a control slide of salivary chromosomes of *D. subobscura* (1000X).

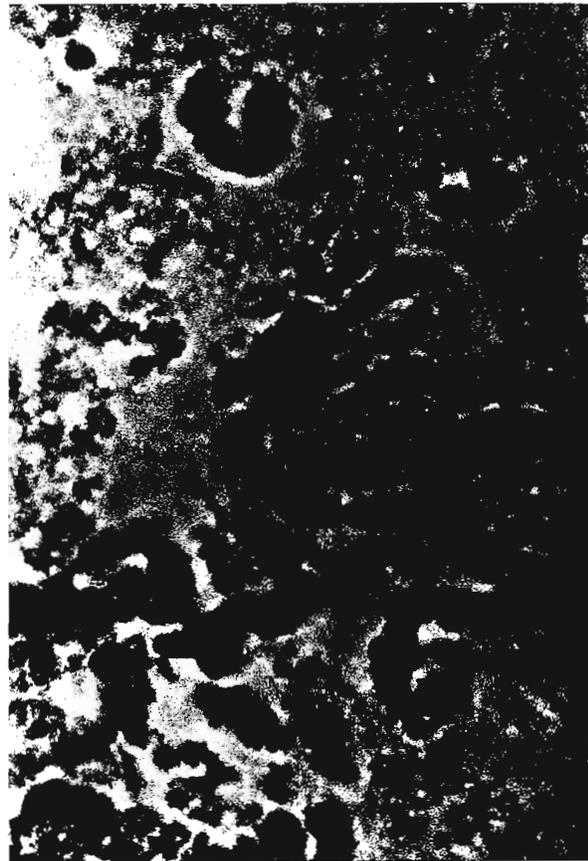


Fig. 2. Partial view of a treated slide (ultrasonication) of salivary chromosomes of *D. subobscura* (1000X).

Since ultrasonication is used to break the bodies of cells such as chloroplasts, it was probable that it also would produce chromosomal breaks in salivary chromosomes *in vitro*. This work gave evidence that this does occur. The unexpected finding was the alteration of staining after ultrasonication with the white bands taking on some color and the dark bands appearing more faint. This resulted in such a difference in the appearance of the chromosome as to render it virtually unidentifiable. If staining is the result of chemical reactions between orcein and chromosomal DNA, then after ultrasonication these reactions take place in

in a different way from the usual. One possible explanation for this could be that ultrasonication alters the chemical and/or physical properties of the chromosomal DNA structure or composition.

Although this work took place *in vitro* and salivary glands were used, these findings require further attention because ultrasonics are utilized in research and applied science such as obstetrical medicine.

Reference: Strickberger, M.W. 1962, in: *Experiments in Genetics with Drosophila*, ch. 18, p. 103, John Wiley & Sons, Inc., New York-London.

Turner, M.E. University of Georgia, Athens, Georgia. A laboratory overwintering experiment with *D. montana* and *D. pseudoobscura*.

*Drosophila* which live at high elevations are subject to low temperature extremes during the winter months. At elevations 7000 ft. and above low temperatures and/or snow cover may last six months or longer. For these populations of *Drosophila* to persist either some

stage (or stages) of the life cycle must overwinter or a new population must be founded each spring from lower elevation populations of the same species. *D. montana* and *D. pseudoobscura* were tested to determine their ability to endure cold temperatures for an extended period of time. *D. montana* were obtained from the University of Texas Stock Center (#1218.8d); this strain was originally captured from Ogden, Utah and has been in the laboratory since 1941. The *D. pseudoobscura* were collected from American Fork Canyon, Utah (elev. 7550) in 1976.

Flies were kept in half-pint milk bottles containing cornmeal-molasses medium. Approximately 50 adults were put in a bottle and allowed to reproduce at 15°C; when pupae appeared the bottles including the parents were put in an incubator at -2°C.

After eight days all *D. pseudoobscura* adults were dead. These bottles were moved to 15°C and no progeny from the original adults appeared; apparently the cold temperature had also killed eggs, larvae and pupae. *D. pseudoobscura* can be kept at 5°C for long periods of time with larvae, pupae and adults surviving.

After six months (184 days) the *montana* bottles (adults still alive) were removed from the incubator, adults were separated by sex and put in new bottles at 15°C. No flies had hatched from the original bottles after one month at 15°C and no living larvae were observed. The other life stages (eggs, larvae, and pupae) had been killed by the cold temperature. Additionally no larvae appeared in the bottles containing surviving females after one month at 15°C. The sexes were combined in a new bottle and larvae, and eventually adult progeny, appeared. The time at the cold temperature had despoiled the "overwintering" females, but had not, at least grossly, affected their fertility.

The ability of *montana* adults to survive this temperature (-2°C) for an extended period of time (6 months) would seem to imply that adults can and probably do overwinter. The death of the *pseudoobscura* individuals does not demonstrate that they do not overwinter, but only that they may overwinter where temperatures do not get this cold. In many forest environments at or above 7000 ft. elevation both *montana* and *pseudoobscura* live in the same area and are attracted to the same banana baits. The greater cold temperature tolerance of *montana* adults should allow them to survive in the more exposed and colder areas of this environment.

Valente, V.L.S., C.C.R. Saavedra, A.M. de Araújo and N.B. Morales. Universidade Federal do Rio Grande do Sul, Porto Alegre, R.S., Brasil. Observations on the attraction of *Drosophila* species for different baits and chromosomal polymorphism in *D. willistoni*.

Present data were obtained in three days of collection from October to November 1978, in the locality of Estação Experimental Agrônômica de Guaíba, Guaíba County, 40 km from Porto Alegre, the capital of the State of Rio Grande do Sul, Brasil. The studied place is a brushwood enclosed in a capon, with some watersheds. Five fermented banana baits were used besides natural available baits: fer-

mented fruits fallen around the original plant, the native palm-tree *Arecastrum romanzoffianum* (Palmae), which fruit is commonly called "coquinho".