results in all wells and produced a profile in the sample tube that was essentially the same as the cycle that had been programmed. Results can be optimized to some extent for most machines, for example in designs 1 and 4 by adjusting for overshoot and undershoot temperatures, in designs 2 and 3 by choosing certain wells, and in all designs that do not incorporate a peripheral thermocouple by adjusting times and temperatures to compensate for the difference between the block and the tube.

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In situ hybridization to pre-fixed polytene chromosomes

As more and more genes are cloned from organisms, interest is increasing in the use of these clones to look at linkage relationships in related species[1], particularly where the band homology is not immediately obvious in the polytene chromosomes. In some dipteran groups, such as the Chironomidae and Simuliidae, where stocks are not easily maintained in the laboratory, the only material available for such analyses is material that has been kept in fixative (3 parts absolute ethanol : 1 part glacial acetic acid), sometimes for more than 10 years[2]. Since I am interested in using clones from *Chironomus* to confirm the identity of chromosome bands in those chromosome arms in which extensive repatterning makes cytological comparison difficult[3], I have experimented with *in situ* hybridization to chromosomes of such pre-fixed larvae. The results have been surprisingly good, with clear hybridization bands on material that had been in fixative for 10–14 years (Fig. 1), although the chromosome morphology was often badly affected by the treatment. This problem can be overcome by photographing the chromosomes before proceeding with the hybridization procedure, as is commonly done even with freshly fixed material[1]. As can be seen in Fig. 1, the bands identified in the long-term fixed chromosomes are identical to those seen in the freshly fixed material, with those on the long-term fixed material sometimes better. The latter may be due to the ease of removal of the solidified salivary secretion from older material. In fresh material the soft, sticky secretion may form a film over parts of chromosomes, which restricts access of the probe DNA and stains.

The technique used here is essentially that of Whiting *et al.*[1] modified according to the earlier technique of Stuart and Porter[4], so that the chromosomal and probe DNA, sealed under a plastic coverglass, are simultaneously denatured in a waterbath for 1 h at 65°C. The waterbath is then turned back to 37°C, allowing it to return slowly to that temperature, and left for about 15 h.

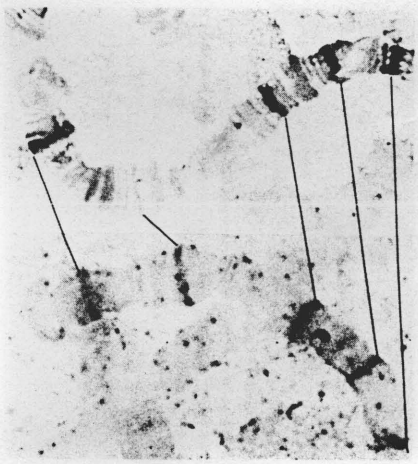

FIG 1

Salivary gland chromosome arm D of *Chironomus duplex* prepared from fresh larva (above) and larva fixed for 14 years (below), hybridized with a histone gene probe, pK611, provided by T. Hankeln and E.R. Schmidt, Institut für Genetik, Johannes Gutenberg-Universität, Mainz, FRG. Lines connect homologous bands of hybridization. Note that the band second from left is much clearer in the pre-fixed chromosome.

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