suggesting a lower production of slow subunits. CS20 was intermediate in parental ADH activity and in F/S ratio. CS4 and CS20 have been crossed to three Adh-Fm homozygous strains, and among the heterozygous progeny the F/S ratio was inversely proportional to the Adh-Sm activity in the parental strain (data not shown). Although we have not yet tested ADH levels directly in the CS strains by immunodiffusion techniques, these results are consistent with the observations on other strains in which differences in ADH activity among strains with the same Adh genotype were shown to be associated with differences in the quantity of ADH (Maroni et al. 1982; Sampsell & Steward 1983).

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Contaminating microorganisms interfere with Southern Blot analysis of Drosophila melanogaster DNA.

During our studies of restriction enzyme polymorphism of the small heat shock genes at the 67B locus in Drosophila we discovered that one of the restriction enzyme fragments which we were observing was actually hybridizing to pBR322, the vector that our heat shock probe was cloned in. In several isofemale lines of Drosophila melanogaster we found one band of about 4.7 kb which showed up when probed with pBR 322 (Fig. 1). We believe our finding may serve as a useful warning to other investigators who are using whole flies as a source of genomic DNA for study of restriction enzyme polymorphism. We would also like to report on the properties of this contaminating fragment which were observed incidentally to our studies of the small heat shock genes.

Our first impression upon discovering this additional fragment was that this was merely a simple case of accidental contamination of some of our genomic DNA stocks by trace quantities of plasmid. If this was true then independent DNA extracts of these lines of flies should be free of the additional fragment. In independent DNA extracts we found that the additional fragment was present in precisely the same lines in which it was found in the first place, and absent in lines in which it was not previously found. This observation could have two possible causes: either there are pBR322 homologous sequences in the Drosophila genome or this fragment was derived from microorganisms which are associated with flies

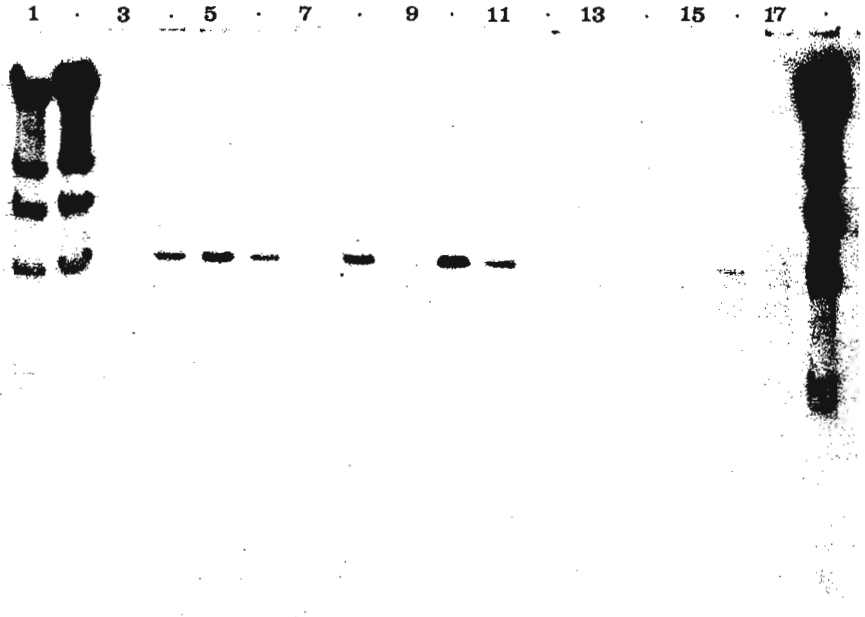

Fig. 1. A Southern Blot of fly DNA from 15 different strains of D.melanogaster. Lane 1, 2 and 18 contain DNA mol. weight markers. Lane 3-9 represent strains from Hamilton, Ontario and lane 10-17 from Brownsville, Texas. The fly DNA is cut with EcoR I and probed with the plasmid (pBR 322) DNA. Migration is from top to bottom. Lane 4, 5, 6, 8, 10, 11 16 show the contaminating 4.7 kb DNA fragment which hybridizes with pBR 322 DNA.

cultured under standard non-axenic conditions. We decided to do a few simple experiments in order to distinguish between these two possibilities.

We attempted to determine whether or not the additional fragment was a part of the Drosophila genome in two different ways. First we tried producing axenic cultures of flies which were known to carry the additional fragment to see if it could be removed. The axenic cultures were produced by surface sterilizing eggs according to the procedure of W.G.Starmer & D.G.Gilbert (DIS 58:170-171). Sterility tests were performed on fly cultures by incubating a fly in bacterial mutrient broth plus 0.2% glucose for several days at 37°C. One line of flies was obtained, which showed no microbial contamination when tested by the above method, and remained sterile throughout the time it was maintained. DNA extracted from these flies did not show the additional fragment when analysed in a Southern blot. We were therefore able to cure the flies of this contaminant by sterilization.

Our second approach was to try to transfer this contaminant from flies which carried it to flies which did not carry it. This was done by taking five males from a contaminated stock, grinding them up in a sterile isotonic buffer and spreading this mixture onto the surface of fresh medium in a clean bottle. Uncontaminated flies were then introduced into the bottle. These flies were collected after one week and their DNA was extracted. Southern Blot analysis of this DNA showed the contaminant band to be present. We were thus able to transfer the contaminant to previously uncontaminated flies.

As we continued our work on restriction enzyme polymorphisms using more enzymes, we identified the contaminating fragment by probing each blot twice, once with just pBR322 and once with pBR322 with the heat shock gene insert. During this process we were also observing digestion patterns of the contaminatn. After using six different restriction enzymes (Bam HI, Eco RI, Pst I, Sal I, Xba I, and Hind III) we found that the contaminating band was the same size in all of the ten lines of flies which carried it. There are three possible explanations for this observation. One is that the fragment is not being cut by any of the six enzymes. The second is that it is a circular fragment with a unique site for all of the enzymes. The third is that it has a unique site for some of the enzymes, is not being cut by other enzymes and that we are unable to distinguish between linear and circular forms of this molecule. After doing a few double digests in an attempt to map some of the heat shock gene restriction fragments, we obtained some results which also helped us distinguish between these three possibilities. We digested one line of flies which carried the contaminant with the following enzyme combinations: Bam HI plus EcoR I, Bam HI plus Pst I and Bam HI plus Xba I. We found no change in size of the contamination fragment following these digestions. This allows us to conclude that the fragment is not being cut by four of the six enzymes we used. The lack of change in mobility of this fragment after cutting it with six different restriction enzymes is therefore not likely to be due to linearizatio of a circular molecule.

The possibility of the contaminating fragment being a circular molecule combined with the fact that it is homologous to pBR322 caused us to speculate that perhaps it is a naturally occurring plasmid of E.coli. At present,however, all that we can conclude from our observations is that since it can be removed by surface sterilization of eggs, the contaminating fragment must be derived from an extracellular microorganism which is associated with some of our fly stocks.

Smith, M.R., G.K.Chambers, L.D.Brooks, F.M.Cohan* and S.C.Cohan.* Museum of Comparative Zoology, Harvard University, Cambridge, Massachusetts USNA. *University of California, Davis USNA. How many Adh clines on the west coast of North America?

Conversations with casual collectors of Adh allele frequencies in the western regions of North America have led us to a growing suspicion that there is no exact western counterpart of the Adh latitudinal cline observed for east coast populations (see Oakesho.t et al. 1982, who also report similar clines in Australasia and Europe/Asia). Recent data we have collected as part of a large geographical survey of genetic variation in D.melanogaster reveal that our suspicions may indeed be well founded.

Field collections were made over a three-month period in 1982 (except for VIN and SEA [Table 1], which were made in 1981). Locations of collections are shown in Figure 1. Isofemale lines were established from wild caught individual females and shipped, usually within 1-2 generations, to the MCZ. Mass cultures were re-established from the separate lines and Adh allele frequencies were determined by cellulose acetate electrophoresis on F1 progeny by the method of Wilks et al. (1980). The data are shown in Table 1.