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.

Table 1. Adh allele frequencies in population samples.

Population	#Iso lines	°Latitude°N	Adh^F	Adh^{Fr}	Adh^S	Sample size (alleles)
LAK	17	32.9	0.44	0.00	0.56	84
WES	21	36.3	0.57	0.00	0.43	28
FNO	23	36.7	0.68	0.00	0.32	196
WAT	26	36.9	0.55	0.00	0.45	28
VIN	55	38.3	0.73	0.01	0.26	196
CAM	28	38.7	0.65	0.01	0.34	196
HAM	37	39.7	0.72	0.00	0.28	196
MED	47	42.4	0.50	0.00	0.50	194
ALO	27	45.6	0.51	0.04	0.45	196
CAR	28	47.6	0.54	0.01	0.45	140
SEA	>50	47.6	0.64	0.05	0.31	42
POC	57	49.3	0.78	0.01	0.21	196

LAK Lakeside,CA; WES Westside Field Station, Five Points, CA; WAT Watson,CA; VIN Vineburg,CA; CAM Camino,CA: HAM Hamilton City,CA; MED Medford,OR; ALO Aloha,OR; CAR Carnation,WA; SEA Seattle,WA; POC Port Coquitlam,B.C.

Table 2. Regression analysis of allele frequency data.

Analysis	r^2	slope	probability
Adh^F vs Latitude (all data)	0.0571	+0.0047	0.455
Adh^F vs Latitude (California)	0.7730	+0.0415	0.009
Adh^F vs Latitude (Oregon & Washington)	0.611	+0.0349	0.118
Adh^{Fr} vs Latitude (all data)	0.383	+0.0019	0.032

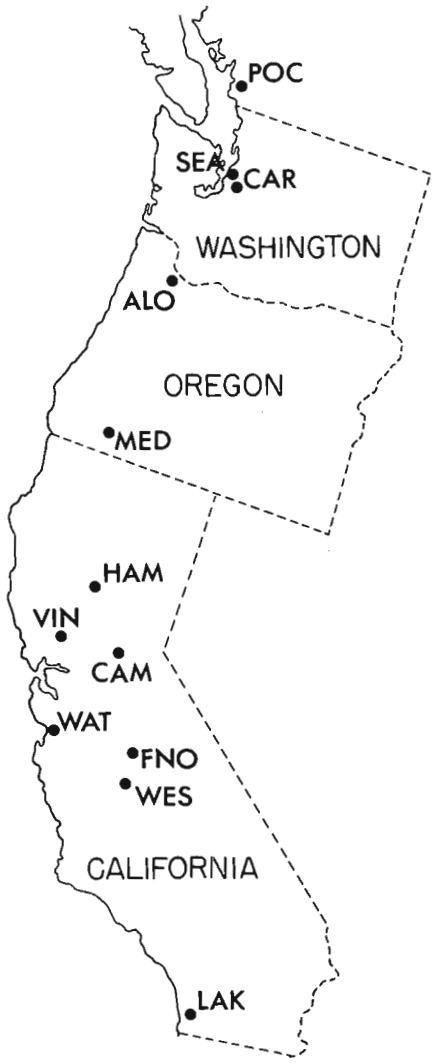

Fig. 1. Location of collections.

It is clear that there is a general trend for Adh^F to increase in frequency as one travels north, but the data do not reveal a uniform pattern of variation. Accordingly, unweighted linear regression analysis of Adh allele frequency on latitude was performed (SAS --General Linear Model). Results are shown in Table 2. The statistical analysis confirmed our initial observation: the regression of Adh^F frequency on latitude does have a positive slope but is non-significant. The data may be better explained by two clines, one in California ($p=0.01$) and one in Oregon, Washington and British Columbia ($p=0.12$). However, we are reluctant to draw firm conclusions from such small selected subsets of the data.

Interestingly, the frequency of Adh^{Fr} is significantly related to latitude ($p=0.03$), but this may be an artifact resulting from its absence over most of California. At this stage we are content to note that Adh^{Fr} is widespread but at low frequency. This distribution resembles that found in Australia by Wilks et al. (1980) and that reported for other U.S. populations by Sampsell (1977).

We conclude that the distribution of Adh alleles on the west coast is complex and it is probable that many more populations will have to be sampled before we can decide between competing descriptions in terms of zero, one or two Adh clines.

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Soutullo, D.[1] and E.Costas.[2] 1-Universidad de Santiago, Espana. 2-Instituto Espanol de Oceanografia, La Coruna, Espana. A method to find the genetic origin of a mutational unstability phenomenon in Drosophila melanogaster.

The present method allows us to tell whether a mutational unstability phenomenon is due to the action of mendelian genes or to mutator polygeneic systems, or even whether it is caused by the presence of transponsable genetic elements.

The procedure consists of substituting every chromosome of the mutator strain--which might eventually be responsible for the unstability--for stable chromosomes marked with dominant markers while making sure that no crossing-over will take place between the different chromosomes (due to the presence of inversions in the marked chromosomes). Previously the 'dot' of the unstable strain has been substituted by means of balancers (it is not described in the Figure), and then it is tested whether the unstability remains. In this way we are able to avoid the taking into account of the IV chromosome in order to establish the possible conclusions of the analysis.

Two series of crosses were performed, one in which the unstability appears initially associated with the II chromosome and another one in which it appears associated with the III chromosome, testing at the end of each series if the unstability remained. This test is performed by checking the sensitive to mutagenic action loci in every major chromosome by means of adequate recessive markers (see Figure).

The persistence of unstability would indicate that its origin is due to the presence of any type of transponsable genetic element, except if a double crossing-over between an unstable chromosome and its stable marked homologous one would have taken place. From the comparison between both series of crosses we observed the hypothetical double crossing-over to have taken place--if it existed--between homologous III chromosomes in the first case, whereas the double crossing-over would have taken place between homologous chromosomes in the second case (noted by squares in the Figure).

From the analysis of results we can get the following conclusions:

Case 1: Presence of mutation at the end of both series of crosses: The unstability would be due to transponsable genetic elements.

Case 2: Presence of mutation at the end of the first series of crosses and absence in the second series: The unstability would be caused by genes of the III chromosome in the unstable strain.