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Letter to the editor

Sir, the by no means new phenomenon of correlation between measurements errors pertaining to heterozygous DNA profiles has recently been rediscovered by Dr. M. M. Shapiro, who remarked on the procedure by which databases of hypervariable DNA fragments are established [1]. Experiments using Southern blot analysis at the FBI contain a constant, known DNA sample used as control, generating replicated sets of band length measurements. Shapiro finds that estimates of band pairs of controls derived from the same electrophoretic lane are correlated with each other. He assumes this to be a general phenomenon destroying whatever independence exists between allelic fragments stored in a database. Thereby he argues that the rule of the double product of single fragment frequencies cannot be applied to calculate the probability of heterozygotes within a population.

A correspondence by I. Evett, recently appeared in one of the latest issues of Nature, replies to Shapiro's arguments by pointing out that the problem is not new and that accuracy with which popualtion frequencies are estimated is a matter of "minor importance" [2].

We substantially agree with Evett's remarks. Furthermore, another point under discussion should be stressed. Measurement errors in predictions of kilobase values basically occur at random, depending on several factors impairing the reproducibility of procedures of Southern blot analysis. However, when numerous aliquots of the same DNA sample are processed as controls in serial experiments, trivial factors (e.g. quality of the DNA extract, salt/contaminants content, and others) introduce error correlations in these particular band pair estimates. This problem is exacerbated if human systematic errors are committed in the process by which autoradiographic images are converted into kilobase values.

We are well aware of this phenomenon, as most laboratories involved in forensic DNA analysis, but we believe that it cannot affect the overall population of kilobase values stored in databases of hypervariable fragments. In fact, nearly each DNA sample contributing one pair of kilobase values for the database is different from another, and the vast majority of measurements are drawn from different experiments. Under these circumstances, error measurements occur at random.

We wish the refer to a very simple measurement test, supporting this statement.

We examined sixty-two family groups, each with one offspring, for one autosomal hypervariable DNA marker (D2S44-pYNH24, also considered by Shapiro). In the resulting autoradiograms, lengths of offspring bands were estimated in two ways: 1) by deducing band lengths from each of the relevant paternal and maternal electrophoretic lanes; 2) by assessing the band pair from one lane, directly from the child's pattern in autoradiograms. The two groups of values were measured by two different technicians in our laboratory. Assuming Shapiro's

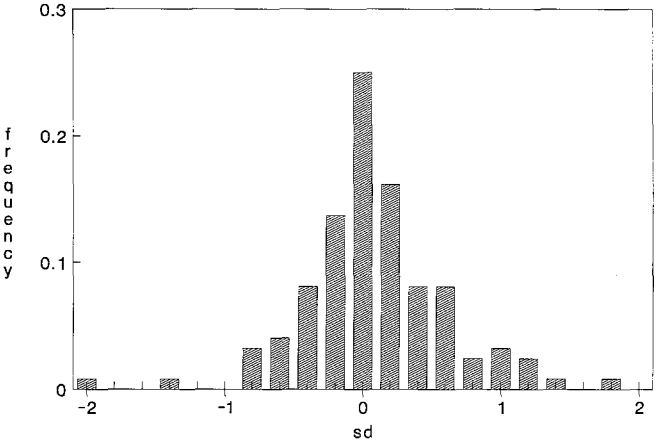

Fig. 1. Regression analysis between replicated sets of measurements of hypervariable DNA fragments form a population of restriction fragments (124 bands). Fragments were scored in 62 family groups, as pairs of bands (62 children) and as single bands (the inherited fragment of parent's assets)

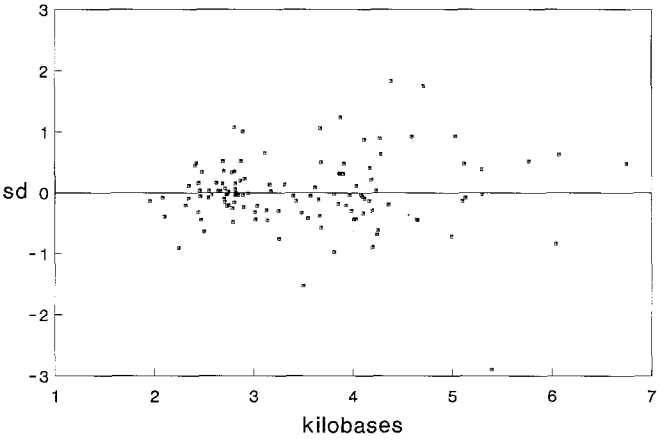

Fig. 2. Distribution of errors (in standard deviation units) in the two populations of kilobases estimates (band pairs vs. single bands)

arguments to be true, we should have found significantly different populations of measurements. In fact (and quite obviously), we found that profiles of relative frequencies from the two groups of data fairly overlapped. This is outlined well by a high correlation coefficient between corresponding estimates of the two populations ($r = 0.998$, 124 values, 122 df) (Fig. 1). Furthermore, errors between corresponding estimates were normally distributed (Fig. 2).

This very simple test proves that: 1) measurements of hypervariable DNA fragments are currently affected by random errors; 2) band pair estimates from the same electrophoretic lane provide unbiased arrays of data; 3) estimates carried out by different people are fully consistent, despite subjective errors in sizing autoradiographic bands; 4) no serious consequences on the independence of allelic fragment measurements (and on the rule of Hardy-Weinberg) can be derived from the process by which the band length estimates are drawn from autoradiograms.

References

1. Shapiro MM (1991) Imprints on DNA fingerprints. Nature 353:121–122 (1991)
2. Evett IW (1991) Trivial error, Nature 354:114

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