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DNA Fingerprinting Loci Do Show Population Differences: Comments on Budowle et al.

To the Editor:

Budowle et al. (1994) assert that some of the results in the study by Krane et al. (1992) describing population differences at DNA fingerprinting (VNTR) loci are misleading and "can be ascribed to statistical artifacts" (p. 533). However, Budowle et al. do not support their statements with any statistical tests with *P*-values, lod scores, or any other rigorous procedures. Indeed, their paper ends just at the point where one would expect a statistical analysis to begin.

The point at issue is whether the observed differences in the frequencies of multilocus VNTR profiles among human subpopulations are significant in the ordinarily accepted sense of statistical significance. For a given data set of VNTR alleles at various loci, the "product-rule" frequency estimate of a VNTR profile of a particular individual is the product of the allele frequencies of the alleles in the profile (with an additional factor of 2 for each locus) using that data set. Krane et al. consider data at the same three VNTR loci from 73 Finnish and 79 Italian individuals. For each complete three-locus profile in the Finnish and Italian databases, a ratio of product-rule frequency

estimates was calculated using the target profile's own (or cognate) database in the numerator and the other database in the denominator. For example, for the complete Finnish profiles, the Finnish data were used to calculate allele frequencies for the numerator and the Italian data for the denominator. These ratios will tend to be large if the VNTR allele frequencies differ between the populations. Krane et al. found that 77% of these ratios were >1 and 34% were >10.

Budowle et al. point out that the distribution of these ratios will have a positive bias because of the fact that the target profile's alleles are in the target database but not in the other database. They compared the observed frequency ratios with the average of similar ratios, calculated in the same manner, for test subpopulations of the same size that were randomly chosen from a larger database consisting of 1,354 U.S. Caucasian profiles. For the simulated data, an average of 73% were >1, and, of those ratios, 17.3% were >10. (This corresponds to ~12.7% of all ratios.) No *P*-values were reported, even for the ratios >10, for which the observed and average values were 34% and ~12.7%.

We repeated the random-sampling procedure of Budowle et al., to compute *P*-values for ratios >1, >10, >50, and >100, as well as for five quantiles from 50% to 99%. Five of these measures were highly significant ($P < .01$), and three others were significant ($P < .05$). The only measure that failed to be statistically significant was the one corresponding to ratios >1.

Budowle et al. further observe that, if the alleles in the target profile are excluded from the target database before the product-rule ratios are calculated, the frequency of ratios >1 becomes 63% for the given data and averages 50.9% in the simulations. Of those ratios that were >1, 23% of the observed ratios and an average of 9.6% of the simulated ratios were >10. We did simulations for this case also. With the target profile alleles excluded in both the observed and simulated ratios, all nine statistics were significant ($P < .05$), and six of the nine were highly significant ($P < .01$; see table 1). In particular, 15% of the observed ratios are >10, in comparison with an average frequency of 3.2% in the simulations ($P = .002$).

The conclusion from table 1 is that there are statistically significant differences with which ratios >1, >10, >50, and >100 are observed according to whether the denominator is from the same ethnic database or the ethnically mixed database. This suggests that the allele frequencies in the two subpopulations vary in a way that significantly affects product-rule frequencies, which undermines the logical foundation for their use in ethnically mixed databases. The effect of subpopulation structure is to make product-rule estimates, on the average, biased against defendants in criminal trials, on the assumption that product-rule estimates are accurate within the subpopulations. While comparison of product-rule estimates for databases from different populations may be useful for some purposes, they cannot be regarded as a replacement for estimates that take possible multilocus statistical dependence into account.

Budowle et al. also expressed concern that samples of 73 Finnish and 79 Italian persons (with 29 and 70 complete profiles, respectively) may be small enough to introduce "unusually large correlations" and other ills (p. 536). However, no departure from Hardy-Weinberg equilibrium was found in the Finnish and Italian data, and only one pairwise linkage equilibrium was statistically significant (Krane et al. 1992). Budowle et al. give no reasons why hidden correlations should be troublesome in samples of this size but not in the population samples of size 200–600 that are normally used for DNA typing in criminal cases.

Table 1

Comparison between the Observed Cognate/Noncognate Database Product-Rule Ratios for the Finnish and Italian Databases, with the Same Ratios for 1,000 Simulated Pairs of Databases of the Same Sizes

	Ratio				Quantile (%)				
	>1	>10	>50	>100	50	75	90	95	99
Observed	62	15	3	1	1.5040	6.6530	19.9092	32.0348	182.8088
Average over simulations	49.47	3.21	.14	.03	1.0233	2.3131	5.0394	8.3735	29.2824
P-value	.0150	.0020	.0000	.0310	.0160	.0000	.0000	.0000	.0050

NOTE.—In each simulation, two sets of 73 and 79 profiles (respectively) were randomly chosen from a data set of 1,347 complete Caucasian profiles (Krane et al. 1992). Alleles in the simulated profiles corresponding to missing alleles in the observed Finnish and Italian profiles were treated as missing in the simulations. There were 99 = 27 + 70 complete profiles in all cases. For each complete profile, the alleles from that profile were excluded from the cognate database. *P*-values are the proportion of simulations for which the simulated value is greater than or equal to the observed value.

Budowle et al. also suggest that the Federal Bureau of Investigation's (FBI) use of binned data may ameliorate the effects of population structure. However, in the FBI's "worldwide survey" of binned VNTR frequencies (1993), ~70% of the comparisons between Caucasian subpopulations are statistically significant as measured by the appropriate $2 \times N$ contingency table tests.

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Editor's Note.—The preceding letter to the editor is a revised version of a letter initially submitted to the *Journal* by the authors. Prior to its external review, the letter was sent to Dr. Budowle and colleagues for reply. The reviewers of the letter from Sawyer et al. asked for changes and clarifications, which were incorporated into the above version. We received a reply from Dr. Budowle to the first version of the letter but returned it, asking that it be revised and that it respond to the final version of the letter from Sawyer et al. Dr. Budowle and colleagues think that their reply to the first version of the letter covers the issues raised there and in the final version and decided to publish it elsewhere. That reply appears as the following publication:

Budowle B, Monson KL. Clarification of additional issues regarding statistics and population substructure effects on forensic DNA profile frequency estimates. In: Sixth International Symposium on Human Identification 1995. Promega, Madison, WI (in press)

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