Hardy-Weinberg equilibrium analysis of DNA polymorphic loci that exhibit a continuous distribution of fragment sizes

I. Balazs, M.L. Baird, K. McElfresh and J. Udey

Lifecodes Corp., Valhalla, NY 10595, USA

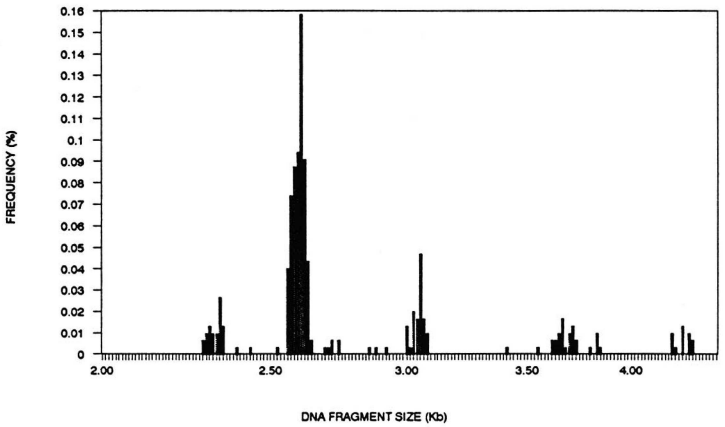
Hardy-Weinberg equilibrium analysis of alleles at a locus can be readily performed for conventional genetic markers (i.e. where each allele can be assigned a specific property to distinguish it from all others). There are however, many DNA polymorphic loci that demonstrate very large number of allele sizes that reflect variations in the number of tandem repeats (VNTR), of short DNA sequences (Baird 1986; Wong 1986; Dykes 1988; Balazs 1989). Complete separation of these alleles by electrophoresis requires very long gels and fractionation times, which is impractical using current technology. Nevertheless, the approximate size of an allele can be determined by measuring its mobility relative to DNA fragments of known size. The estimation of the size of these alleles allows them to be subjected to the same type of analysis as conventional genetic markers. One such analysis is to determine whether the alleles are in Hardy-Weinberg equilibrium. The following examples illustrate the effect of the allele frequency distribution pattern and the size measurement error in the Hardy-Weinberg equilibrium analysis of DNA polymorphic loci that exhibit a continuous distribution of DNA fragment sizes.

SIZE MEASUREMENT ERROR AND THE DETERMINATION OF ALLELE FREQUENCY.

Repeated measurement of the DNA fragment sizes, after multiple independent fractionations of the same DNA sample, show that the value of the measured sizes follow a normal distribution. Under our conditions of DNA fractionation and size measurement (Balazs 1989) the standard error of measurement is approximately 0.6% of the DNA fragment size, and the individual measurements fall within +/- 1.8% of the average fragment size. Therefore, in order to account for all the observations that represent a particular allele size it is necessary to sum all the events falling within a window or bin size. The boundary of the window is in turn determined by the size measurement error.

When polymorphic DNA fragments that are well resolved from each other are sized (e.g. such as those detected for the HRAS locus), the frequency of each allele can be obtained by summing the events that fall within this 3.6% bin size (+/- 1.8% of the average fragment size). It is important to note that it is not the resolution of the gel that defines the allele size range, but the measurement error. The results presented in Fig. 1 illustrate this type of analysis as applied to alleles of the HRAS locus. Using this criteria, a test for Hardy-Weinberg equilibrium for the HRAS locus does not reveal a significant difference between the expected vs observed number of homozygotes or heterozygotes (see Table A, below Fig. 1). In contrast, an apparent excess of homozygotes is obtained if the measurement error is ignored and the size measurements of a DNA fragment are arbitrarily subdivided into two or more size intervals (see Table B,C below Fig. 1).

Advances in Forensic Haemogenetics 3
Edited by H. F. Polesky and W. R. Mayr



SIZE RANGE(KB) 3.6% increments	>2.27 2.35		>2.54 2.64		>2.96 3.07	(A)
EXPECTED	1.2		53.3		2.2	
OBSERVED	2		54		2	

SIZE RANGE(KB) 3.6% increments	>2.49 2.59	>2.59 2.69	(B)
EXPECTED	13.5	13.8	
OBSERVED	24	30	

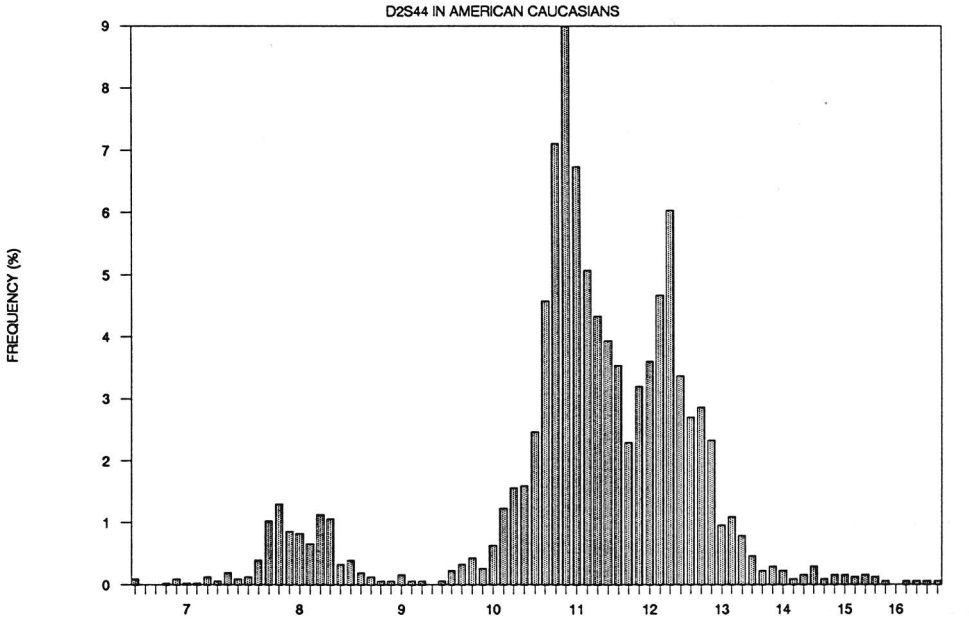
SIZE RANGE(KB) 0.6% increments	>2.55 2.56	>2.56 2.58	>2.58 2.59	>2.59 2.61	>2.61 2.62	>2.62 2.64	(C)
EXPECTED	0.2	0.8	5	3.8	2.2	0	
OBSERVED	3	5	16	14	13	2	

Fig. 1

ESTIMATION OF ALLELE FREQUENCY FOR LOCI HAVING A CONTINUOUS DISTRIBUTION OF DNA FRAGMENT SIZES.

Polymorphic DNA fragments can be assigned to discrete alleles as long as the resolution between DNA fragments exceeds the size measurement error. As the number of alleles, within a given distance in a gel increases, the allele frequency distribution becomes a sum of the overlapping normal distribution curves that represent each allele. As boundaries between alleles overlap the distinction between alleles disappears and the result is a continuous distribution of DNA fragments.
When loci exhibit increments in allele size smaller than our ability to measure them, the size measurement error becomes the parameter that best defines the limits of the alleles. In addition, the subdivision of the distribution of DNA fragment sizes should not be arbitrary. The position of the most common alleles has to be taken into account. As shown above for the HRAS locus, the

subdivision of the data representing the distribution of DNA fragment sizes, has to be centered around the most frequent DNA fragment sizes. Otherwise, the allele frequency obtained may underestimate of the actual allele frequency and the test for Hardy-Weinberg equilibrium may artificially result in an excess of observed vs expected homozygotes.
When this type of analysis was applied to several VNTR containing loci (e.g. D2S44, D14S13, D17S79), the observed frequency of homozygote and heterozygote genotypes was that predicted from the frequency of the individual alleles. One example of such analysis is illustrated in Fig. 2 for D2S44 using unrelated American caucasian individuals. The accompanying table shows no significant difference between the observed and expected number of homozygotes when the data is divided into bins that encompass the most frequent DNA fragment sizes.



SIZE RANGE(Kb)	7.72 8.00	8.00 8.29	8.29 8.59			10.25 10.62	10.62 11	11 11.4	11.4 11.81	11.81 12.23	12.23 12.67	12.67 13.13	13.13 13.6	
Expected	1.24	1.51	0.12			10.22	75.6	31.37	12.46	30.37	13.94	2.85	0.51	
Observed	1	1	0			13	82	34	13	34	14	5	2	

Fig. 2

REFERENCES

Baird M, et al. (1986) Allele frequency distribution of two highly polymorphic DNA sequences in three ethnic groups and its application to the determination of paternity. Am J Hum Genet 39:489-501

Balazs I, Baird M, Clyne M and Meade E (1989) Population genetic studies of five hypervariable loci. Am J Hum Genet 44:182-190

Dykes DD (1988) The use of biotinylated DNA probes in parentage testing: Non-isotopic labeling and non-toxic extraction. Electrophoresis 9:359-368

Wong Z, Wilson V, Jeffreys AJ and Thein SL (1986) Cloning a selected fragment from a human DNA 'fingerprint': isolation of an extremely polymorphic minisatellite. Nucleic Acid Res 14:4605-4616