Multimodal Frequency Distribution Analysis of Peripheral Nerves

Yves Usson Dipl. Biol.
Gerard Drouet d'Aubigny PhD

In morphometric studies of peripheral nerves, the statistical analysis of such data as axon diameters is complicated by the presence of multimodal distributions. Nerve fiber diameters, for example, cannot be analyzed by classical parametric tests, and such decriptive statistics as mean or variance lose much of their usefulness.

The recent development of stochastic catastrophe models offers a new parametric tool with which to describe multimodal distributions. This paper describes our development of a PASCAL computer program that permitted the modelling, comparison and segmentation of multimodal distributions. The method is based on a description of the multimodal frequency distributions by probability density functions of the canonical exponential family.

Statistical analysis of biologic measurements is an important feature of morphologic studies. In most instances, classical univariate analysis is adequate and easy to use; unfortunately, it fails in some particular cases. Many authors who have conducted experiments on peripheral nerves, such as comparing one set of nerves to a control set, have pointed out the problem that arises from the bimodal frequency distributions of myelinated axon diameters.[1,3,9,12]

Nonparametric testing procedures, such as the chi-square or Kolmogorov-Smirnov tests, are commonly used in these instances. Because of its great sensitivity to interclassing and to the sample range, the chi-square test is not entirely satisfactory. The Kolmogorov-Smirnov test does not possess the same defect but is time consuming.

Another approach to the problem is to consider bimodal distributions as mixtures of normally distributed subpopulations. After dividing frequency distributions into their Gaussian components, it is possible to compare these by a parametric statistical test, such as the nested analysis of variance.[3] Various methods for dividing bimodal frequency distributions, including graphic methods[2,10] and computing methods,[6-8,11] have been described. This paper proposes a method for dividing bimodal distributions that is based on recent applications of stochastic catastrophe models.[4,5]

Materials and Methods

Tissue Preparation

Specimens of superficial peroneal nerves were obtained from people who died of traumatic injuries at the Regional Hospital Center of Grenoble. Three-centimeter-long segments were dissected from the third inferior part of the leg. The specimens were fixed for one hour in 2.5% glutaraldehyde in cacodylate buffer, pH 7.4, at room temperature. After

---

From the Laboratory of Animal Biology and Zoology, Scientific and Medical University of Grenoble, St. Martin d'Heres, and the Department of Statistics, University of Grenoble, Grenoble, France. senior researcher

Dr. Usson is Y, Neurobiologic Development Section, Laboratory of Animal Biology and Zoology, Scientific and Medical University of Grenoble. U.A 682 CNRS assistant professor

Dr. Drouet d'Aubigny is Y, Laboratory for Information and Mathematics in the Social Sciences, Department of Statistics, University of Grenoble.

Address reprint requests to: Yves Usson, Groupe de Neurobiologie du Développement, Laboratoire Biologie Animale et Zoologie, Université Scientifique et Médicale de Grenoble, B.P. 68, F-38402 St. Martin d'Heres CEDEX, France.

This study was supported by INSERM grant 836026.

This paper was presented at the Third International Conference on Morphometry in Morphological Diagnosis, Delft, the Netherlands, September 13-15, 1984.

postfixation for half an hour in 1% osmium tetroxide in the same buffer, the pieces were dehydrated in a graded ethanol series and embedded in Epon. The nerves were cut as close as possible to the transverse. All thin cross sections were stained with uranyl acetate and lead citrate. Electron micrographs were taken with a Philips 201 transmission electron microscope. The final magnification was 2,800×.

Measurement of External Axon Caliber

Using a Hipad digitizing tablet (Bausch & Lomb) connected to an Apple III microcomputer, the external contours of myelinated axons were recorded and their areas were computed. From these values, assuming the axons to be cylindrical, the external diameter of the fibers was calculated as the diameter of the equivalent circle (equal area)[13]. All of the data was stored on a disk file for further computations by means of the PASCAL program.

Mathematical Tools

The principle on which this method for dividing bimodal distributions is based is the description of multimodal frequency distributions by probability density functions of the canonical exponential family. These provide at least four parameters that can be employed in a new statistical parametric tool.

Modelling. The general expression of canonical exponential models is

$$f(x) = \xi.\exp(g(x))$$

where ξ is a normalization coefficient and g(x) is a polynomial, called a "shape polynomial" for the density f.

The degree k of the polynomial g is directly related to the number of distribution modes; for a bimodal distribution, k should be at least equal to three. Various classes of multimodal density functions can be defined by modifying the expression of the shape polynomial. Four of these multimodal families, specified by the latters N, G, I and B, are of particular interest because the common unimodal frequency distributions (normal, gamma, inverted gamma and beta) can be considered as special cases of these. The expression of the shape polynomial for the N-density class is:

$$g(X) = a_1*X+a_2*X^2+\ldots+X^{k+1}$$

The estimation of the parameters a_1 through a_{k+1} requires the solution of a system of linear equations involving the $2k$ first empirical moments of the distribution. The formula of the moments is

$$M_i = \sum_{j=1}^{n} X_j^i/n$$

where n is the sample size. Let b_{i-1} be the estimates of the parameters a_i such that $a_i = -b_{i-1}/i$. For an N-density function of degree 3, the b_{i-1} are solutions of the following system:

$$M(i+j-2) * b(i-1) = R((i-1)*M(i-2))$$

$$\begin{bmatrix} 1 & M_1 & M_2 & M_3 \\ M_1 & M_2 & M_3 & M_4 \\ M_2 & M_3 & M_4 & M_5 \\ M_3 & M_4 & M_5 & M_6 \end{bmatrix} * \begin{bmatrix} b_0 \\ b_1 \\ b_2 \\ b_3 \end{bmatrix} = \begin{bmatrix} 0 \\ 1 \\ 2M_1 \\ 3M_2 \end{bmatrix}$$

The solvability of this system depends on the invertibility of the square matrix $M(i+j-2)$. The estimators b_{i-1} are consistent and asymptotically normal.[4] The normalization parameter ξ is obtained by integrating the density function. Afterward, the goodness of fit for a calculated model function may be examined with the chi-square test.

Comparison of Multimodal Distribution. Because they are consistent, the b_{i-1} estimates can be used in a two-sample test. Let N_a and N_b be the density functions calculated for two sets of data and b_a and b_b be the respective estimates of their parameters. Under the H_0 null hypothesis, we assume N_a to be equal to N_b, and b_a must consequently be equal to b_b. The purpose of the two-sample test is to determine whether or not the difference $b_a - b_b$ is significant. Because the b_{i-1} are asymptotically normal, the quadratic form of the matrix of differences, denoted

$$\left\| b_a - b_b \right\|_{\mathrm{I}}^2$$

follows asymptotically a chi-square distribution with $k+1$ degrees of freedom (k is the degree of the shape polynomial):

$$\left\| b_a - b_b \right\|_{\mathrm{I}}^2 = {}^t(b_a - b_b)*\mathrm{I}*(b_a - b_b) \rightarrow \chi^2_{k+1}$$

where the t superscript denotes the transposition and I is the identity matrix (a square matrix with unity on the main diagonal and zero everywhere else).

The significance of the difference can be expressed by the probability of exceeding the theoretical chi-square value with $k+1$ degrees of freedom.

Table I *Goodness-of-Fit Test for Calculated Density Functions of Increasing Polynomial Degree (K)*

	Superficial peroneal nerves											
	A			B			C			D		
K	χ^2_o	a	P	χ^2_o	a	P	χ^2_o	a	P	χ^2_o	a	P
3	46.5	19	<.001	23.4	17	0.137	181.8	41	<.001	110.6	36	<.001
4	29.2	19	.063	17.6	17	0.416	50.5	41	.147	42.6	36	.150
5	33.5	18	.015	13.9	17	0.669	59.4	40	.025	53.5	34	.018

χ^2_o is the calculated chi-square and P is the probability of exceeding the theoretical chi-square value with a degrees of freedom.

Results

Modelling

To test the adequacy of density functions computed from real bimodal histograms, chi-square values were calculated for a set of four nerves (A, B, C and D). Under the H_0 null hypothesis, the goodness of fit is expressed by the P value, which is the probability of exceeding the theoretical chi-square value with a degrees of freedom. The results for curves of the N family with increasing polynomial degree are shown in Table I. Except for nerve B, the optimum degree of the shape polynomial appeared to be four. Below this threshold, the goodness of fit was poor ($P<.001$). A higher degree did not increase the quality of the models. This is illustrated for nerve C (a set of 1,078 fibers) in Figure 1. The bimodality was well depicted with degrees 3 and 5, but the goodness of fit was severely eroded, with the overestimation of the first mode and underestimation of the second. Furthermore, for degree 3, the second mode and the antimode were displaced towards high values of the diameter. Further calculations, therefore, were based only on shape polynomials of degree 4.

Scale invariance of modelling was tested by computing the N-density function for nerve C from the transformed data and comparing it with the previously calculated density function. The transformed data were obtained by dividing one hundred times the diameter values of the original data file. Table II shows the parameter estimates of the two functions. The ratio of corresponding estimates shows that these are related by a "ten to the h power" law, where h is equal to the ratio of data unities multiplied by the parameter order. The slight bias on the second decimal of ratios must be attributed to a numerical rounding limitation of our microcomputer.

Comparison of Bimodal Distributions

To test the method of comparison, we used the N-density functions calculated for the previous set of nerves (A, B, C and D). These curves are presented in Figure 2 and illustrate the spreading of the spectra, the locations of modes and antimodes and the relative amplitudes of the peaks. The quadratic differences of parameter estimates and the associated probabilities of exceeding the theoretical chi-square value were calculated for each possible pair of nerves; all of the results are presented in Table III. Under the H_0 null hypothesis, if the probability exceeds 0.95, the

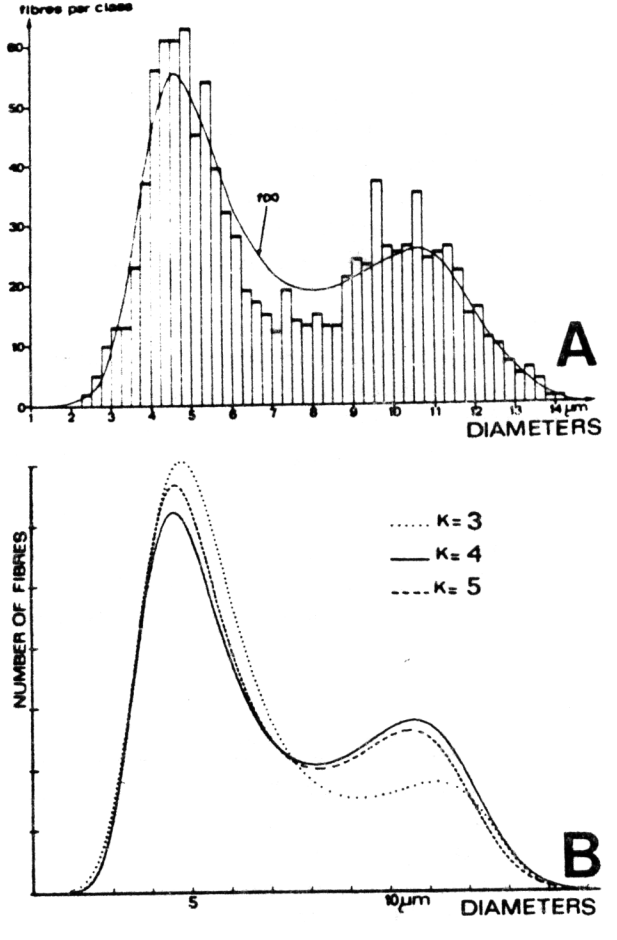

Figure 1
(A) Histogram of myelinated axon calibers of the superficial peroneal nerve C. The fitting curve of the N-density function of degree k=4 is superimposed. (B) Calculated density curves with increasing degrees (3 to 5) for nerve C.

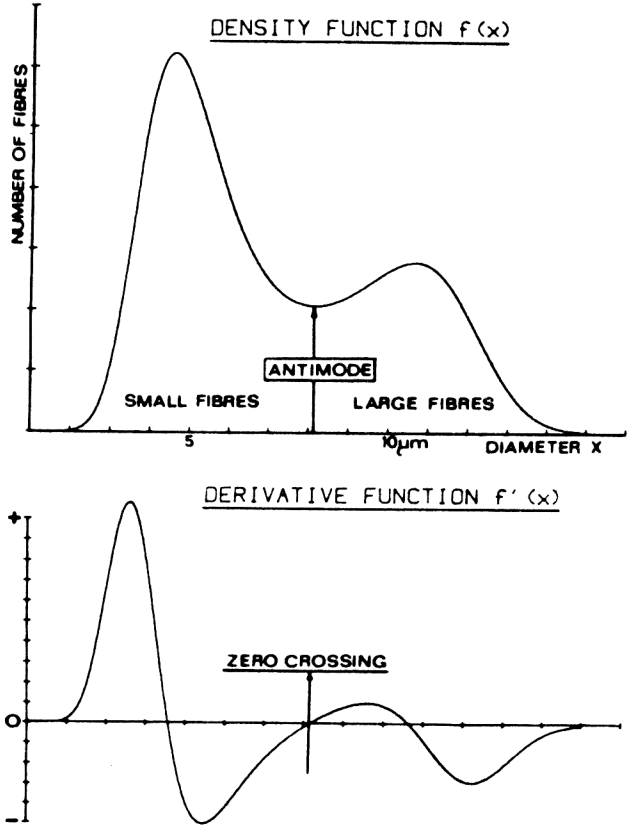

Figure 3
Localization of the antimode of a bimodal distribution for the segmentation of the subpopulations of the large and small fibers.

References

1. Barioz CM, Stoebner P, Bourdin A, Vila A: Methode d'analyse morphometrique optique et ultrastructurale du nerf musculocutane humain normal. Biol Cell 45:289, 1982
2. Bhattacharya CG: A simple method of resolution of a distribution into Gaussian components. Biometrics 23:115-137, 1967
3. Bronson RT, Bishop Y, Hedley-Whyte TE: A contribution to the electron microscopic morphometric analysis of peripheral nerve. J Compar Neurol 178:117-186, 1978
4. Cobb L: Stochastic catastrophe models and multimodal distributions. Behav Sci 23:360-374, 1978
5. Cobb L, Koppstein P, Chen NH: Estimation and moment recursion relations for multimodal distributions of the exponential family. J Am Stat Assoc 78:124-130, 1983
6. Court A: Separating frequency distributions into two normal components. Science 110:500-501, 1949
7. Day NE: Estimating the components of a mixture of normal distributions. Biometrika 56:463-474, 1969
8. Gregor J: An algorithm for the decomposition of a distribution into Gaussian components. Biometrics 25:79-93, 1969
9. Grosse R, Burlet C, Floquet A, Floquet J: Morphometric study of the postnatal maturation of human superficial peroneal nerve sampled by biopsy achieved using automatic image analysis. Stereol Iugosl [suppl] 3:533-538, 1981
10. Harding JP: The use of probability paper for the graphical analysis of polymodal frequency distribution. J Mar Biol Assoc 28:141-153, 1949
11. Hosmer DW: A comparison of iterative maximum likelihood estimates of the parameters of a mixture of two normal distributions under three different types of sample. Biometrics 29: 761-770, 1973
12. Mira JC: Etudes quantitatives sur la regeneration des fibres nerveuses myelinisees. Arch Anat Microsc Morphol Exp 65: 255-284, 1976
13. Stoebner P, Roche C, Ianeselli JC, Mezin P, Laurent A, Vila A, Saxod R: Medex-nerf: Progiciel d'analyse quantitative semi-automatisee des biopsies nerveuses en microscopie electronique. Biol Cell (~~in press~~) , 52, 120a 1984