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# Protein Bioinformatics and Mixtures of Bivariate von Mises Distributions for Angular Data

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SUMMARY. A fundamental problem in bioinformatics is to characterize the secondary structure of a protein, which has traditionally been carried out by examining a scatterplot (Ramachandran plot) of the conformational angles. We examine two natural bivariate von Mises distributions—referred to as Sine and Cosine models—which have five parameters and, for concentrated data, tend to a bivariate normal distribution. These are analyzed and their main properties derived. Conditions on the parameters are established which result in bimodal behavior for the joint density and the marginal distribution, and we note an interesting situation in which the joint density is bimodal but the marginal distributions are unimodal. We carry out comparisons of the two models, and it is seen that the Cosine model may be preferred. Mixture distributions of the Cosine model are fitted to two representative protein datasets using the expectation maximization algorithm, which results in an objective partition of the scatterplot into a number of components. Our results are consistent with empirical observations; new insights are discussed.

KEY WORDS: Bivariate angular data; Bivariate circular mixture; Directional statistics; Distribution on torus; Myoglobin; Protein conformational angles; Ramachandran plots.

## 1. Introduction

Protein shapes are often described using the (3-d) coordinates of the backbone chain, which consists of an ordered sequence of nitrogen and carbon atoms known as peptide units. These coordinates can be used to obtain a sequence of conformational angles, known as *torsional* angles, which form a natural pairing $(\phi, \psi)$ with each angle in $(-\pi, \pi]$. These angles can be plotted in a scatterplot—now known as a Ramachandran plot (Ramachandran, Ramakrishnan, and Sasisekharan, 1963)—and these have been used in the understanding of protein secondary structure which describes a protein in terms of $\alpha$-helices, $\beta$-sheets, and loop motifs, etc. Branden and Tooze (1998, p. 9) describe in more detail the uses of these very familiar plots, including an indication of the approximate segmentation into various motifs, which was initially empirically observed by Ramachandran et al. (1963).

Recently, there have been attempts to parameterize the joint distribution of $(\phi, \psi)$ (Pertsemlidis et al., 2005) and this has been done using Fourier basis functions to represent the bivariate distribution of $(\phi, \psi)$ on the torus. This parameterization requires about a hundred parameters and does not seem to allow an easy interpretation from a biological (or statistical) perspective.

One of our motivations for this article is to describe the probability distribution on the torus, but our approach is to use a different parameterization, which is based on a mixture of bivariate von Mises distributions. The von Mises distribution on the circle is well known; it has two parameters (analogous to the normal distribution), the mean ($\mu$) and

concentration parameter ($\kappa$), which is antivariance. The density is given by (see, e.g., Mardia and Jupp, 1999, p. 36)

$$(2\pi I_0(\kappa))^{-1} \exp\{\kappa \cos(\phi - \mu)\},$$

where $I_0(\cdot)$ denotes the modified Bessel function of the first kind and order 0, the parameter $\mu$ is the mean direction, and $\kappa$ is the concentration parameter. This distribution can be approximated by the normal density for small "variance" (large $\kappa$). In Section 2, we introduce two bivariate von Mises distributions which, like the bivariate normal distribution, have five parameters: two means, two concentrations (antivariance), and a parameter controlling "correlation." In Section 3, we describe some key properties and make some comparisons. In Section 4, we return to our motivating example of fitting an appropriate distribution to $(\phi, \psi)$ angles obtained from a protein backbone. The model is extended to mixtures of distributions which are fitted using an expectation maximization (EM) algorithm and some results for two proteins are described. A final discussion section describes further possible extensions using a Markov chain Monte Carlo procedure.

## 2. Bivariate von Mises Distributions

For the study of conformational angles $(\phi, \psi)$, we need a bivariate angular distribution which extends the univariate von Mises distribution and has analogy with the bivariate normal distribution, namely, two mean parameters, two parameters for variance (antivariance), and a parameter that determines the correlation. Mardia (1975) introduced a bivariate circular

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distribution for $(\Phi, \Psi)$, with the probability density function (PDF) proportional to

$$\exp\left[\kappa_1\cos(\phi-\mu)+\kappa_2\cos(\psi-\nu)\right.$$
$$\left.+\{\cos(\phi-\mu),\sin(\phi-\mu)\}A\{cos(\psi-\nu),\sin(\psi-\nu)\}^T\right], \tag{1}$$

where $A$ is a $2\times 2$ matrix. This has more parameters $(4+2+2=8)$ than we would often require, there are difficulties of interpretation, and, at least for large concentrations, there is redundancy. A subclass has been introduced by Rivest (1988), which has PDF proportional to

$$\exp\{\kappa_1\cos(\phi-\mu)+\kappa_2\cos(\psi-\nu)+\alpha\cos(\phi-\mu)\cos(\psi-\nu)$$
$$+\beta\sin(\phi-\mu)\sin(\psi-\nu)\}. \tag{2}$$

However, both equations (1) and (2) are overparameterized in the sense that there should only be five parameters by analogy with the bivariate normal. To overcome this defect, Singh, Hnizdo, and Demchuk (2002) have recently concentrated on a special case of equation (2) when $\alpha=0$ and $\beta=\lambda$. We call this the *Sine model* whose PDF is given by

$$f_{\rm S}(\phi,\psi)=C\exp\{\kappa_1\cos(\phi-\mu)+\kappa_2\cos(\psi-\nu)$$
$$+\lambda\sin(\phi-\mu)\sin(\psi-\nu)\}, \tag{3}$$

where the normalizing constant is given by

$$C^{-1}=4\pi^2\sum_{m=0}^{\infty}\binom{2m}{m}\left(\frac{\lambda^2}{4\kappa_1\kappa_2}\right)^m I_m(\kappa_1)I_m(\kappa_2),$$

and $I_r(\kappa)$ denotes the modified Bessel function of the first kind and order $r$.

Another such submodel of equation (2) with similar characteristics is when $\alpha=\beta=-\kappa_3$. We will call this the *Cosine model* and its PDF is given by

$$f_{\rm C}(\phi,\psi)=\{c(\kappa_1,\kappa_2,\kappa_3)\}\exp\{\kappa_1\cos(\phi-\mu)$$
$$+\kappa_2\cos(\psi-\nu)-\kappa_3\cos(\phi-\mu-\psi+\nu)\}, \tag{4}$$

for $\kappa_1, \kappa_2\geq 0$. Here the normalizing constant is given by

$$c(\kappa_1,\kappa_2,\kappa_3)^{-1}$$
$$=(2\pi)^2\left\{I_0(\kappa_1)I_0(\kappa_2)I_0(\kappa_3)+2\sum_{p=1}^{\infty}I_p(\kappa_1)I_p(\kappa_2)I_p(\kappa_3)\right\}.$$

The marginal probability density of $\psi$ for the Cosine model (4) is given by

$$f_{\rm C}(\psi)=c(\kappa_1,\kappa_2,\kappa_3)2\pi I_0(\kappa_{13}(\psi-\nu))\exp\{\kappa_2\cos(\psi-\nu)\}, \tag{5}$$

where $\kappa_{13}(\psi)^2=\kappa_1^2+\kappa_3^2-2\kappa_1\kappa_3\cos\psi$. The marginal probability density of $\phi$ is given by an analogous expression. This distribution is symmetric about $\nu$ and is approximately a von Mises distribution for small values of $\kappa_3$. For $\kappa_1=\kappa_2=\kappa_3=0$, the distribution is uniform. For $\kappa_1=\kappa_2=0$, the distribution is von Mises. Further, the conditional density $f_{\rm C}(\phi\,|\,\psi)$ is von Mises M $(\psi_\nu, \kappa_{13}(\psi))$, where $\tan\psi_\nu=-\kappa_3\sin(\psi-\nu)/(\kappa_1-\kappa_3\cos(\psi-\nu))$. An analogous expression holds for $f_{\rm C}(\psi\,|\,\phi)$.

Singh et al. (2002) have given the marginal and conditional distributions for the Sine model, which we state here for comparison. The marginal density of $\phi$ (when $\mu=0$) is proportional to

$$f_{\rm S}(\phi)=I_0(\kappa_{2\lambda}(\phi))\exp\{\kappa_1\cos\phi\}, \tag{6}$$

where $\kappa_{2\lambda}(\phi)^2=\kappa_2^2+\lambda^2\sin^2\phi$. This is symmetric about $\mu=0$ but not von Mises. Using equations (3) and (6) we have the conditional PDF of $\Psi$ given $\Phi=\phi$ as M $(\phi_\mu, \kappa_{2\lambda}(\phi))$ where $\tan\phi_\mu=(\lambda/\kappa_2)\sin\phi$.

## 3. Properties of Bivariate Circular Models

### 3.1 *Bimodality Conditions*

In this section, we state some key results for the Sine and Cosine models. The proofs are given in Web Appendix A.

Note that the joint density function $(\Phi, \Psi)$ in equation (4) (with $\mu=\nu=0$) is also a bivariate von Mises for $f_{\phi,-\psi}$ and $f_{-\phi,\psi}$. Also $f_{\phi,0}$ and $f_{0,\psi}$ are univariate von Mises, and for the Sine model we have similar results.

THEOREM 1: *For the Cosine density, when $\kappa_1$ and $\kappa_2$ are large, the random variable $(\Phi, \Psi)$ is approximately bivariate normal distributed if and only if $\kappa_3\leq\kappa_1\kappa_2/(\kappa_1+\kappa_2)$.*

REMARK: Derivations for bivariate normal approximations for the Sine model yield similar results and can be found in Singh et al. (2002).

THEOREM 2: *The joint density function $(\Phi, \Psi)$ of the Cosine model in equation (4) is unimodal if $\kappa_3<\kappa_1\kappa_2/(\kappa_1+\kappa_2)$ and is bimodal if $\kappa_3>\kappa_1\kappa_2/(\kappa_1+\kappa_2)$ when $\kappa_1>\kappa_3>0$, $\kappa_2>\kappa_3>0$.*

THEOREM 3: *The joint density function of the Sine model in equation (3) is unimodal if $\kappa_1\kappa_2>\lambda^2$ and is bimodal if $\kappa_1\kappa_2<\lambda^2$, when $\kappa_1>0$, $\kappa_2>0$, and $-\infty<\lambda<\infty$.*

REMARK: We note that Singh et al. only discuss bimodality for the marginal distributions of the Sine density, and not the joint distribution as given here.

The marginal distribution of $\psi$ for the Cosine model is given by equation (5). It is easy to construct an example ($\kappa_1=1.5, \kappa_2=1.7$, and $\kappa_3=1.3$) for which the marginal distribution is unimodal even though the bivariate density is bimodal for the same $\kappa$ values. This feature may be surprising at first: that, for a given set of parameters such that the Cosine density is bimodal, the corresponding marginal density may be unimodal. The following theorem describes the conditions under which this occurs.

THEOREM 4: *For the Cosine model (4) with $\kappa_3\neq 0$, the marginal distribution of $\Phi$ is symmetric around $\psi=\mu$ and unimodal (respectively bimodal) with mode at $\mu$ (respectively with the modes at $\mu-\phi^*$ and $\mu+\phi^*$) if and only if $A(|\kappa_1-\kappa_3|)\leq$ (respectively $>$)$|\kappa_1-\kappa_3|\kappa_2/(\kappa_1\kappa_3)$, where $\phi^*$ is given by the solution to $\kappa_1\kappa_3A(\kappa_{13}(\phi))/\kappa_{13}(\phi)-\kappa_2=0$, and $A(\kappa)=I_1(\kappa)/I_0(\kappa)$.*

### 3.2 *Choosing a Model*

*Analytical comparisons.* Clearly, a change in the mean values ($\mu$ and $\nu$) will just shift the origin, so we will consider $\mu=\nu=0$ in what follows. We note the following comparisons,

which can all be obtained by considering the joint PDF and conditional distributions:

| Sine model | Cosine model |
|---|---|
| (1) Changing the sign of $\lambda$ simply reflects in an axis. | Changing the sign of small $\kappa_3$ gives an approximate reflection, but for $|\kappa_3|$ large (relative to $\kappa_1$, $\kappa_2$), bimodality occurs only for positive $\kappa_3$. |
| (2) The expected value of $\phi \,\vert\, \psi = 0$ is always 0 for any values of $\kappa_1$, $\kappa_2$, and $\lambda$. | The expectation is zero only when the $\kappa_i$, $i = 1, 2, 3$ give a unimodal PDF. |
| (3) The expected value of $\phi \,\vert\, \psi$ is always constrained to lie in the range $[-\pi/2, \pi/2]$ for any values of $\kappa_1$, $\kappa_2$, $\lambda$, and $\psi$. | For large (negative) $\kappa_3$ the expected value of $\phi \,\vert\, \psi$ approximates $\psi$ over the full range of $(-\pi, \pi)$. |
| (4) A bimodal density occurs for large $|\lambda|$ (relative to $\kappa_1$, $\kappa_2$). | A bimodal distribution occurs only for large positive $\kappa_3$. |
| (5) Transforming $(\phi, \psi)$ to $(\psi, -\phi)$ is equivalent to changing the sign of $\lambda$. | This transformation allows for rotations of the PDF (which cannot be achieved by changing $\kappa_3$). |

The bivariate densities can be represented by contour plots that can be used to illustrate the above statements. However, the key features are more easily compared by plotting the log of the density and omitting the normalizing constant. In numerical comparisons we noted that for small $\lambda \approx \kappa_3$ the two models are very similar.

Overall, we could conclude that, if we allow for the possibility of transformations of the form $(\phi, \psi)$ to $(\psi, \pi + \phi)$ and $(\phi, \psi)$ to $(\psi, -\phi)$ then the Cosine model gives a richer set of possible contour plots (see point 5 above), and hence should be able to fit more closely a larger class of distributions.

*Numerical comparisons.* Another approach for comparing the Sine and Cosine models is to examine their moments. Characteristic functions can be used to estimate the moments of the two distributions and we numerically computed the correlations for the Cosine and Sine models in order to study their behavior in relation to the parameters $\kappa_3$ and $\lambda$. The parameter $\kappa_3$ for the Cosine model and $\lambda$ for the Sine model were varied for fixed values of $\kappa_1$ and $\kappa_2$. Using some selected values of $\kappa_1$ and $\kappa_2$, we observed that the correlation between $\cos \phi$ and $\cos \psi$ and between $\sin \phi$ and $\sin \psi$ were seen to be (mostly) decreasing functions of $\kappa_3$ for the Cosine model, whereas for the Sine model the correlation between $\sin \phi$ and $\sin \psi$ was a monotonic increasing function of $\lambda$, and between $\cos \phi$ and $\cos \psi$ was always nonnegative and has a U-shaped relationship with $\lambda$. Thus we believe that the Cosine model may be better able to capture the correlation between $\phi$ and $\psi$ as ei-

ther a correlation of $(\cos \phi, \cos \psi)$ or $(\sin \phi, \sin \psi)$. In the Sine model, $\lambda$ does not measure the correlation of $(\cos \phi, \cos \psi)$, and his would seem to give the Cosine model an advantage over the Sine model.

It is also of interest to empirically explore the relationship between the bivariate normal distribution and the Cosine and Sine models when the data are highly concentrated. We estimate the correlation under the assumption of normality induced by high concentration. We compare this estimated correlation under the assumption of normality with the correlation evaluated from the distributions by integration. Under high concentration, the approximate correlation between $\phi$ and $\psi$, for the Cosine model, is

$$\rho_c = \frac{-\kappa_3}{\sqrt{(\kappa_1 - \kappa_3)(\kappa_2 - \kappa_3)}}, \qquad 3.2.2$$

and for the Sine model Singh et al. (2002) obtain $\rho_s = \lambda/(\kappa_1 \kappa_2)^{1/2}$. We compared the values of $\rho_c$ and $\rho_s$ with the correlation of $(\cos \phi, \cos \psi)$ and $(\sin \phi, \sin \psi)$ for various large $(\kappa_1, \kappa_2)$. Overall, we found that the Cosine model has some advantages over the Sine model. Sometimes the Sine model can explain the presence of correlation only in the range of $(0, 0.4)$. In the Cosine model, the "estimated correlation" tracks the values of the parameter $\kappa_3$ very well for the entire range of values between $(-1, 1)$. For further details, see Subramaniam (2005).

## 4. Application: Mixture Models for Proteins

### 4.1 *Protein Data*

Protein structures can be determined to an atomic level by X-ray diffraction and neutron-diffraction studies of crystallized proteins, and more recently by nuclear magnetic resonance (NMR) spectroscopy of proteins in solution. In a protein, the backbone chain $N$-$C_\alpha$ and $C_\alpha$-$C$ bonds are relatively free to rotate. These rotations are represented by the torsion angles $\phi$ and $\psi$, respectively.

A scatter planar representation of such torus data has come to be known as a *Ramachandran plot* (Ramachandran et al., 1963), and a study of these angles through directional statistics methods has been one of our goals. Ramachandran used computer models of small polypeptides to systematically vary $\phi$ and $\psi$ with the objective of finding stable conformations. For each conformation, the structure was examined for close contacts between atoms. Atoms were treated as hard spheres with dimensions corresponding to their van der Waals radii. Therefore, $\phi$ and $\psi$ angles that cause spheres to collide correspond to sterically disallowed conformations of the polypeptide backbone.

We consider two datasets that correspond to these conformational angles from the proteins Malate dehydrogenase (7mdh in the protein database http://www.rcsb.org/pdb) and Myoglobin (protein 101m). Myoglobin is one of the smallest proteins, consisting mainly of $\alpha$-helices, and the first protein whose structure was determined (Bennett and Kendrew, 1952). Malate dehydrogenase is an "average" protein, with various motifs.

The Ramachandran plot and the circular histograms of the marginal variables for Malate dehydrogenase are shown in Figure 1. These clearly indicate that a mixture distribution
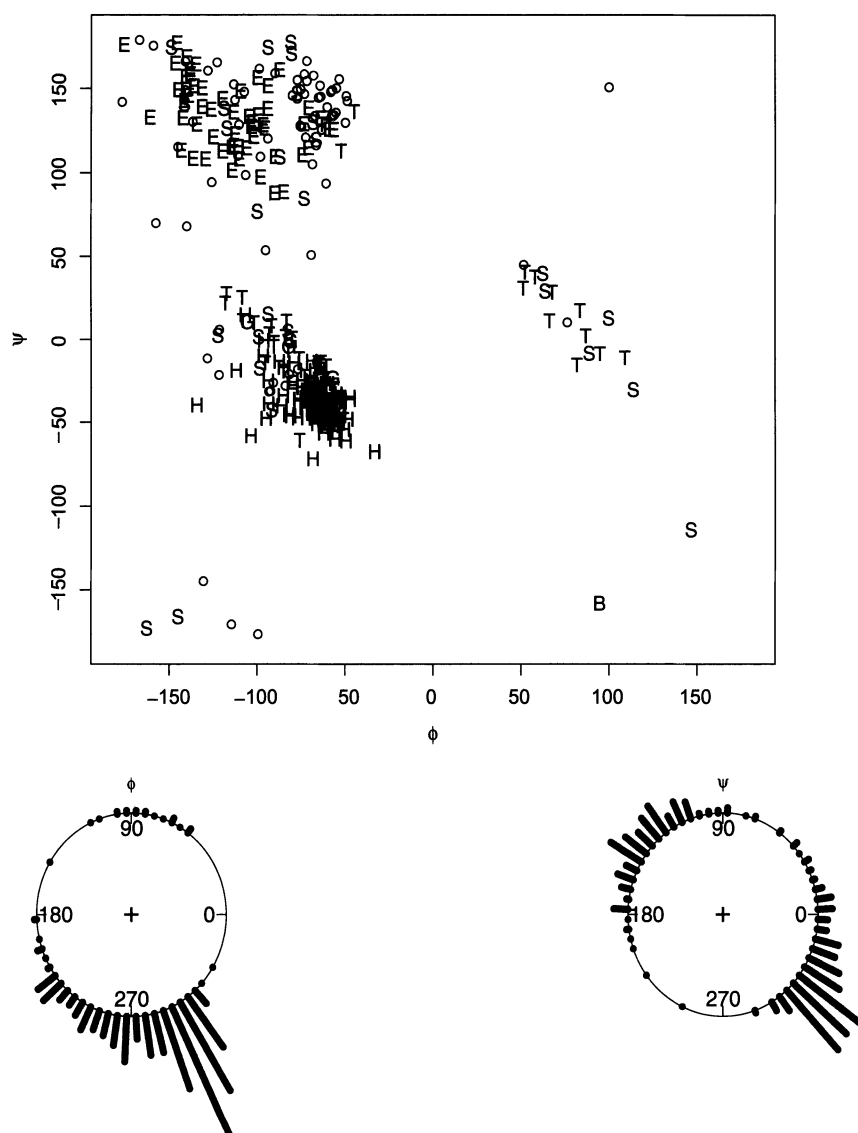
*Biometrics, June* 2007



**Figure 1.** Ramachandran plot of Malate dehydrogenase (top) and circular plots of $\phi$, $\psi$ (bottom). The symbols used in the plot correspond to the dssp classifications; see Section 4.4.

may be appropriate because there are obviously a number of distinct clusters corresponding to the secondary structure of the protein. The marginals show departure from the von Mises distribution that only has one mode.

### 4.2 *Mixture Models*

We can use the method of maximum likelihood to estimate the parameters of a Cosine density. The log-likelihood function for equation (4) is

$$\sum_{i=1}^{n} \left( \kappa_1 \cos(\phi_i - \mu) + \kappa_2 \cos(\psi_i - \nu) \right.$$

$$\left. - \kappa_3 \cos(\phi_i - \psi_i + \nu - \mu) \right) + n \log(c(\kappa_1, \kappa_2, \kappa_3)), \quad (7)$$

and we can obtain starting values under the assumption that the marginal distributions are von Mises. Then, for example, starting values for $\mu$ are given by

$$\bar{\phi} = \begin{cases} \tan^{-1}(\bar{S}/\bar{C}) & \text{if } \bar{C} \geq 0; \\ \tan^{-1}(\bar{S}/\bar{C}) + \pi & \text{if } \bar{C} < 0, \end{cases}$$

where $\bar{C}$ and $\bar{S}$ are the means of the cos $(\phi)$ and sin $(\phi)$, respectively, and starting values for $\nu$ are similarly found from the $\psi$'s. For $\hat{\kappa}_1$ an approximate solution due to Dobson (1978) is used. The starting values are obtained for $\nu$ and $\hat{\kappa}_2$ in the same way. For $\hat{\kappa}_3$ we use the mean of $(\hat{\kappa}_1, \hat{\kappa}_2)$. Our optimization program for maximizing the (log) likelihood was tried and tested using data simulated from the bivariate Cosine distribution. For details of the simulation method, see Web Appendix B.

However, it is clear from Figure 1 that a single Cosine model will not fit these data—even though a bimodal distribution can sometimes be obtained. The plot suggests $K \geq 3$ components in a mixture model, which can be parameterized by:

$$f_M(\phi, \psi) = \sum_{j=1}^{K} \pi_j f_j(\phi, \psi), \quad (8)$$

**Table 1**

*Estimates ($\mu$ and $\nu$ are given in radians) and standard errors for the mixture components fitted to Myoglobin (top; three components) and Malate dehydrogenase (bottom; five components). The final two columns indicate: $R = Y$ that the component parameters are fitted to rotated data (otherwise $R = N$) and modes $= B$ that the density is bimodal (otherwise unimodal [$U$]).*

| Component | | $\kappa_1$ | $\kappa_2$ | $\kappa_3$ | $\mu$ | $\nu$ | $\pi$ | $R$ | Modes |
|---|---|---|---|---|---|---|---|---|---|
| | | | | Myoglobin | | | | | |
| 1 | est. | 8.459 | 13.544 | 8.081 | −0.191 | 0.093 | 0.138 | Y | B |
| | SE | 1.881 | 3.243 | 1.819 | 0.129 | 0.073 | 0.028 | | |
| 2 | est. | 8.340 | 3.989 | 2.872 | −1.858 | 1.843 | 0.124 | Y | B |
| | SE | 2.546 | 1.340 | 1.230 | 0.094 | 0.220 | 0.027 | | |
| 3 | est. | 89.075 | 39.679 | −38.603 | −1.124 | −0.689 | 0.738 | Y | U |
| | SE | 14.700 | 10.181 | 10.108 | 0.009 | 0.012 | 0.036 | | |
| | | | | Malate dehydrogenase | | | | | |
| 1 | est. | 27.759 | 9.491 | −8.669 | −1.136 | 2.449 | 0.115 | Y | U |
| | SE | 7.283 | 4.407 | 4.325 | 0.028 | 0.040 | 0.017 | | |
| 2 | est. | 2.495 | 2.434 | −2.119 | −2.063 | 2.367 | 0.281 | Y | U |
| | SE | 0.509 | 0.504 | 0.484 | 0.058 | 0.059 | 0.024 | | |
| 3 | est. | 7.075 | 3.741 | −5.405 | −1.486 | −0.335 | 0.219 | Y | U |
| | SE | 1.529 | 1.215 | 1.354 | 0.039 | 0.046 | 0.022 | | |
| 4 | est. | 134.897 | 83.831 | 28.405 | −1.08 | −0.699 | 0.336 | N | U |
| | SE | 18.960 | 12.900 | 7.769 | 0.01 | 0.013 | 0.025 | | |
| 5 | est. | 70.840 | 62.464 | 34.960 | 1.378 | 0.197 | 0.050 | N | B |
| | SE | 22.557 | 19.917 | 11.525 | 0.061 | 0.069 | 0.012 | | |

where $f_j$ denotes a Cosine density with parameters $\theta_j = (\kappa_{jk}, k = 1, 2, 3, \mu_j, \nu_j)$, $j = 1, \ldots, K$, and $\pi_1, \ldots, \pi_K$ are the mixing proportions (with $\sum \pi_i = 1$).

### 4.3 EM Algorithm

We will investigate the use of the EM algorithm (McLachlan and Krishnan, 1997, p. 71–72) to fit equation (8). As usual, there are several steps that are iterated to convergence:

(1) Estimation of membership probabilities using

  (a) $p_{ij} = \pi_j f_j(\phi_i, \psi_i)$ for $i = 1, \ldots, n$, $j = 1, \ldots, K$,
  (b) Normalization $p_{ij} = p_{ij} / \sum_j p_{ij}$ for $i = 1, \ldots, n$.

(2) Use maximum likelihood to find the $\theta_j = (\kappa_{j1}, \kappa_{j2}, \kappa_{j3}, \mu_j, \nu_j)$ that maximizes the weighted likelihood function

$$\prod_{i=1}^{n} p_{ij} f_j(\phi_i, \psi_i)$$

for $j = 1, \ldots, K$.

(3) Obtain the mixing proportions $\pi_j = \sum_i p_{ij}$.

However, in our use of the Cosine density we have increased the ability to model the data by also considering transformations of all the data of the form $(\phi, \psi) \rightarrow (\psi, -\phi)$ for estimating the parameters of each mixture component. In practice this means checking the likelihood (for each $j$) at step 2, and choosing the rotation that gives the larger value.

It is well known that the EM algorithm can get stuck in local solutions, and there can also be a problem with singularities in which one of the components consists of only a single observation. In our implementation, we tried several starting values and chose the best final solution, excluding any solutions with very high concentrations (large $\kappa$).

The choice of the number of components $K$ will obviously affect the final likelihood. Adding another component will increase the number of parameters by six, and this was done

incrementally (starting with $K = 1$) until the first minimum of the Akaike information criterion (AIC), and it is this value of $K$ which is then reported. After convergence to the final solution we can note the mixing proportions $\pi_1, \ldots, \pi_K$, the parameter estimates $\theta_j$, $j = 1, \ldots, K$, and the membership probabilities $p_{ij}$. Then, for each observation $(\phi_i, \psi_i)$ we can assign it to the group that maximizes $p_{ij}$.

### 4.4 Results

The EM algorithm has been used to fit mixtures of the Cosine model to both Malate dehydrogenase and Myoglobin conformational angles.

For Myoglobin, the algorithm selected $K = 3$ components, all of which used the rotated parameterization. The contour

**Table 2**

*Crossclassification based on the labels from the dssp program (columns) and the clusters obtained from the K-mixture Cosine distribution model. Top: $K = 3$ for Myoglobin. Bottom: $K = 5$ for Malate dehydrogenase.*

| | | | Myoglobin | | |
|---|---|---|---|---|---|
| Gp | | G | H | S | T |
| 1 | 3 | 2 | 4 | 1 | 10 |
| 2 | 14 | 2 | 0 | 2 | 1 |
| 3 | 0 | 8 | 104 | 0 | 1 |

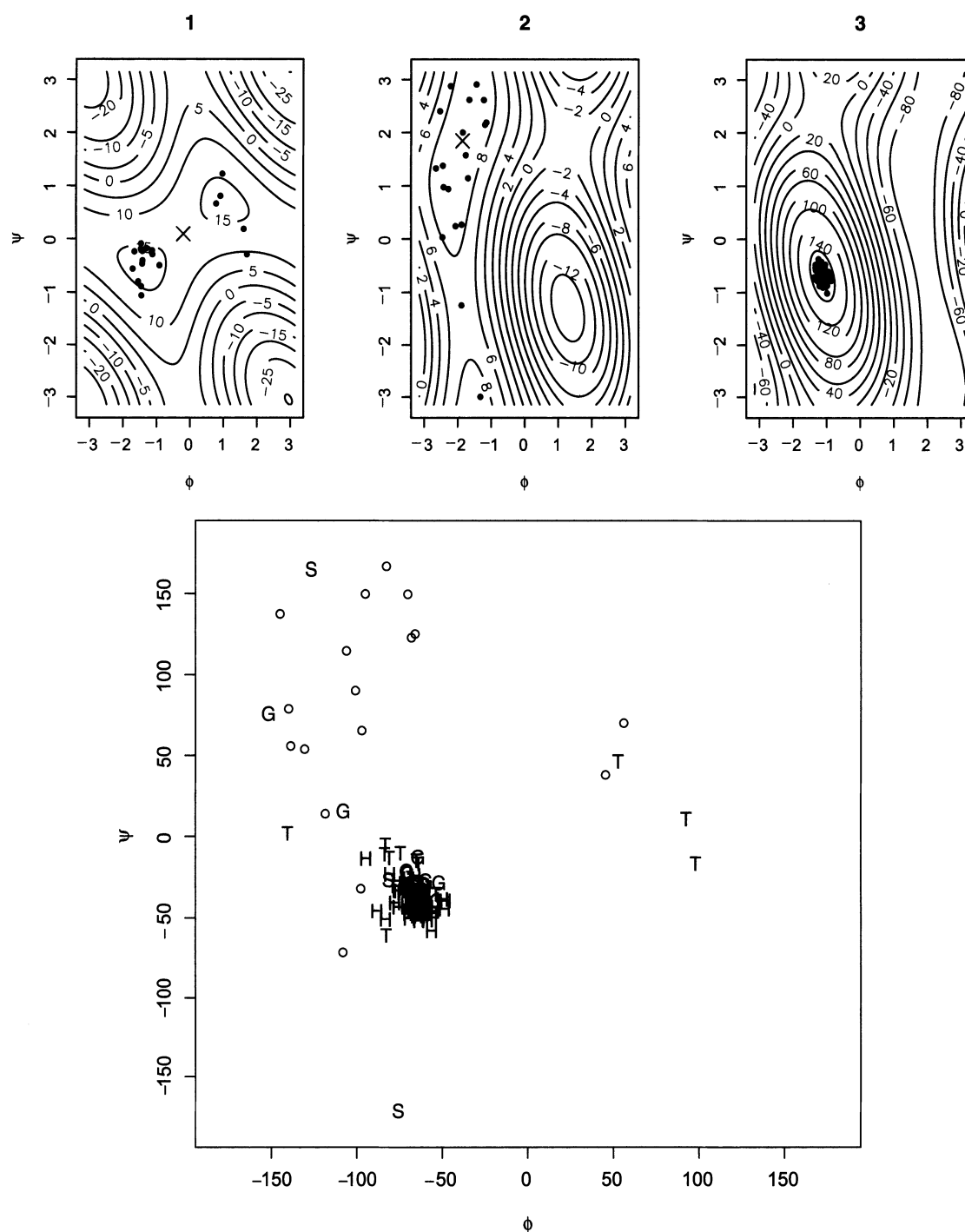| | | | Malate dehydrogenase | | | |
|---|---|---|---|---|---|---|
| Gp | | B | E | G | H | S | T |
| 1 | 29 | 0 | 5 | 0 | 0 | 5 | 4 |
| 2 | 30 | 1 | 51 | 0 | 0 | 11 | 0 |
| 3 | 9 | 0 | 1 | 4 | 26 | 6 | 20 |
| 4 | 0 | 0 | 0 | 2 | 113 | 1 | 8 |
| 5 | 2 | 0 | 0 | 0 | 0 | 5 | 10 |

**Figure 2.** Mixture model for Myoglobin. The top three panels correspond to the three components, with contour plots of the log density and points allocated to the most probable mixture. The location × (sometimes obscured in a cluster of points) marks the mean $(\mu, \nu)$ for each mixture. Bottom: Ramachandran plot of Myoglobin, with dssp classifications. (The unlabeled points, given by open circles, are "blank" in dssp.)

plots of the log densities are shown in Figure 3, and the parameter estimates are given in Table 1. We note that the first and second components have fit a Cosine model, which results in a bimodal density, and in this case Theorem 4 can be used to locate the modes exactly. Further, we note that the third component (which contains nearly 3/4 of the observations) has formed a very tight cluster about its mean.

(As a rough guide, $\kappa$ values around 5 are considered as "moderate concentration," and values over 10 are considered as "high concentration.") Myoglobin is known to contain nearly all helices, and this dominance of one of the components is consistent with this fact.

Historically, describing protein secondary structure has been somewhat subjective, but the dssp program (Kabsch and
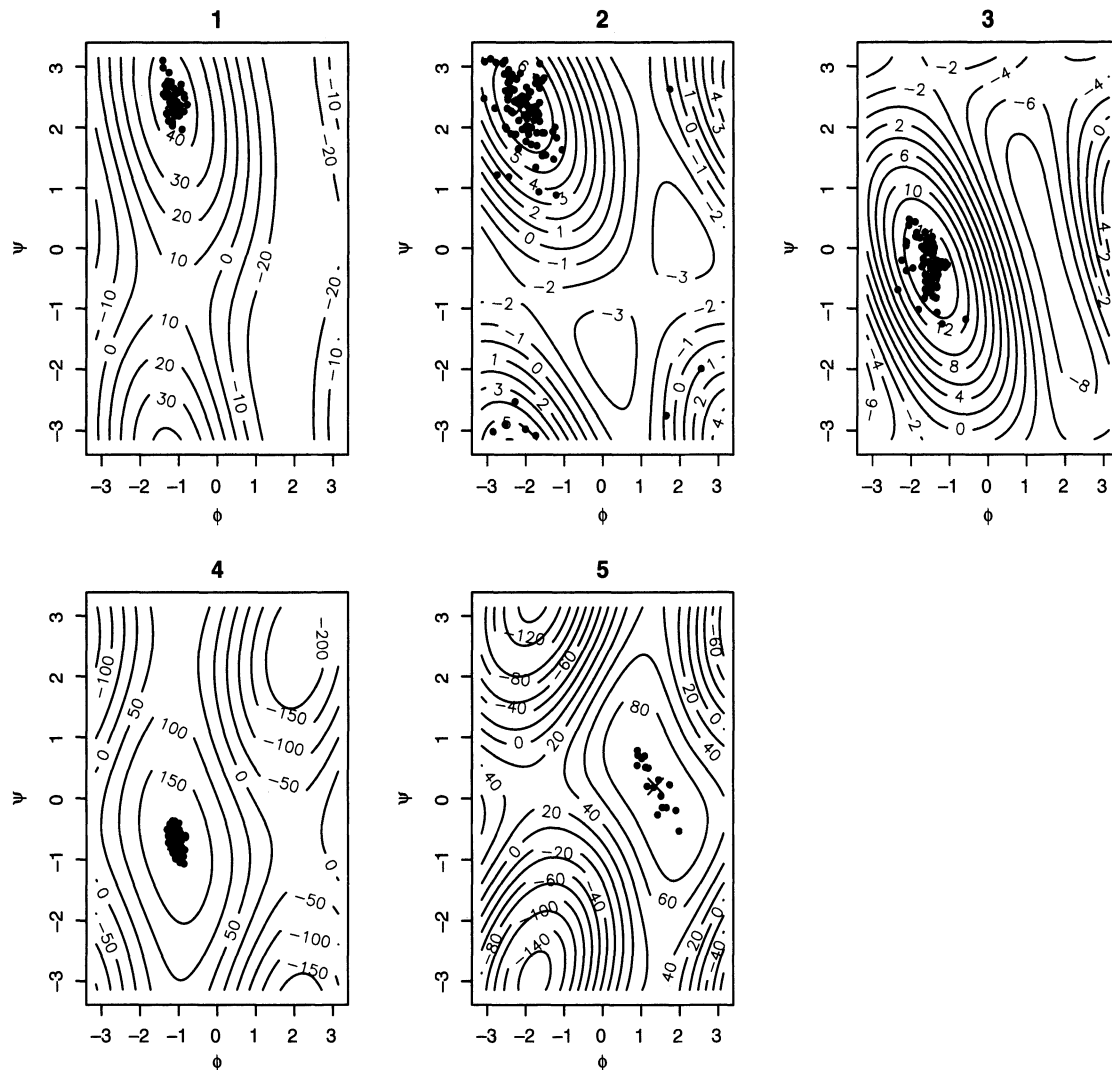
**Figure 3.** Mixture model for Malate dehydrogenase. The five panels correspond to the five components, with contour plots of the log density and points allocated to the most probable mixture. The location × (sometimes obscured in a cluster of points) marks the mean $(\mu, \nu)$ for each mixture.

Sander, 1983; http://swift.cmbi.kun.nl/swift/dssp) was designed to standardize the assignment from protein database coordinates to secondary structures. This program will convert a protein database file to $(\phi, \psi)$ angles and assigns each pair of angles to one of:

"loop or irregular" (for which no label is given, i.e., blank)
**B** residue in isolated $\beta$-bridge
**E** extended strand, participates in $\beta$-ladder
**G** 3-helix (3/10-helix)
**H** $\alpha$-helix
**I** 5-helix ($\pi$-helix)
**S** bend
**T** hydrogen bonded turn

It is of interest to compare the clusters formed by our three-mixture model with the classifications given by dssp and these are shown in Table 2. Almost all of the "helix" (H) angles are contained in the third cluster, which is the most highly

concentrated group; indeed the third cluster (which has very high concentration) contains only angles labeled H ($\alpha$-helix) or G (3/10-helix), and one hydrogen bonded turn. Figure 2 shows the Ramachandran plot for Myoglobin with the dssp classifications used as labels.

The second protein for which we present results is Malate dehydrogenase, which is somewhat larger (343 angles), with several motifs, including $\beta$-sheets. The dssp classifications are shown in a Ramachandran plot in Figure 1. In this case, the EM algorithm was used to find solutions for $K = 1, 2, \ldots, 6$ in which AIC selected $K = 5$ components, of which three used the parameter rotations. The contour plots of the log densities are shown in Figure 3, and the parameter estimates are given in Table 1. Again, we note that one of the components (4) has formed a very tight cluster about its mean, and that component 5 has a bimodal density. Comparison of our cluster labels with those obtained from dssp is given in Table 2. In this table we see that cluster 4 is almost all $\alpha$-helix, but that many helices are also in cluster 3.

For both proteins we observe that the mean values of the components in the mixture models are consistent with the traditional partitions given to the Ramachandran plots based on an empirical distribution analysis (e.g., Branden and Tooze, 1998, p. 9). Moreover, we note that the model selection has fit more components to Malate dehydrogenase (which contains more types of secondary structures) than for Myoglobin (which does not contain any $\beta$-sheets [dssp class E]).

## 5. Discussion

There are various future directions that are possible. For example, we could take account of any serial correlation between the conformational angles $\psi_i$ and $\psi_{i+1}$, or between $\phi_i$ and $\phi_{i+1}$, because we may not have i.i.d. data. This serial correlation could be modeled (e.g., by some sort of Markov chain) or a simpler solution would be "thinning" in which only every fourth (say) observation was retained. Serial correlation models could also be used to adjust the posterior probabilities of membership $p_{ij}$, so that isolated angle membership was made less likely than an independence assumption would permit. These are subjects of ongoing research. Note also the possibility of allowing for two additional classifications: a class "doubt" if all $p_{ij} < t$ for some threshold $t$, and a class "uncertain" if the two largest probabilities are almost equal.

Clearly other submodels are also possible such as the bivariate wrapped normal, Cauchy, but these are not members of the exponential family. However, when applied to the protein data, it is clear that several mixture components will be required. In our case the number of parameters for $K$ mixtures is $6K - 1$, so for the larger five-component model we have 29 parameters. However, we think that there is still good interpretability—and standard errors are also available. This contrasts with the model of Pertsemlidis et al. (2005) who used about 100 parameters that cannot easily be interpreted. The final mixture model and corresponding set of clusters is objective, and has much similarity with both initial subjective classifications and the output from dssp.

Extending to more than two dimensions is clearly possible both for the Sine model and Cosine model, but is somewhat easier to express for the Sine model. Further work in higher dimensions is in progress. Finally, we note that the methods of this article can be used in other applications with bivariate angular data, for example, wind directions (Fisher, 1993).

## 5. Supplementary Materials

Web Appendices referenced in Sections 3 and 4 are available under the Paper Information link at the *Biometrics* website http://www.tibs.org/biometrics.

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