5 Dalton, H., Ammonia oxidation by the methane oxidising bacterium *Methylococcus capsulatus* strain bath. Archs Microbiol. *114* (1977) 273–279.
6 Drozd, J. W., Bailey, M. L., and Godley, A., Oxidation of ammonia by methane-oxidizing bacteria. Proc. Soc. gen. Microbiol. *4* (1976) 26.
7 Egli, Th., (An)aerobic breakdown of chelating agents used in household detergents. Microbiol. Sci. *5* (1988) 36–41.
8 Egli, Th., Bosshard, Ch., and Hamer, G., Simultaneous utilization of methanol-glucose mixtures by *Hansenula polymorpha* in chemostat: influence of dilution rate and mixture composition on utilization pattern. Biotechnol. Bioeng. *28* (1986) 1735–1741.
9 Egli, Th., Weilenmann, H.-U., El-Banna, T., and Auling, G., Gram-negative, aerobic, nitrilotriacetate-utilizing bacteria from wastewater and soil. Syst. appl. Microbiol. *10* (1988) 297–305.
10 Gottschal, J. C., de Vries, S., and Kuenen, J. G., Competition between the facultatively chemolithotrophic *Thiobacillus* A2, an obligately chemolithotrophic *Thiobacillus* and a heterotrophic *Spirillum* for inorganic and organic substrates. Archs Microbiol. *121* (1979) 241–249.
11 Hamer, G., A biotechnological approach to the treatment of wastewater from petrochemicals manufacture. Instn chem. Engrs Symp. Ser. *77* (1983) 87–101.
12 Hamer, G., and Zwiefelhofer, H. P., Aerobic thermophilic hygienization – a supplement to anaerobic mesophilic waste sludge digestion. Chem. Engng Res. Des. *64* (1986) 417–424.
13 Humphrey, A. E., Moreira, A., Armiger, W., and Zabriskie, D., Production of single cell protein from cellulose wastes. Biotechnol. Bioeng. Symp. *7* (1977) 45–64.
14 Hutton, W. E., and ZoBell, C. E., Production of nitrite from ammonia by methane-oxidizing bacteria. J. Bact. *65* (1949) 216–219.
15 Leisinger, Th., Cook, A. M., Hütter, R., and Nüesch, J., (Eds) Microbial Degradation of Xenobiotic and Recalcitrant compounds. Academic Press, London 1981.
16 Linton, J. D., and Stephenson, R. J., A preliminary study on growth yields in relation to the carbon and energy content of varous organic growth substrates. FEMS Microbiol. Lett. *3* (1978) 95–98.
17 Mason, C. A., Microbial death, lysis and 'cryptic' growth: fundamental and applied aspects. Doc. Diss. ETHZ No. 8150 (1986).
18 Monod, J., Recherches sur la croissance des cultures bactériennes. Hermann, Paris 1942.
19 O'Neill, J. G., and Wilkinson, J. F., Oxidation of ammonia by methane-oxidizing bacteria and the effects of ammonia on methane oxidation. J. gen. Microbiol. *100* (1977) 407–412.
20 Prochazka, G. J., Payne, W. J., and Mayberry, W. R., Clorific contents of microorganisms. Biotechnol. Bioeng. *15* (1973) 1006–1011.
21 Robertson, L. A., and Kuenen, J. F., *Thiospaera pantotropha* gen. nov. sp. nov., a facultatively anaerobic, facultatively autotrophic sulphur bacterium. J. gen. Microbiol. *129* (1983) 2847–2855.
22 Robertson, L. A., and Kuenen, J. F., Aerobic denitrification: a controversy revived. Archs Microbiol. *139* (1984) 351–354.
23 Schneider, R. P., The NTA-monooxygenase from *Pseudomonas* sp. ATCC 29600. Doc. Diss. ETHZ No. 8824 (1989).
24 Senn, H. P., Kinetik und Regulation des Zuckerabbaus von *Escherichia coli* ML 30 bei tiefen Zuckerkonzentrationen. Doc. Diss. ETHZ No. 8831 (1989).
25 Stumm-Zollinger, E., Substrate utilization in heterogeneous bacterial communities. J. Wat. Pollut. Control Fed. *40* (1968) R213–R229.
26 Wehrli, E., and Egli, Th., Morphology of nitrilotriacetate-utilizing bacteria. Syst. appl. Microbiol. *10* (1986) 306–312.
27 Wilkinson, T. G., Interactions in a mixed bacterial population growing on methane in continuous culture. Doc. Diss. Univ. London (1972).
28 Wilkinson, T. G., An environmental programm for offshore oil operations. Chem. Indust. (1982) 115–123.
29 Wilkinson, T. G., and Hamer, G., The microbial oxidation of mixtures of methanol, phenol, acetone and isopropanol with reference to effluent purification. J. chem. Technol. Biotechnol. *29* (1979) 56–67.
30 Wilkinson, T. G., Topiwala, H. H., and Hamer, G., Interactions in a mixed bacterial population growing on methane in continuous culture. Biotechnol. Bioeng. *16* (1974) 41–59.
31 Zlokarnik, M., Bioengineering aspects of aerobic waste water purification. Ger. Chem. Engng. *6* (1983) 183–197.

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Research Articles

The use of Bose-Einstein statistics in analysing the distribution of intracellular organelles: The development of a Bose-Einstein probe

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Summary. Bose-Einstein statistics have been used to study the distribution patterns of organelles within cells, for example, invertebrate smooth muscle filaments, microtubules in axons and micropinocytic vesicles in capillary endothelial cells. These and other intracellular structures were found to occur non-randomly within cells. An index of redundancy (R) was used as a measure of the other of their two-dimensional distributional pattern and as an estimate of the extent to which mechanical or other processes might be inducing the occurrence of an otherwise non-random distribution. It is suggested that Bose-Einstein statistics could be of considerable value in analysing not only the distribution patterns of organelles in cell biology but also two dimensional patterns in many other fields of study such as histology, pathology, autoradiography, or epidemiology.
Key words. Spatial distribution; randomness; entropy; Bose-Einstein statistics; patterns; order; hyperdispersed populations.

Introduction

Studies on the distribution pattern of intracellular organelles are important since they indicate how such structures come to lie in relation to each other. The finding that any type of intracellular organelle possesses a non-random distribution pattern naturally leads to speculation and experiment as to the functional and structural

causes. For example, it is known that thick filaments of snail smooth muscle [1], microtubules of nerves [2], pig muscle mitochondria [3], type I skeletal muscle fibres [4], Rh antigens on erythrocyte membranes [5] and micropinocytic vesicles of capillary endothelia in rats [6] all form hyperdisperse populations, i.e. each organelle occurred further away from each of its nearest neighbours than if they formed randomly distributed populations.

Two basic types of procedure can be used for studying spatial point patterns in two dimensions (in R^2), distance sampling and quadrat sampling [7–11]. In distance sampling, the mean distances between test objects and their nearest neighbours are measured whereas in conventional quadrat sampling the numbers of objects found within randomly placed single sampling areas (quadrats) are counted. The ratio of the sample variance to the mean of the number of objects per quadrat can be used as an index of spatial dispersion [7, 9, 12]. Variance to mean ratios not significantly different from one indicate a Poisson (complete spatial randomness, CSR) distribution of points whilst values significantly greater or less than one indicate a clumped or dispersed distribution respectively.

In conventional quadrat sampling two major types of difficulty often prevent a comprehensive description of the processes responsible for the generating patterns [10]. First there are technical problems such as the selection of appropriate quadrat size of the distribution of quadrats to be followed during the sampling exercise. Second, the assumption of a particular probability distribution function may not necessarily be indicative of the actual process governing the formation of a particular pattern. In order to overcome some of these difficulties Bose-Einstein statistics have previously been used in such subjects as geography and ecology to give alternative definitions of randomness [13, 14].

Bose-Einstein statistics describe one of a series of classical occupancy problems commonly encountered in statistical mechanics in which the number of different ways in which any number of r identical particles can be placed into n boxes is under study. Alternatively, where identical particles are limited to one per box or the particles are different and any number can be placed in any box, then the distributions are described by Fermi-Dirac and Maxwell-Boltzman statistics respectively. Bose-Einstein statistics are relevant to many other problems including investigations into the number of data blocks required to answer queries in computing science [15]. The relation of Bose-Einstein statistics to the concept of entropy has been widely explored and used in information theory [16, 17]. It has been proposed that Bose-Einstein statistics provide a link between spatial and numerical distributions. The spatial pattern determines the numerical distribution and the probability of seeing a specific numerical distribution depends on the number of spatial patterns giving rise to that distribution.

The practical implementation of Bose-Einstein statistics depends upon the probability distribution

$$P_m = \frac{\binom{n+r-m-2}{r-m}}{\binom{n+r-1}{r}}$$

where $m = 0, 1, 2, \ldots . r$, P_m is the probability of finding a specified cell in a quadrat census that contains m points and where r is the total number of points lying in the n cells of the census. A scale free redundancy index (R) is then used to give a measure of the extent to which a process governs the formation of a pattern using the relation

$$R = 1 - \left(\frac{\sum_{m=0}^{r} \frac{n_m}{n} \log\left(\frac{n}{n_m}\right)}{\sum_{m=0}^{r} P_m \log\left(\frac{1}{P_m}\right)} \right)$$

where n_m is the number of cells containing m points.

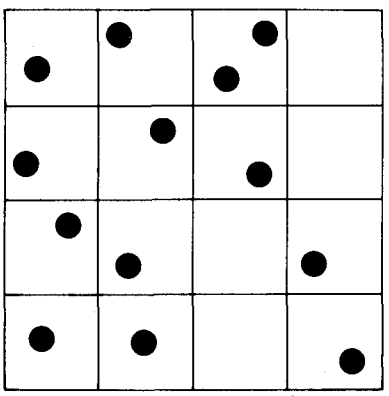

Figure 1. Typical example of a 4×4 sampling grid superimposed on synthetic but non-random population of test objects. Note that some square cells (quadrats) of the sampling grid (which would be placed randomly on test micrographs) contain one test object whilst a few quadrats contain either none or two test objects.

Materials and methods

Several sources of material were used for analyses of spatial patterns. Electron micrographs of transverse sections of smooth muscle fibres of the snail, *Helix aspersa* [1] and guinea pig axons were reanalysed to study the distribution of thick paramyosin filaments and microtubules respectively. Freeze fracture preparations previously used to measure the mean nearest neighbour distances between micropinocytic vesicles [6] and previously obtained data on the distribution of Rhesus antigens on red cell membranes were also reanalysed [5].

Transparent plastic sheets each bearing a test grid consisting of a square (4×4) lattice of $n = 16$ cells (fig. 1) were superimposed randomly on micrographs and the number of test objects (paramyosin filaments, vesicles etc.) lying in each cell was recorded. Values for the redundancy index were computed as shown above. Two artificial populations were also created empirically, one con-

sisting of a regular distribution of points occurring further from each other than in a Poisson distribution and another having a markedly clumped pattern of points. These were also subjected to analyses and some typical values are recorded in the table. Statistical hypothesis testing was carried out using the modified single value-small sample comparison *t*-test of Student and critical Bonferroni *t* values for 7 comparisons found in tables of Sidak's multiplicative inequalities [18].

Results of comparative Bose-Einstein, nearest neighbour and random quadrat testing on various biological tissues[a]

Tissue	Redundancy index	Dispersion index	Quadrat testing
Snail paramyosin filaments	0.47	1.61	0.38
Rat micropinocytic vesicles	0.42	1.57	0.45
¶ Erythrocyte Rh antigens	0.31	1.48	0.57
¶ Axonal microtubules (neurotubules)	0.53	1.51	0.37
Synthetic random population	0.00	1.00	1.00
Synthetic dispersed population	1.00	2.15	0.50
Synthetic clumped population	0.83	0.25	1.00

[a] The dispersion index is given by the ratio of the empirically determined mean distance between objects to the mean distance expected in a random population. The mean distance for a random population is given by $1/2\sqrt{N_A}$ and with standard error $\sqrt{(4-\pi)/(4\pi n N_A)}$ where n is the number of distances measured and N_A their density per unit area. Items indicated by ¶ subjected to meta-analysis [24].

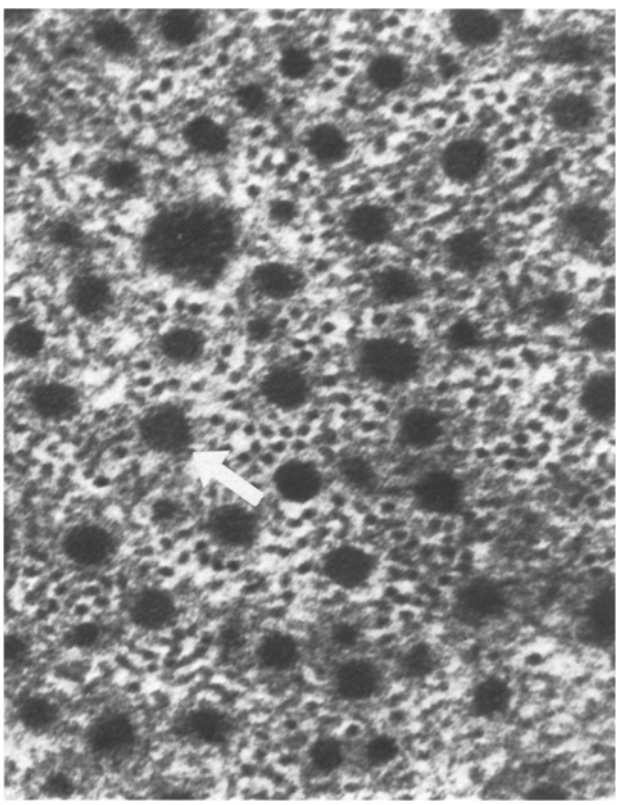

Figure 2. Sample electron micrograph of transverse section of snail smooth muscle fibre. The large electron dense paramyosin filaments (arrowed) appear to be non-randomly distributed. Specimens of muscle were fixed in 3% glutaraldehyde, post fixed in osmium tetroxide and embedded in Araldite®. Thin sections were cut for electron microscopy and prints prepared at a final magnification of ×86,000.

Results

The results of the Bose-Einstein analyses given in the table clearly indicate the high redundancy values that are associated with the dispersed patterns of both organelles and antigen sites previously studied by nearest neighbour and quadrat techniques. Values for maximum dispersion and clumping for the size of test grids used in the present study give an indication of the maximum values possible. The present author has not been able to find any two-dimensional distribution of organelles which appears to be random when using conventional methods of analysis and consequently values obtained by the analyses of synthetic patterns has been included as a measure of the lowest level of redundancy.

Discussion

It is important to note that the values obtained for R, unlike those for nearest neighbour and other conventional quadrat methods, yield a measure of the extent to which processes are tending to reduce the disorder (entropy) of the system. R is given by the relationship $R =$ (1 – relative entropy) when the relative entropy is a measure of the extent to which the distribution is free to vary. Bose-Einstein statistics are not intended to replace other measures of spatial pattern but to provide alternative information and be entirely complementary.

It must be noted that redundancy does not differentiate between uniform and clustered patterns unlike conventional analyses. For perfectly uniform and perfectly clustered patterns the redundancy will equal unity. However, in practice, for a clumped distribution R will always be less than one as a non-maximally clumped distribution can be realised in an analysis by the presence of all the points equivalent to an experimental procedure all being placed in a single cell of finite size.

Conventional nearest neighbour measures do not contain as much information as a quadrat census since short distances only are involved. Longer range interactions do not usually contribute to the pattern information. Similarly, single quadrat studies yielding variance to mean ratios are size dependent and inevitably sample smaller regions.

The majority of point patterns studied by conventional methods in disciplines other than cell biology, e.g. geography and ecology, have been clustered distributions with variance/mean ratios significantly greater than unity. It is not surprising to find that the negative binomial theorem has been fitted successfully to a wide range of patterns. When the constant of the negative binomial distribution (k) is unity, the negative binomial distribution is identical to a geometric distribution which is a limiting form of Bose-Einstein statistics. This is an im-

portant theoretical link between conventional methods and those of Bose-Einstein statistics. In the present study, variance to mean ratios of the studied populations are significantly less than one. In these circumstances the technique of using the data to see if it fits a negative binomial distribution with $k = 1$ is not applicable. An alternative procedure in the presence of a hyperdispersed distribution is to compare the empirical frequency array of all the values with that predicted by Equation 1 using a Kolmogorov-Smirnov type procedure[19].

Speculation on the biological significance of the findings reported in the present study would seem appropriate. That all types of the organelle examined occurred further from each other than would be expected in a random distribution and that clearly some causative process must exist suggests further topics for investigation. For example, it is possible that some structural features have yet to be identified which are responsible for the increased spacing between organelles. The relatively regular spacing between the paramyosin filaments in snail muscle clearly indicate some structural spacing support not yet identified by electron microscopy and which also suggests contraction mechanisms more closely resembling skeletal muscle than those of smooth muscle. The spacing between axonal microtubules may similarly be related to an unidentified structural support system. The mechanisms of axonal transport certainly require a regular and directionally consistent vesicular transport system without microtubular entanglement. Alternatively, their regular spacing could reflect a more economical distribution of neurotubules necessary for structural support or rigidity. It is tempting to speculate that the newly described filamentous system associated with neurotubules[20] may be the cause of the characteristic pattern. The non-random distributions of micropinocytic vesicles in endothelia suggest specific structural mechanisms for the siting of their origins on the cell membrane though mutual repulsion by surface borne electrical charges remains a possibility.

It should be noted that in the present study the distributions analysed have either been on plane surfaces or their equivalent in that very long structures (filaments and tubules) are distributed spatially only in the transverse plane (in R^2). Several types of problem naturally suggest themselves for future investigation, for example the distribution of nuclear pores where some nearest neighbour analyses have already been carried out[21]. Though there is also considerable interest in the distribution of organelles in three dimensions (in R^3) generally, for example, coated pits in phagocytes[22] and membrane particles[23], it is not yet clear if the use of Bose-Einstein statistics can be extended to such distributions. Such limitations, however, are also applicable to other spatial pattern measures in R^2 and further theoretical studies are necessary.

The approach identified in the present study using Bose-Einstein statistics as a test probe of two-dimensional distributions is potentially of widespread use in that it is both magnification and subject independent (interdisciplinary). Its potential use includes estimating the order of pattern in the organisation of cells in histology and pathology in which processes are particularly characterised by increased disorder of cell distributions. As a specific example, the disordered pattern of microtubules seen in association with transport abnormalities induced by vinblastine and vincristine administration could be analysed. Similarly, rapid tests of whether autoradiographic grains seen in micrographs could be of some interest particularly in relation to the quantification associated with specific test structures[25]. Epidemiological phenomena are distributed two-dimensionally and the occurrence of non-random distributions is of prime interest in the aetiology of some pathological processes.

1 Meek, G. A., and James, N. T., Electron Microsc. *2* (1980) 16.
2 James, N. T., J. Anat. *130* (1980) 665.
3 James, N. T., J. neurol. Sci. *23* (1974) 565.
4 James, N. T., J. neurol. Sci. *14* (1971) 381.
5 James, N. T., and James, V., Br. J. Haemat. *40* (1978) 657.
6 Bullock, G. R., Goodall, M., James, N. T., Meek, G. A., Quine, H., Robards, A. W., and Sibbons, P., J. Anat. *133* (1982) 696.
7 Getis, A., and Boots, B., Models of Spatial Processes. Cambridge University Press, Cambridge 1978.
8 Bartlett, M. S., The Statistical Analysis of Spatial Pattern. Chapman and Hall, London 1975.
9 Cliff, A. D., and Ord, J. K., Spatial Processes, Models and Applications. Pion Press, London 1981.
10 Rogers, A., Statistical Analysis of Spatial Dispersion. The Quadrat Method. Pion Press, London 1974.
11 Pielou, E. C., An Introduction to Mathematical Ecology. Wiley-Interscience, New York 1969.
12 Greig-Smith, P., Quantitative Plant Ecology, 2nd edn. Butterworths, London 1964.
13 Thomas, R. W., and Reeve, D. E., Geog. Anal. *9* (1976) 113.
14 Wilson, L. J., Young, J. H., and Folks, J. L., Commun. Statist. Theory Meth. *16* (1987) 445.
15 Lam, K., Am. Stat. *37* (1983) 213.
16 Fast, J. D., Entropy, Netherlands: Phillips Technical Library 1968.
17 Levine, R. D., and Tribus, M., The Maximum Entropy Formalism. MIT Press, Cambridge, Mass. 1968.
18 Sokal, R. R., and Rohlf, F. J., Biometry, 2nd edn. W. H. Freeman, San Francisco 1981.
19 Conover, W. J., Practical Non-parametric Statistics, 2nd edn. John Wiley, 1980.
20 Ochs, R. L., and Burton, P. R., J. Ultrastruct. Res. *73* (1980) 169.
21 Pedro, N., Fonseca-Carmo, M., and Fernandes, P., J. Microsc. *134* (1984) 271.
22 Pfeiffer, J. R., Oliver, J. M., and Berlin, R. D., Nature *286* (1980).
23 Appleyard, S. T., Witowski, J. A., Ripley, B. D., Shotton, D. M., and Dubowitz, V., J. Cell Sci. *74* (1985) 105.
24 Hedges, L. V., and Olkin, I., Statistical Methods for Meta-Analysis. Academic Press, London 1985.
25 Boast, C. A., Snowhill, E. W., and Altar, C. A., Quantitative Receptor Autoradiography. Alan R. Liss, New York 1986.

0014-4754/89/11-12/1078-04$1.50 + 0.20/0