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Detection and characterization of spatial pattern in chimaeric tissue

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SUMMARY

The mosaic pattern of patches of crypts of Lieberkühn in chimaeric C57BL/6JLac (B6)↔DDK mouse small intestine, demonstrated using *Dolichos biflorus* agglutinin as strain-specific marker, is quantitatively examined using the Greig-Smith analysis of variance. This analysis, widely used in ecological research, provides a method to detect and characterize pattern at various scales. The analysis demonstrates that B6 patches are non-randomly distributed at all scales examined. A consistent increase in the intensity of pattern at one particular scale over all replicate samples identifies 'clusters of clusters' which probably are territories of 'descendent' clones. The sizes of descendent clones, either in terms of numbers of patches or total numbers of crypts, are highly variable. A steady reduction in the strength of pattern from proximal to distal is found. The Greig-Smith analysis of variance provides a valuable method for the analysis of pattern in chimaeric tissue.

INTRODUCTION

The present paper is a contribution towards the detection and characterization of patterns in chimaeric tissues. The majority of studies using chimaeras have reported on some form of pattern (McLaren, 1976 for references; Green, Durham, Mayer & Hoppe, 1977; Ezine, Weissman & Rouse, 1984; Gardner, 1984; Mikoshiba *et al.* 1984), although the term 'pattern' has consistently been used in a broad general sense and without verification. Less often, a random arrangement (and therefore lack of pattern) of the material under study (e.g. cells or coherent patches of like cells) has been suggested (Mullen, 1977; Rossant, Gray, Clark & Chapman, 1983), but again only in descriptive, undefined terms (e.g. 'apparently randomly distributed', Rossant *et al.* 1983). Wolpert & Gingell (1970) suggested that 'much more attention should be given to the reliability of spatial patterns in general'. Their own considerations regarding the stripey pattern in the coat of chimaeric mice, however, led to the seemingly contradictory conclusion 'that an essentially random mechanism might be a major aspect of the mechanism involved in striping' (and therefore pattern formation). The issue may be resolved by looking at different scales of pattern: on a small scale, pattern exists in the form of

Key words: mouse chimaera, pattern, Greig-Smith analysis of variance, descendent clones, *Dolichos biflorus* agglutinin, intestinal crypts.

stripes, but on a larger scale, the distribution of the stripes themselves may be random. The qualitative distinction between 'coherent' and 'diffuse' patterns made by Gardner (1984) further emphasizes the need for a method which will identify the various scales at which spatial pattern may occur, or alternatively, identify lack of pattern, i.e. randomness.

An appropriate method is widely used in ecological research (Greig-Smith, 1952 and 1983; Angel & Angel, 1967; Mead, 1974). Originally suggested by Greig-Smith (1952), it is known as the Greig-Smith analysis of variance (see Materials and Methods). This method has advantages over other methods, e.g. 'nearest neighbour distance tests', which are frequently used to define pattern in terms of departures from a random distribution (cf. Vandermeer, 1981; Aherne & Dunhill, 1982; Schmidt, 1982): not only does the analysis identify the scales at which patterns occur, but by testing at different scales, one is also able to detect 'clusters of clusters' (Mead, 1974). Hence, such methods may be capable of identifying territories of groups of clones related by descent from a common progenitor ('descendent clones': West, 1978) in chimaeric tissues.

In the present paper we have used the Greig-Smith analysis of variance to define and characterize the two-dimensional spatial pattern of intestinal crypt populations in a mouse aggregation chimaera (cf. Schmidt, Wilkinson & Ponder, 1984; Schmidt, Garbutt, Wilkinson & Ponder, 1985). For clarity, we have limited ourselves in this paper to the data obtained from a single intestinal sheet. Our primary intention is to illustrate the application of the method.

MATERIALS AND METHODS

The analysis was carried out for a 17-week-old DDK↔C57BL/6JLac (B6) mouse aggregation chimaera which was constructed by Dr M. Wood, MRC Laboratories, Carshalton (Ponder, Wilkinson & Wood, 1983). A sheet of entire small intestinal mucosa was prepared and stained with *Dolichos biflorus* agglutinin-peroxidase (DBA) conjugate as described previously (Schmidt *et al.* 1984). In adult chimaeras, intestinal crypts of Lieberkühn are always composed of a single parental genotype (Ponder *et al.* 1985*b*). DBA binds to the epithelium of B6 but not of DDK crypts (Ponder, Festing & Wilkinson, 1985*a*). Using DBA as a strain-specific marker it is therefore possible to demonstrate the mosaic distribution of intestinal crypt populations (Schmidt *et al.* 1984, 1985).

The percentage contribution of the minority component (B6) was calculated by the line interception method (Aherne & Dunhill, 1982). The entire intestinal sheet was scanned under ×60 magnification (Leitz, Laborlux 12, Wetzlar; fitted with a 10×10 eyepiece graticule, Graticule Ltd, Tonbridge, Kent), and the number of interceptions overlying the B6 component was scored. This number, divided by the total number of points sampled (6384)×100, gave the percentage of B6 crypts present in the intestinal sheet.

The Greig-Smith analysis of variance

The principle of the Greig-Smith analysis of variance is to examine the distribution of numbers of objects (in the present case, mosaic patches) scored by means of a grid. If the objects are randomly distributed the numbers of objects per square of the grid will conform to a Poisson distribution (see below); if they are clustered or regularly spaced, the distribution will depart from this expectation. By repeating the analysis for larger blocks obtained by combining adjacent grid squares, the occurrence of pattern at different scales can be sought. The mathematical analysis is based on the analysis of variance, as outlined in Table 1 and discussed in more detail below.

From a photomontage (cf. Schmidt *et al.* 1985) of the stained intestinal sheet, we took eight samples approximately 8×8 mm in original size, which contained at least 14 patches per sample (Fig. 1A). Five of the samples were taken from the proximal part of the small intestine and three from the mid-gut region (see Table 6). As the gut was cut open along the mesentery, orientations of the samples were comparable. The patches were mapped by recording the co-ordinates of patch centres with the aid of a graphics tablet (MOP Videoplan, Kontron). The edges of the gut were curved to varying degrees owing to unavoidable stretching when they were pinned out during preparation (Fig. 1). These edges were disregarded in the analysis which requires that sample areas have straight outlines (Figs 1B and C; see below).

1. *Detection of pattern*

A computer program was written for the analysis using Genstat (The Numerical Algorithms Group Ltd, Oxford) and run on an Amdahl machine at the University of Reading. An 8 × 8 grid

Table 1. *Calculations carried out for the Greig-Smith analysis of variance of numbers of patches at different block sizes (cf. Fig. 1)*

Source of variation (block size)	Sum of squares (SS) * G = total (64 quadrats); F = half total (32 quadrats); etc	Degree of freedom (d.f.)	Mean square (MS)	$\frac{MS}{Mean}$
32	x-direction: $\Sigma\frac{Fx^2}{32} - \frac{G^{2*}}{64}$	1		
	y-direction: $\Sigma\frac{Fy^2}{32} - \frac{G^2}{64}$	1		
16	$\Sigma\frac{E^2}{16} - \Sigma\frac{Fx^2}{32} - \Sigma\frac{Fy^2}{32} + \frac{G^2}{64}$	1		
8	x: $\Sigma\frac{Dx^2}{8} - \Sigma\frac{E^2}{16}$	4		
	y: $\Sigma\frac{Dy^2}{8} - \Sigma\frac{E^2}{16}$	4		
4	$\Sigma\frac{C^2}{4} - \Sigma\frac{Dx^2}{8} - \Sigma\frac{Dy^2}{8} + \frac{E^2}{16}$	4		
2	x: $\Sigma\frac{Bx^2}{2} - \Sigma\frac{C^2}{4}$	16		
	y: $\Sigma\frac{By^2}{2} - \Sigma\frac{C^2}{4}$	16		
1	$\Sigma A^2 - \frac{Bx^2}{2} - \frac{By^2}{2} + \frac{C^2}{4}$	16		

The table follows the standard structure of the analysis of variance (Bailey, 1976, although the technique does not involve computation of the components of variance [in the usual sense]). A distinction between x- and y-direction was made, allowing us to test for differences of pattern in the longitudinal and transverse directions of the small intestine (for further explanation, see text). The 'total' referred to is the total number of patches for the respective block sizes. The blank columns on the right will contain the numbers calculated after substituting the appropriate values for numbers of patches in the sum of squares: a worked example is given in Table 2.

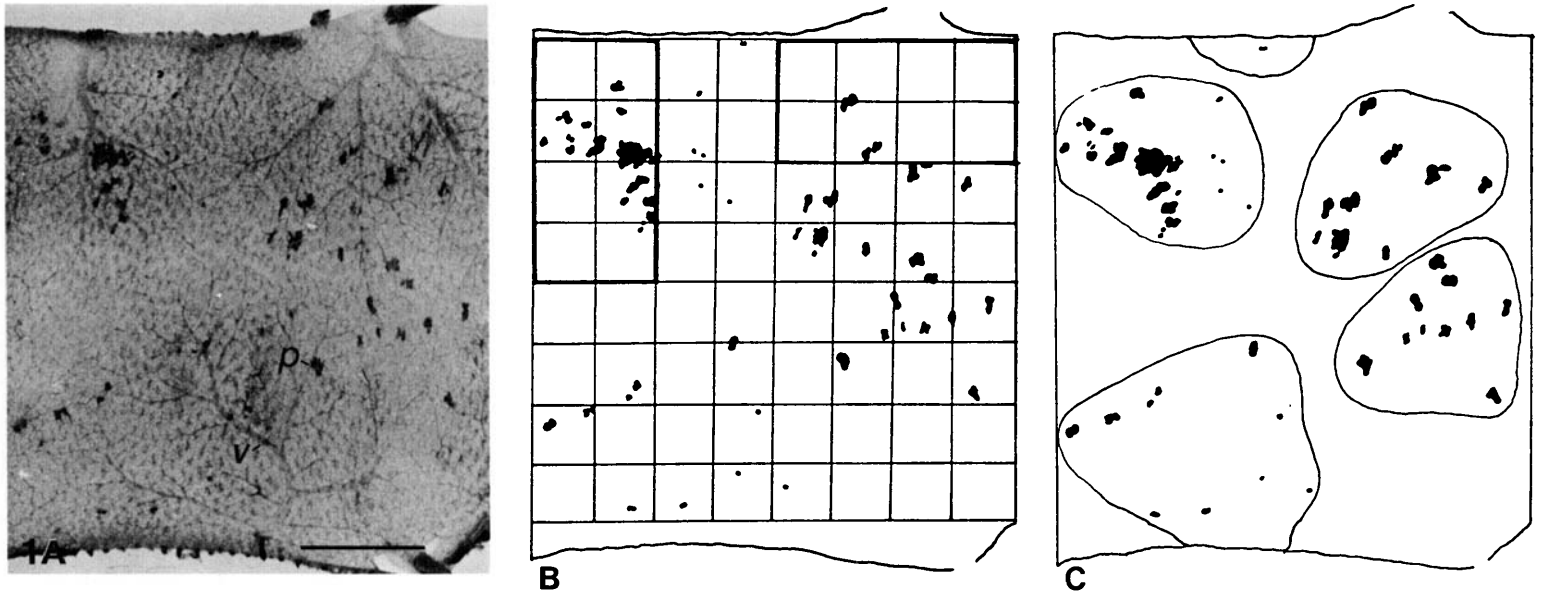

Fig. 1. (A) Sample area (No. 2, duodenum) used for the Greig-Smith analysis of variance. Patches of B6 crypts (*p*) in a sheet of intestinal mucosa of a B6↔DDK chimaera (viewed from abluminal side; muscles of gut wall have been removed). Stained with *Dolichos biflorus* agglutinin-peroxidase. DDK crypts do not stain, although DDK blood vessels (*v*) do. The regular background pattern is formed by the cores of villi on the other side of the sheet. Bar equals 2 mm. (B) Diagrammatic drawing of (A) to show the position of patches and the superimposed 8×8 grid which was used in the analysis. Heavy lines show block size 8. The y-direction is indicated at the left half, the x-direction on the right. The pattern increased at this scale (Fig. 2), indicating clustering of the clusters found at the smaller block sizes. (C) We interpret the secondary clusters as territories of 'descendent clones'. Using block size 8 as a guide we were able to identify the probable territories of four descendent clones in this sample area, although the outlines will of course only be approximate. As the tissue is cylindrical, the B6 patches at the bottom left and top left may belong to the same descendent clone.

of quadrats was superimposed over each sample (Fig. 1B) and counts of patches were obtained for each quadrat from the known x-y co-ordinates of the patches. It is advisable to set the quadrat size so as to obtain a mean number of patches per quadrat of approximately 1 or over, as evidence of clustering may disappear if smaller units are used (Greig-Smith, 1983). For data sets which contain more patches than the present one a smaller grid size may of course be used. The restriction regarding the minimum size of the quadrat, i.e. the 'grain size' of the grid, therefore also sets the lower limit at which pattern may still be detected. Greig-Smith (1952) showed that contiguous quadrats of the grid can be blocked together into consecutive blocks of pairs, fours, eights, etc., and the variability for each block size analysed statistically by means of an analysis of variance. (We therefore have different *sizes* of blocks (cf. Table 1), the smallest size, 1, being equal to the basic unit of the grid, i.e. the quadrat.) For each block size we thus obtain a mean square for the variation between blocks. The mean square values of the block data can then be used as an indication of the spatial distribution of patches: contagious (clustered), random or regular. Under the hypothesis that patches are randomly (Poisson) distributed, the mean squares at each block size should be equal to the mean number of patches per quadrat, i.e. the ratio, mean square/mean, should be 1 and conform to an expected χ^2-distribution. We call this the Index of Distribution (IOD). If the patches are clustered, the IODs will be consistently higher than 1, but if they are regularly spaced the IODs will be lower than 1. The IOD values are conveniently plotted against block size (cf. Fig. 2). The block size gives the scale of the pattern, e.g. large scale or small scale, and the values of the IODs indicate the intensity of the pattern, i.e. the higher the values the stronger is the pattern. Hence, pattern at a particular scale is often indicated by a peak in the graph (cf. Greig-Smith, 1983). We must, however, emphasize at this point that the block sizes have different degrees of freedom (d.f. = one less than the numbers of observations; cf. Table 1) and that the variability of the IODs is therefore greater at large block sizes, for which particularly high values are often found (cf. Table 3). We therefore require replicate samples and need to demonstrate consistencies between them in order to verify any observed pattern.

2. *Characterization of pattern*

Having completed the first step in the analysis and determined whether the patches are non-randomly distributed (using the χ^2-distribution for each scale for each set of replicate samples) we proceed to further characterize the scale at which pattern is found, i.e. to confirm observed peaks or, if peaks are not apparent, to define stepwise increases of IODs. Mead (1974) compared several appropriate test statistics and concluded that increases or decreases of IODs may be identified by examining successive IOD values using the F-statistic. In this test, if the IODs for one block size are *consistently* larger than the IODs for the immediately smaller block size, then clustering at about the order of the larger block size may be inferred. Therefore, if clustering is identified at, for example, block sizes 4 and 8 using the χ^2-distribution (see above), the F-statistic is used to examine the ratio of successive IODs at the two block sizes taking the numbers of degrees of freedom into account.

RESULTS

The minority (B6) component of the chimaera constituted only 0·8% of the crypt population and occurred in discrete patches (Fig. 1). The Greig-Smith analysis yielded almost invariably IOD values above 1 at all scales: only 2 out of 48 values were less than 1 (Table 3). This implies that there was pattern, i.e. non-random distribution of patches, at all scales examined. This conclusion was validated by comparing the IODs with the χ^2-distribution. The whole set of eight replicate samples at each scale was compared with the values expected for a χ^2-distribution, taking the different degrees of freedom into account, as illustrated in Table 4. Table 4 gives an example for two scales, showing that at scale 1 values

were clearly overall higher than expected. Even the IOD values of scale 4 which were relatively close to 1 differed from those expected from the χ^2-distribution; instead, they were similar to the values of the other scales, which suggests that the spatial arrangement of patches was non-random also at scale 4.

In order to detect an increase of clustering at a particular scale it was necessary to identify consistencies of increases of IODs, a consistent increase over all eight samples at a particular scale indicating a further level of clustering, i.e. 'clusters of clusters' (Mead, 1974). This was carried out in two steps, the first being a subjective assessment of IOD values (Table 3) : they appeared to show a consistent increase at level 8. In the second step, the F-statistic (Mead, 1974) was used to provide an objective assessment of the strength of the evidence for our conclusion. The ratios of successive IODs were determined and analysed for different levels of comparison by examining complete sets of samples (similar to the χ^2-procedure above). The results (Table 5) showed a consistent increase at one

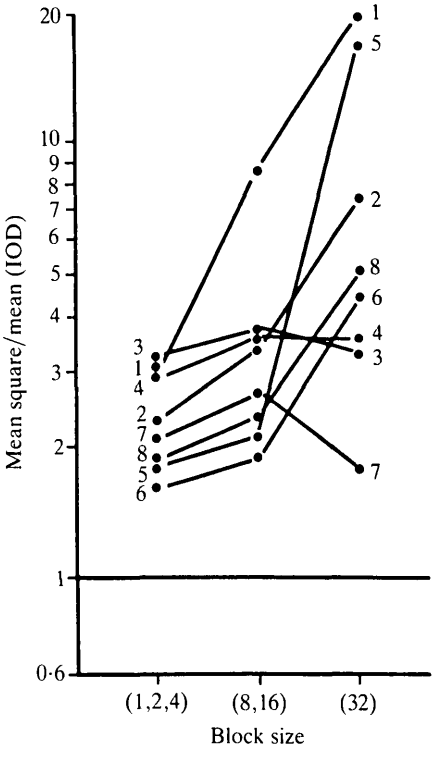

Fig. 2. Summary of the spatial analysis of pattern in patches of the minority (B6) component in eight 8×8 mm samples of small intestine from a B6↔DDK chimaera. The IODs (Table 3) are plotted (on a log scale) against block size. The different gut positions (numbers in the graph) refer to the numbers in Table 6. The straight line at IOD 1 indicates the value expected under randomness (Greig-Smith, 1952). A non-random distribution of patches occurs at all scales. By testing the ratios of successive IODs for each of the eight replicas we were able to show a consistent increase in pattern at block sizes 8, and to combine block sizes 1, 2, 4 and 8 and 16 (see text for further explanation).

scale only: scale 8. This was true regardless whether one considered ratios of scales 8 and 4, or the average of (8,16) and (1,2,4). We conclude that at scale 8 the pattern became more intense, i.e. the smaller clusters found at scales 1, 2 and 4 formed higher order clusters recognizable at scale 8. The F-statistic also justified the procedure of combining levels (1,2,4) and (8,16) for a summarizing graphical representation (Fig. 2) as successive IODs between 1, 2 and 4, and 8 and 16 respectively were low and did not show consistent increases (Table 5).

Having demonstrated the non-random spatial arrangement of patches and the scale of the secondary pattern, we proceeded to search for 'patchiness' at this level in the entire intestinal epithelial sheet. An example of this procedure is given in Fig. 1C. It might reasonably be argued that the consistent increase at block size 8 provided evidence of underlying clusters of patches at that block size, and that the patches in these discrete groups were related by origin from a single source. On the basis of this hypothesis we were able to identify a total of 41 clusters of clusters, possibly representing 'descendent clones' of B6 crypts, in the entire sheet of small intestinal epithelium.

The sizes of these putative descendent clones, measured in terms of total numbers of crypts, were highly variable. They ranged from containing as many as 23 patches (cf. Fig. 1C) with one patch containing 134 B6 crypts, to a single patch with a solitary crypt. B6 crypts were relatively more frequent in the duodenum than the jejunum, and in the posterior (ileal) 108 mm of the small intestine only one putative descendant clone was found which comprised two patches with two and four crypts respectively.

It was noted on the intestinal preparation that pattern appeared more intense in the anterior part of the gut. To examine this, the eight samples were arranged according to their position in the gut and the corresponding IODs were compared for each of the six different block sizes (including the x- and y-splits when they

Table 2. *Greig-Smith analysis of variance table for one of eight replicate samples*

Source of variation (block size)	SS		d.f.	MS	MS/ Mean (IOD) (Mean = 0·94)
32	x-direction:	5·063	1	5·063	5·34
	y-direction:	9·000	1	9·000	9·57
16		5·063	1	5·063	5·39
8	x:	6·875	4	1·719	1·83
	y:	17·12	4	4·281	4·55
4		8·625	4	2·156	2·29
2	x:	54·00	16	3·375	3·59
	y:	26·00	16	1·625	1·73
1		24·00	16	1·500	1·60

All IOD (mean square/mean) values were above 1, the highest found for the large scales which have the lowest degrees of freedom (1). The differences between x- and y-directions were not consistent in this nor any of the other samples (not shown); x- and y-values were therefore averaged for further data analysis (Tables 3 to 5).

Table 3. *Index of distribution (IOD) values obtained from the Greig-Smith analysis of variance for eight replicate samples and six different scales*

Scale (block size)	d.f.	IOD values (mean square/mean) Sample number (1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)
32	2	19·97	7·48	3·29	3·56	16·31	4·54	1·78	5·66
16	1	6·78	5·39	5·26	4·55	3·26	1·81	1·09	0·43
8	8	8·85	3·19	3·62	3·55	1·95	1·92	2·91	2·60
4	4	1·54	2·29	1·97	0·71	1·72	1·71	1·35	1·09
2	32	2·92	2·66	3·46	2·98	2·13	1·82	2·13	1·19
1	16	3·76	1·60	3·13	3·55	1·13	1·11	2·13	3·36

were performed separately (cf. Table 1), thus giving nine levels at which comparisons could be made). The results are given in Table 6. The Table shows that position 1 (the most anterior position) had generally higher IODs than succeeding samples (2 to 8), position 2 had overall higher values than those posterior to position 2 (i.e. 3 to 8), etc. The results show a clear and steady change, i.e. reduction in the strength of pattern along the set of eight samples, and therefore from anterior to posterior.

DISCUSSION

The Greig-Smith method involves a subjective element in the initial search for consistent increases of IODs in the data. Because of this, the technique is generally regarded as a 'descriptive statistic' (Mead, 1974). However, the subjective interpretation is subsequently tested by statistical analysis which provides an objective

Table 4. *Comparison of IOD values with the χ^2 distribution*

	Points of the distribution			
	Lower quartile	median	Upper quartile	
IOD values expected for the χ^2 distribution Scale 1; 16 d.f.	>0·75>	<0·95>	<1·2>	
Numbers of observations: expected	2	2	2	2
observed	0	0	2	6
Scale 4; 4 d.f.	>0·48>	<0·84>	<1·35>	
numbers of observations: expected	2	2	2	2
observed	0	1	2	5

IOD values of Table 3 were compared with χ^2-distribution. Under the hypothesis that patches were randomly distributed, IODs of the eight samples at each scale should have conformed to the χ^2-distribution, e.g. at scale 1: two values should have been less than 0·75, two values between 0·75 and 0·95, two between 0·95, and 1·2 and two above 1·2. The distributions of observed values clearly differed from expectation, the excess of high IOD values confirming that patches were non-randomly distributed. All eight scales were compared with the χ^2-distribution in this way and an overall non-random spatial arrangement of patches was thereby detected.

Table 5. *Testing for scale of pattern by examination of successive IOD ratios*

Level of comparison (block sizes)	d.f.	Successive IOD ratios Sample number (1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	Summary >1	<1	Consistent outcome
2:1	32,16	0·78	1·66	1·10	0·84	1·89	1·64	1·00	0·35	4½	3½	
4:(1,2)	4,48	0·48	1·00	0·60	0·22	0·96	1·08	0·64	0·52	1½	6½	
8:4	8,4	5·75	1·39	1·84	5·00	1·13	1·12	2·16	2·39	8	0	8>4
8:(1,2,4)	8,48	2·88	1·60	1·12	1·19	1·09	1·21	1·41	1·42	8	0	8>(1,2,4)
16:8	1,8	0·77	1·69	1·45	1·28	1·67	0·94	0·38	0·17	4	4	
(8,16):(1,2,4)	9,48	2·81	2·15	1·17	1·23	1·17	1·20	1·31	1·29	8	0	(8,16)>(1,2,4)
32:(8,16)	2,9	2·45	2·18	0·87	0·97	9·26	2·38	0·66	2·40	5	3	
32:16	2,1	2·95	1·39	0·63	0·78	1·94	2·51	1·63	13·16	6	2	

The figures in the Table are the ratio of the IOD values for each sample (Table 3) for each of the block sizes shown (e.g. the IOD for sample 1 at block size 2 is 2·92, at block size 1 is 3·76 (Table 3), giving a ratio of 2·92:3·77 = 0·78 in the top left hand corner of the present table). If the pattern does not change at successive scales, the expected ratio is 1 (similar to the Poisson expectation, see above). An increase in pattern is identified when successive IODs are *consistently* (i.e. over all eight samples), above 1. This occurred at scale 8 and at no other scale. The probability (P) of observing the 8-0 split by chance alone is very small, approximately: $P = 2^8 = \frac{1}{256}$.

Table 6. *Testing for differences in the strength of pattern related to distance from pylorus*

Sample no:	1	2	3	4	5	6	7	8
Distance from pylorus (mm):	(33)	(46)	(58)	(72)	(83)	(153)	(166)	(183)
2	7							
3	6·5	5						
4	8	6	6					
5	6·5	7	8	6				
6	8	7	7	7	5			
7	8	8	7	7	6	5		
8	9	6	6	7	7	6	5	

The figures in the table are the number of comparisons (out of nine) at different block sizes for which the IODs indicate a stronger pattern in each of the samples (indicated across the top of the table) versus each of the more distal sample areas (listed vertically). For example, the first figure in the table indicates that 7/9 IOD values showed stronger pattern in sample 1 compared with sample 2. Half (0·5) was allocated when IODs had equal values. Distances from the pylorus are given for each position (mm in brackets; total length of pinned out small intestine from pylorus to ileo-caecal junction = 436 mm). Positions 1 to 5 were duodenum, 6 to 8 jejunum.

assessment of the strength of the evidence for the conclusions made. In ecological research the method has therefore become a reliable, established tool for the detection and characterization of pattern (Greig-Smith, 1983).

We have shown that the technique is also suitable for the analysis of chimaeric tissues. Highly unbalanced tissues are particularly useful for clonal analysis, as aggregation of clones into larger patches is minimal, and each discrete patch therefore represents essentially a single coherent clone (West, 1975; Whitten, 1978; Schmidt *et al.* 1985). Our analysis of the spatial arrangement of such discrete patches shows clustering at all scales, but a consistent increase in clustering at a block size of $8\,mm^2$ (block size 8). It is probable that the strengthening of the pattern at this scale is due to descendent clones (cells, or crypts, which although no longer contiguous are related by descent from a common progenitor (West, 1978)). The arrangement of patches suggests that considerable fragmentation of descendent clones occurs during development of the gut, even though the progeny of these clones remain in identifiably distinct clusters.

The considerable size range of descendent clones is consistent with theoretical models for clonal growth which predict that a skewed clone size frequency (i.e. geometric) distribution will be obtained, even if all clones proliferate with equal probability and do not fragment (Schmidt *et al.* 1985).

If it is assumed that the proportions of B6 and DDK cells have not changed during development, the observed number of descendent clones and the overall proportion of B6 and DDK components can be used to estimate the total number of descendent clones in the small intestinal epithelium. Thus, 41 B6 'descendent clones' were found in the entire small intestine, and B6 crypts formed 0·8 % of the total. The total number of descendent clones of either genotype is then $41 \times \frac{100}{0{\cdot}8} = 5028$. This value must, however, be regarded with caution as differences in proliferation rates are known to occur in some strain combinations (McLaren, 1976). Indeed, the decline in the strength of pattern of B6 patches from proximal to distal (Table 6) despite consistency in the scale of pattern, and the general dominance of the DDK component in the distal region (Schmidt *et al.* 1985), indicate that DDK crypts may have a proliferative advantage in at least the distal zone of the small intestine in the DDK↔B6 combination used here. In this case the estimate of 5028 descendent clones will be too high. Making the assumption of equal proliferation, an estimate of a minimum number of progenitors for the gut epithelium (120, or $\frac{100}{0{\cdot}8}$) may be obtained from the proportion of B6 crypts, assuming the extreme case that all B6 crypts (comprising 0·8 % of the total crypts) were descended from a single progenitor. However, this estimate must be treated with caution because the extent of proliferation and hence the size of the clone which arises from any single progenitor will be a matter of chance: one progenitor in 120 will not necessarily provide 1/120th of the adult tissue (see above; Schmidt *et al.* 1985).

Although we have illustrated the Greig-Smith analysis of variance for a tissue with highly unbalanced proportions of the chimaeric components, the method is not restricted to such material. Pattern can also be detected and characterized

even if discrete patches cannot be defined, as would be the case if the components of the chimaera were more nearly balanced. In this case, % cover per quadrat rather than discrete patches would have to be scored. The Poisson expectation then no longer applies, but the distribution would conform reasonably well to the normal distribution, and no modification to the method is required when using the F-statistic (see below; Mead, 1974). Binomial has been used in some cases when scoring % cover (Greig-Smith, 1983), however, it is not a valid model for balanced chimaeric tissues as there is no fixed number of possibilities occurring with constant probability. The analysis we describe is widely applicable to definition of pattern, and may provide a quantitative method to gain insight into clonal histories during development.

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