AN UNEXPLAINED FEATURE OF THE VASCULAR CAMBIAL ACTIVITY IN FOSSIL AND LIVING GYMNOSPERMS

by G. T. CREBER

ABSTRACT. When traced across a growth ring in both fossil and Recent gymnosperm wood, from the earlywood to the latewood, the radial diameters of the tracheids exhibit a variation which is not random. Instead they show a series of gradual increases to a maximum and decreases to a minimum with periodicities of about 6, 9, 11, and 18 cells. Work is described which attempts to explain this effect in terms of differential expansion cells or polyploidy.

THE trunk wood of trees is the secondary xylem tissue formed by the activity of the vascular cambium. This tissue, known as a lateral meristem, is arranged in the form of a cylinder just within the secondary phloem and bark of a tree. It has the capacity to go through the process of cell division more or less indefinitely until the tree dies. Cells produced towards the exterior become phloem tissue whilst those towards the interior develop into secondary xylem (text-fig. 1*a*). In the temperate regions of the world the cambium ceases its activity during the winter and the last-formed cells of the autumn tend to be small and thick-walled (text-fig. 2*a*, *b*). In the following spring the cells produced by the cambium expand considerably, but as the season progresses the cells attain successively lower maximum diameters. This decline is, however, not a gradual one in any given file of cells but proceeds in a series of cycles (text-fig. 1*c*) until the ring boundary is reached. Although this phenomenon was first described in fossil wood it has since been established as occurring in the wood of many diverse genera of living and fossil trees (Creber 1972, 1975; Creber and Chaloner 1984*a*, *b*)

Although the cambium produces very uniform files of cells (angiosperms excepted) (text-fig. 2*a*, *b*) it is a fact that each cell may not be in all respects identical to its neighbours in the file. Because some of the cells (xylem mother cells) produced by the cambium may themselves undergo further division before maturing, a given file may be composed of different generations of cells (t_1, t_2; text-fig. 1*b*). Mahmood (1968) showed that these further divisions undergone by the xylem mother cells result in the formation of multiple walls around the daughter cells (text-fig. 1*b*). The significance of the irregular occurrence of multiple walls along a file is discussed below.

MATERIALS AND METHODS

Transverse sections of both fossil and living gymnosperm wood have been used in the present investigation. The fossil material studied in detail was *Metacedroxylon scoticum* (Holden 1915, pl. 3) from the Kimmeridgian of East Sutherland but other species are illustrated (text-fig. 2). The living genera studied include *Pinus* and *Cupressus*. The radial diameters of files of cells across entire growth rings (text-fig. 1*c*) were measured with an eyepiece micrometer. Early attempts (Creber 1975) to study the cyclic effect made use of a moving average technique which tended to exaggerate the cyclicity by what is known to statisticians as the Slutzky effect. A different approach was therefore adopted in order to process the data without introducing spurious effects. An SPSS bivariate regression program (Nie *et al.* 1975) was used to fit a straight line to the data (for a detailed description of the operation of the program see Appendix).

The program also calculated the amount of the variation in cell diameter which was attributable to the linear decline (r^2) and expressed this as a percentage of the total variation (%) (r^2, % Table 1). In Table 1, r^2 is

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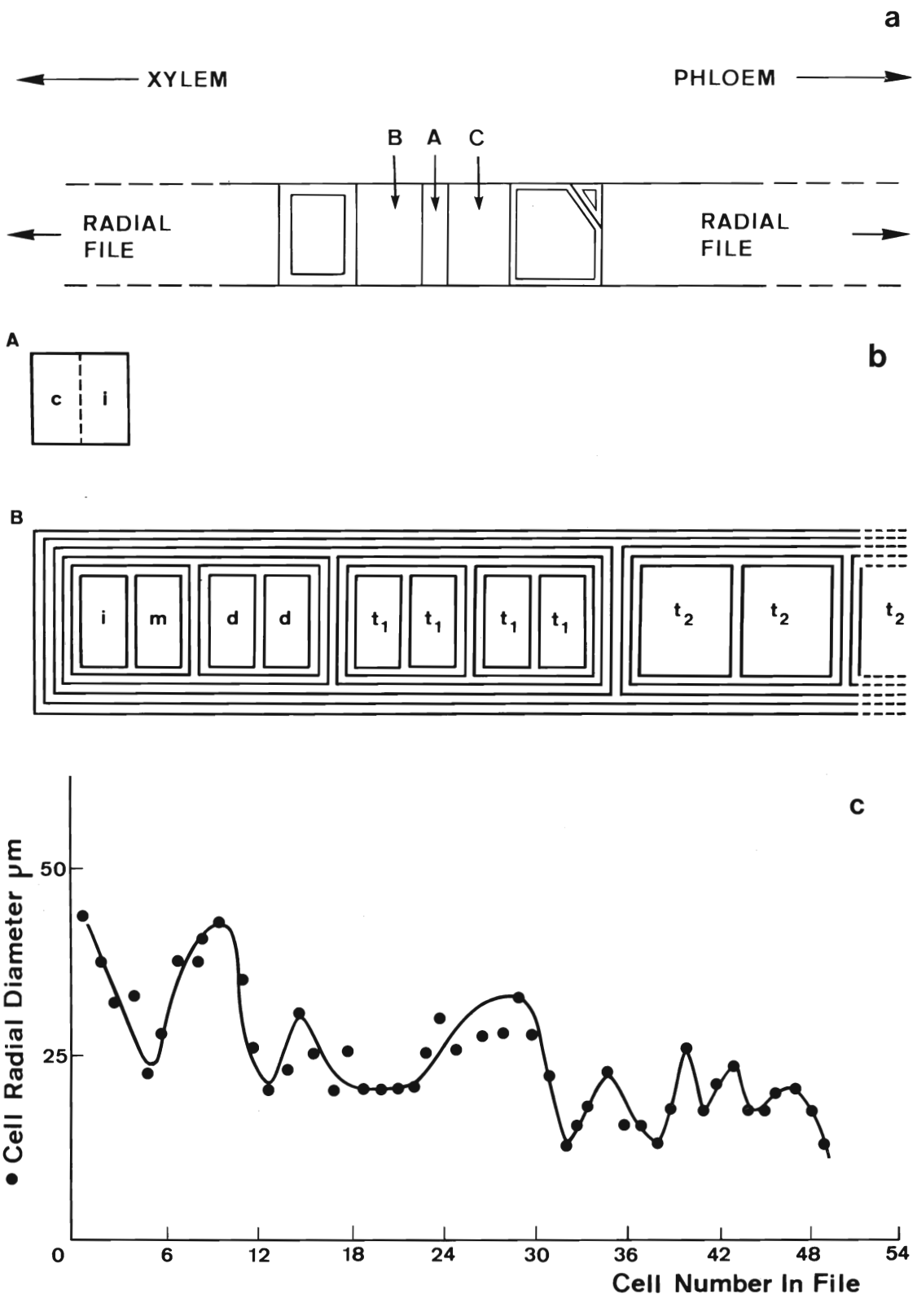
a
XYLEM
PHLOEM
B A C
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RADIAL FILE
A
c i
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B
i m d d t1 t1 t1 t1 t2 t2 t2
c
• Cell Radial Diameter µm
50
25
0 6 12 18 24 30 36 42 48 54
Cell Number In File

TABLE 1. Periodicities in the files of cells

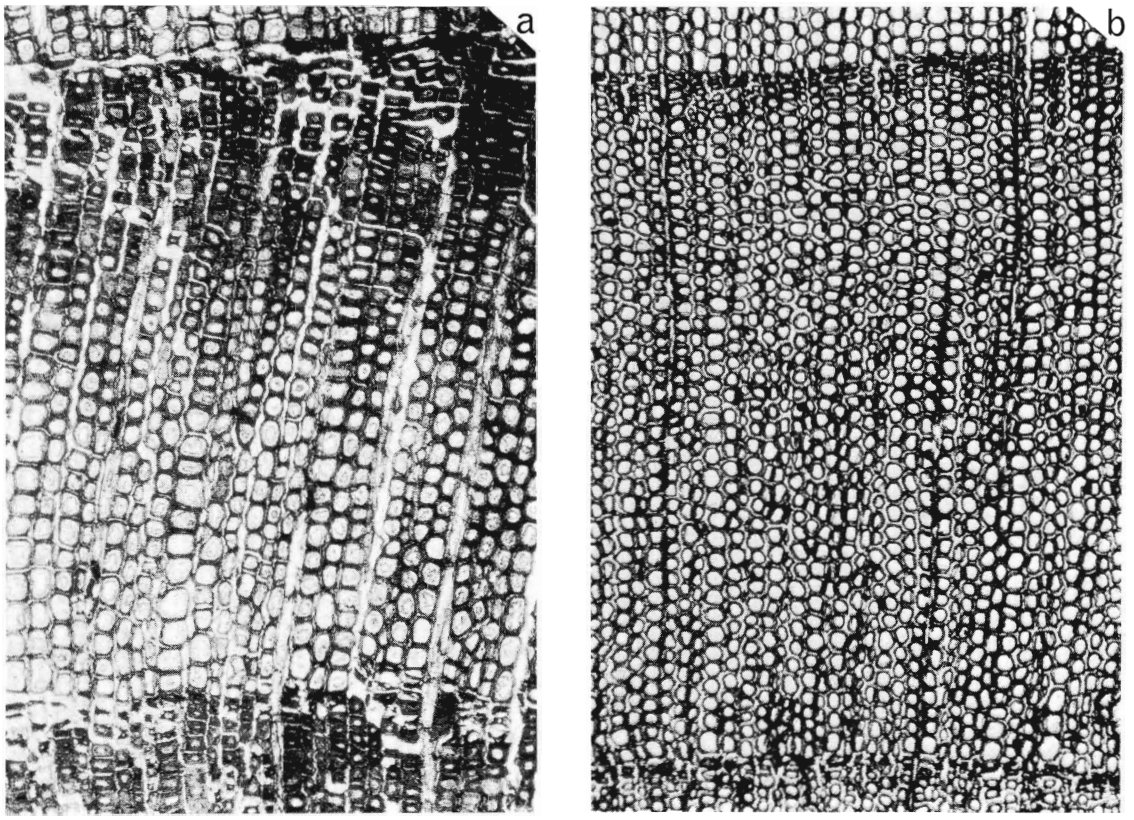
Name	File	Cells	r^2	%	Periodicities 4–7	7–10	10–13	13–25	Above 25
Metacedroxylon scoticum	1	70	0·739	73·9	5·98	8·5	12·4	—	41·6
	2	70	0·846	84·6	5·5	—	10·3	22·2	—
	3	70	0·899	89·9	5·96	—	—	20·8	—
Cupressus, Ring 1	1	91	0·273	27·3	6·0	8·2	12·1	20·6	55·5
	2	91	0·32	32·0	6·0/6·9	—	10·5	15·7	39·9
	3	91	0·154	15·4	4·5/6·9	—	11·0	19·0	41·6
	4	91	0·383	38·3	4·6/5·5	9·1	—	—	40·7
Cupressus, Ring 2	1	66	0·562	56·2	6·3	8·3	—	13·1	34·4
	2	66	0·485	48·5	—	7·6	—	21·5	—
	3	66	0·277	27·7	4·7/6·6	—	11·2	23·5	—
	4	66	0·269	26·9	—	7·1	—	15·9	30·2
Pinus strobus Ring 2	1	51	0·755	75·5	4·7	9·76	—	—	37·0
	2	51	0·757	75·7	—	8·4	—	18·9	—
	3	51	0·837	83·7	—	7·3	—	18·8	—
Theoretical cell data	1	112	—	—	5·98	8·95	—	17·8	—

given for groups of contiguous files of cells in the wood of a fossil species, *M. scoticum* and in the wood of some living genera. It is evident that in some cases, e.g. the fossil wood, much of the variation (up to 89·9 %) is explained by the regression line. In the case of the *Cupressus* specimens, however, the unexplained variation may be as high as 84·6 % (100 −15·4 %).

The use of this program, however, was not limited to obtaining a measure of r^2 for the data. A further object was to pass the residuals into a Maximum Entropy Spectral Analysis (MESA) program which was designed to search for periodicities. It was thought better to use the residuals for this purpose rather than the raw cell data since it was evident (Table 1) that, at least for the fossil wood and the specimen of *P. strobus*, a high proportion of the significance of the cell data can be explained by a straight-line decline in cell size. The 'residuals' then represent departures in the form of cyclic periodicities from the straight-line decline in cell size calculated by the program. The results from the MESA program are shown in Table 1 under 'Periodicities'; it is seen that they differ from one contiguous file to the next and may be grouped into a number of categories.

In an attempt to simulate data for the radial diameters, Mahmood's (1968) paper was used. Here, as already mentioned, he was able to show that the file of immature cells left behind as the cambium proceeds in an outward direction is not homogeneous but consists of cells surrounded by differing numbers of wall layers (text-fig. 1*a*). Assuming that those cells with a larger number of layers might not attain such a size as those not quite so constrained, a sequence of cell diameters has been estimated. The sequence was then converted to the residuals of the straight-line regression which in turn were passed through the MESA program. The periodicities thus found appear in Table 1 as 'Theoretical Cell Data'. Whilst it is accepted that their similarity

TEXT-FIG. 1. *a*, a diagrammatic representation of part of a radial file of cells in conifer secondary vascular tissue. Cell A is a cambial initial cell and B and C have arisen from it by cell division. B and C will differentiate into xylem and phloem elements respectively whilst A will divide again. *b*, diagrams illustrating the formation of parental primary walls as a result of the divisions of the cambial initial cell (*c*) (= A, text-fig. 1*a*) and its daughter cells. A. The division of the cambial initial cell (*c*) giving rise to the xylem initial (*i*). B. A theoretical construction of successive divisions of the xylem initial (*i*) showing the origins of parental primary walls surrounding each daughter cell. *d*, daughter cells of xylem mother cell; *i*, xylem initial cell; *m*, xylem mother cell; t_1, t_2, cells formed from daughter cells after further divisions. If the cells t_2 do not divide again, they will be surrounded by fewer primary wall layers than the cells t_1 (redrawn after Mahmood 1968). *c*, A graph of the radial diameters of the tracheids in one file across a growth ring in *Metacedroxylon scoticum* Holden from the Kimmeridgian of East Sutherland. The earlywood tracheids extend from cell 1 to about cell number 30, the remainder being latewood cells. The cell diameters pass through a series of cycles as the ring is traversed from earlywood to latewood. The amplitude of the cycles decreases as the cell diameters diminish (specimen K. 613, Sedgwick Museum, Cambridge).



TEXT-FIG. 2. *a*. part of a transverse section of *Cedroxylon hornei* Seward and Bancroft from the Kimmeridgian of East Sutherland (Seward and Bancroft 1913). One entire growth ring is shown with parts of two others at the top and bottom of the photograph. In the process of permineralization the files of cells have separated slightly from one another. The diminution in cell size from the earlywood at the bottom to the latewood at the top can be clearly seen. Also it is evident that in some of the files there are cells towards the latewood part of the ring which are larger than the first cells in the earlywood (text-fig. 1*c*). × 70. (Specimen BM(NH) V62302.) *b*, part of a transverse section of *Cupressinoxylon* sp. from the Bathonian of Robin Hood's Bay. This exhibits the same features as in *a* above but there is much less latewood in this growth ring. × 70. (Specimen BM(NH) V62284.)

to those of the actual cell data is no proof of Mahmood's phenomenon being the causal mechanism, it is also true that the hypothesis is not in conflict with the results of the analysis.

VARIATION IN CELL SIZE DUE TO ENVIRONMENTAL INFLUENCE

A possible interpretation of the variability of the radial diameters of tracheids within the growth rings of gymnospermous wood was published by Ford *et al.* (1978). They described work on a dominant tree in a Forestry Commission Sitka spruce plantation in Dumfriesshire. Samples of cambial zone tissue and immature secondary xylem were taken at 12 hourly intervals for 15 days (27 June–11 July). This frequent sampling interval had not been used by any previous workers and it represented an attempt to seek changes in xylem production and maturation that might be due to short-term changes in the weather. Their resulting data was in the form of measurements of tracheidal tangential and radial diameters together with wall thicknesses.

Their main methods of analysis were correlation and autocorrelation which showed a widespread, significant positive correlation of radial tracheid diameter and wall thickness. They found positive values of autocorrelation along three contiguous files of cells which were interpreted as an indication that consecutive cells along the radial files had similar diameters (as I have also found). Their results were not used to explore the groups of cells involved but from their fig. 4 it could be seen that the autocorrelation fell to zero at about the 5th–6th cell distance along the files. This would correspond with the 4–7 group (Table 1) which was demonstrated by the MESA program in my data and may represent the progeny of one xylem mother cell.

They also presented results of simple correlations between contiguous files of cells using the measurements (radial diameters) of cells that had most or all of their radial walls in contact with one another across the boundary of two contiguous files (text-fig. 2*a*, *b*). This was done in preference to correlating the entire lists of cell data from two complete files; the loss of cell data inherent in using this method was accepted. It was argued that the remaining data achieved a greater significance because the pairs of cells so selected were those that had expanded together radially at the same time when a certain environmental factor was acting on the tree, whereas if one takes cell no. 5, say, in each file, these may well not be in contact with one another in the two files and were therefore not expanding at the same time. The results of their correlations produced significant positive coefficients between files which they claimed as an indication of a simultaneous causal mechanism affecting all of the files together.

However, an alternative explanation might well be that this particular region of the growth ring had a similar capacity for expansion in all of the files due to their simultaneous ability to expand. Ford (pers. comm.) was critical of my original procedure (Creber 1975) of superimposing the graphs of moving averages of cell diameters to show apparently out-of-phase situations along the files. Instead, he recommended the use of his technique of correlating only those cells which shared a substantial common boundary in contiguous files. This suggestion was adopted and the very conflicting results that were obtained are shown in Table 2.

It is seen that for some pairs of files the correlation coefficient may have a very high positive value (e.g. *P. strobus* Files 2/3, +0·901, but for others it may be as low as +0·381). From this admittedly rather limited database it would appear that no definite conclusion can be drawn as to the validity of Ford *et al.*'s claim. In Table 2 there are also included some results from *M. scoticum* in the form of the residuals from the SPSS regression program. These results show no similarity at all with those from the raw data (Files 1/2 and 2/3); in the case of Files 1/2 a negative figure results (−0·067) as against +0·705 for the raw data. This kind of anomaly may also be resolved when a much larger database is available.

TABLE 2. Correlations between files of cells

Name and file numbers			
Metacedroxylon scoticum	Residuals	1/2	−0·067
	Files	1/2	+0·705
	Residuals	2/3	+0·202
	Files	2/3	+0·870
Pinus strobus	Files	1/2	+0·776
	Files	2/3	+0·901
Cupressus Ring 1	Files	1/2	+0·388
	Files	2/3	+0·487
	Files	3/4	+0·393
Cupressus Ring 2	Files	1/2	+0·542
	Files	2/3	+0·381
	Files	3/4	+0·528

Files 1/2 indicates File 1 correlated with File 2.

VARIATION IN CELL SIZE DUE TO POLYPLOIDY

Winkler (1916), in a general survey of cell sizes and chromosome numbers in higher plants, concluded that there was a close correlation between cell size and chromosomal mass. He found, too, that many differentiated plant cells were polyploid. In more recent work it has been shown that in *Zea mays* root metaxylem cells the DNA content of the nuclei falls into a frequency distribution with peaks at the 4-, 8-, 16-, and 32-ploid equivalent. The DNA content had a high positive correlation with nuclear volume in the overall growth of the metaxylem cells (List 1963; Barlow 1985). List also studied widely diverse genera such as *Acorus* and *Peltandra* (Araceae) and *Marsilea* and *Dennstaedtia* (Filicales) and found positive correlations of cell volumes and polyploidy in differentiating xylem. The widespread occurrence of polyploidy in differentiating cells (not only xylem) led Evans and Van't Hof (1975) to investigate whether or not it was an essential feature of plant cell differentiation. They found that in *Pisum sativum* polyploidy was present in roots, sepals, pods, pistils, and stamens but not in petals or leaves. In *Triticum aestivum* some leaf cells exhibited polyploidy but the root cells did not. No polyploid cells could be found in any of the tissues of *Helianthus annuus*. They therefore came to the conclusion that polyploidy should not be considered essential to cell differentiation in spite of its widespread occurrence.

It still remains a possibility therefore that the variation in cell radial diameter, observed along files of cells in gymnosperm xylem, might be due to different levels of polyploidy in the initials during differentiation. However, the apparently cyclic variation exhibited is less likely to be due to variation in the ploidy state as the latter seems to occur in rather a random fashion. At the moment the matter remains unresolved because the techniques for the investigation of polyploidy in primary tissues are not usable in a tissue such as maturing secondary xylem.

CONCLUSION

The occurrence in both Mesozoic and living gymnosperms of the phenomenon described in this paper leads to the conclusion that it results from a fundamental aspect of the production of secondary xylem cells by the vascular cambial layer. The out-of-phase nature of the periodicities in neighbouring files of cells would seen to rule out an environmental cause. Instead, it appears more likely that it results from a periodic pattern of inhomogeneity in the capacity of the xylem initial cells to expand. It is hoped that with the current increase in availability of rapid image analysers a much larger number of wood specimens may be examined to enable a more definite conclusion to be drawn from a more extensive database.

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APPENDIX

In this paper an SPSS bivariate regression program (Nie *et al.* 1975) was used to fit a straight line to the data. In the program, values of the dependent variable are predicted from a linear function of the form:

$$Y^1 = A + BX, \tag{1}$$

where Y^1 is the estimated value of the dependent variable Y (the radial diameter of the tracheid), B is a constant by which all values of the independent variable X are multiplied, and A is a constant which is added to each case. The independent variable X is the cell's numbered position in the file (text-fig. 1*c*), starting with the first earlywood cell. The difference between the actual and the estimated value of Y for each case is called the residual, i.e. the error in prediction, and may be represented by the expression:

$$\text{Residuals} = Y - Y^1. \tag{2}$$

The regression technique involves the selection of A and B such that the sum of the squared residuals is smaller than any possible alternative, i.e. it is a 'least squares' solution. This may be expressed as:

$$(Y - Y^1)^2 = SS_{\text{res}} = \text{minimum}. \tag{3}$$

The total sum of squares in Y (which is the total variability of the dependent variable Y) can be partitioned into components that are (*a*) explained or accounted for by the regression line, denoted by SS_{reg} and (*b*) unexplained (the sum of the squared residuals) SS_{res} which may be expressed as:

$$SS_{\text{res}} = (Y - Y^1)^2. \tag{4}$$

This partition may be written as:

$$SS_{\text{y}} = SS_{\text{reg}} - SS_{\text{res}}. \tag{5}$$

A measure of prediction accuracy and strength of linear association is the ratio of explained variation in the dependent variable Y to the total variation in Y. Thus:

$$r^2_{\text{xy}} = \frac{SS_{\text{reg}}}{SS_{\text{y}}} = \frac{SS_{\text{y}} - SS_{\text{res}}}{SS_{\text{y}}}. \tag{6}$$

The ratio r^2 is the amount of variation in cell diameter which is explained by a linear decline in cell size; it is also called the coefficient of determination.