KAWASAKI MED. J. VOL. 10 : NO. 4, 245-254 (1984)

The Spatial Pattern Analysis of the Regenerating Axons of Peroneal Nerves in the Rat

Seizaburo ARITA

Department of Mathematics, Kawasaki Medical School, Kuraskiki 701-01, Japan
Accepted for Publication on December 13, 1984

ABSTRACT. Changes in the spatial patterns of transverse sections of regenerating nerves observed in the electron micrographs seemed to be dependent upon the stage of nerve regeneration. The aim of this study was to classify the spatial distribution of axons into regular, random and clustered patterns by spatial pattern analysis and to describe the stages of regeneration in terms of these spatial patterns. Based on analysis of experimental data from free grafts of common peroneal nerves of rats, it is suggested that the spatial pattern of regenerating nerve fibers changes from the clustered to random to regular as they approach a normal condition.

Key words : Spatial patterns — Nerve regeneration — Regenerating axons

The success of operative suturing of nerves has been quantitatively evaluated by the number of regenerating axons and their diameters. Sanders[1] first measured the diameters of regenerating axons, Nomura,[2] Matsuda[3] and Arita[4] discussed the number of regenerating axons and Arita[5] and Miyamoto[6] studied these qualities as well as the location of axons.

Observation of transverse sections of regenerating nerves suggests that the spatial patterns of axons change during the various stages of regeneration. Spatial pattern analysis classifies spatial distribution into three patterns; clustered, random and regular. The distance method of this analysis was applied to the transected nerve fibers in tissue photographs taken by means of an electron microscope.

In this paper we have attempted to classify the spatial patterns of regenerating axons during the sequential stage of nerve regeneration using experimental data from rats, and to discuss whether the spatial pattern of axons may be considered as a satisfactory index for describing the state of regeneration.

MATERIALS AND METHODS

The right peroneal nerves of 84 male rats weighing from 250 to 300 g were used. Nerve segments of four different lengths, 1.0, 1.5, 2.0 and 2.5 cm, designated as A, B, C and D respectively, were taken and then resutured orthotopically with the aid of an operating microscope to the original site using 10-0 threaded nylon sutures. The state of regeneration was observed at four week intervals up to the 24th week after surgery.

有田清三郎

Transverse sections were taken as specimens from three sites; 5 mm distal to the proximal suture site (designated as "a"), 5 mm proximal to the distal suture site (designated as "b") and at the distal cut end (designated as "c"). Using a transmission electron microscope, these specimens were placed on a single hole grid. Then photomicrographs of 6300 magnification were prepared for computer analysis with a computer system having the necessary hardware and program for input and analysis of the photomicrographs. The input device was a Graf/Pen Model GP3 binary digitizer (Scientific Accessories Corporation) with an effective tablet area of 350×350 mm. By placing the photos on this tablet, the coordinates of the central points of axons were input. The data obtained were analyzed using another personal computer (If-800 model 20, OKI Co.). The computer programs used were developed specifically for this purpose.

The distance method of spatial pattern analysis was applied to this data. Two kinds of distances were calculated ; one being the squared distance from a randomly selected axon to its nearest neighboring axon and the other being that from a random point to its nearest neighboring axon. The flow chart of this analysis is shown in Figure 1.

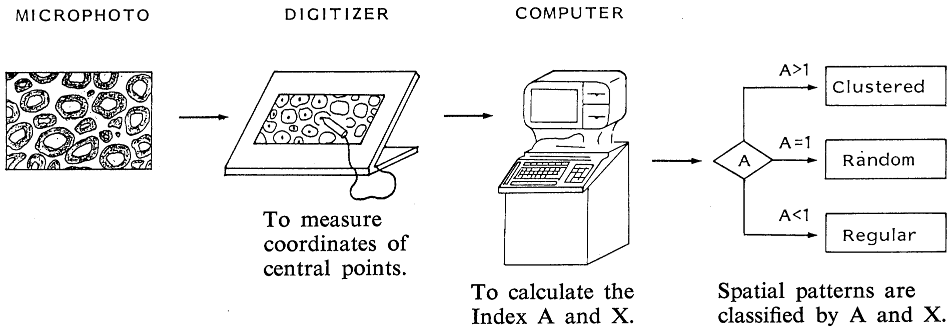


Fig 1. The flow chart of analysis of spatial patterns.

ANALYSIS OF SPATIAL PATTERNS

Spatial pattern analysis can classify spatial distribution into three patterns; regular, random and clustered, and it has been applied to many fields of science, i.e., to the study of plant population, the territories of birds and so on. In this analysis two methods are mainly employed; the quadrat and distance methods. The quadrat method deals with the number of individuals in a quadrat. On the other hand, the distance method deals with the distances between individuals and distances from random points to individuals. These methods were originally proposed as a test for the randomness of spatial distribution.

The distance method was introduced by Hopkins and Skellam[7] in 1954. Since then many researchers have proposed Indexes.[8-10] Recently Ripley,[11] Diggle[12] and Byth and Ripley[13] have discussed the precision of these Indexes. Ogata and Tanemura[14] have proposed a potential method by the maximum likelihood.

Axons are represented by the points, $M_1, M_2, \ldots, M_m$ and other random points are represented by $P_1, P_2, \ldots, P_n$. The distance method analyzes spatial distribution by two types of distances U and V. U_i is the distance from the random point P_i to its nearest neighboring axon. V_j is the distance from the randomly selected axon M_j to its nearest neighboring axon, for $j=1, 2, \ldots, n$ $(n \leq m)$.

Hopkins and Skellam classified spatial patterns by the following index

$$A = \sum_{i=1}^{n} U_i^2 \ / \ \sum_{j=1}^{n} V_j^2 , \qquad (1)$$

where the numerator of the index is the sum of distances between axons, the denominator is that from random points to axons, and n is the sample size.

For this index, the following lemma are known.

Lemma 1 (Hopkins' Index)

If the distribution of axons is random, then the expected value of index A is equal to 1. If the distribution is clustered or regular, the value of A is larger than 1 or less than 1, respectively.

The typical distributions of the three patterns are shown in Figure 2.

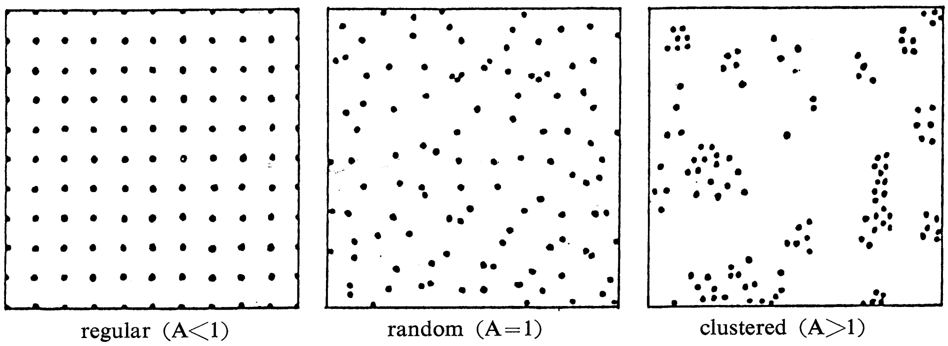


Fig. 2. The spatial pattern of axons was classified into regular, random and clustered. Using Hopkins' index, random distribution is A=1, clustered A>1 and regular A<1.

As a test for the difference of A from 1, the following statistic is used

$$x = A \ / \ (A+1). \qquad (2)$$

Then the following theorem holds for the property of the statistic x.

Theorem 1

If $M_1, M_2, \ldots, M_m$ are randomly distributed aud n is sufficiently large, then the distribution of x *is well approximated by the normal distribution having the mean value of*

$E(x) = 1/2$

and the variance of

$V(x) = 1/4\,(2n+1)$.

The proof of this theorem has been provided by Skellam and Pielou. From Theorem 1, for large n, the distribution of the statistic

$$\tilde{Z} = \frac{x - 1/2}{1/2\sqrt{2n+1}} \qquad (3)$$

is well approximated by the standard normal distribution.

Therefore, the spatial patterns are classified as follows.

Theorem 2 (Hopkins' Test)

At a significance level of α, we can classify spatial patterns as follows.

If $|\tilde{Z}| \leqq Z_\alpha$, then the spatial pattern is judged as random. If $\tilde{Z} > Z_\alpha$, then the pattern is considered to be clustered and if $\tilde{Z} < -Z_\alpha$, then the pattern is classified as regular. Z_α is the Z-value at the significance level α of the standard normal distribution.

A simple method of classification is used in the following procedure.

Corollary 1

Based on the sample size n and the statistic x, *we can classify the spatial patterns of axons as follows. For large n, at the significance level $\alpha = 0.05$, if*

$$x < 1/2 - 1/\sqrt{2n+1} \quad ,$$

then the spatial pattern is regular ; if

$$1/2 - 1/\sqrt{2n+1} \leqq x \leqq 1/2 + 1\sqrt{2n+1} \quad ,$$

then the pattern is random, and if

$$x > 1/2 + 1/\sqrt{2n+1} \quad ,$$

then it is clustered.

In this paper, the significance level of classification was set at $\alpha = 0.05$.

RESULTS

Four photos will be cited as examples, and their respective analyses will be described.

Figure 3(i) shows a normal common peroneal nerve. In this photo, there were 22 axons, and the coordinates of the central points of axons were measured, while other random points were generated by the computer. Using the above mentioned distances of the data of the axons and random points, index A, statistic x and the standard deviation of x were calculated ; $A = 0.2244$, $x = 0\ 1833$ and $1/\sqrt{2n+1} = 0.0715$. From these quantities, the $\tilde{Z}$ value was found to be

$$\tilde{Z} = -4.423 < -Z_\alpha = -1.96 \quad .$$

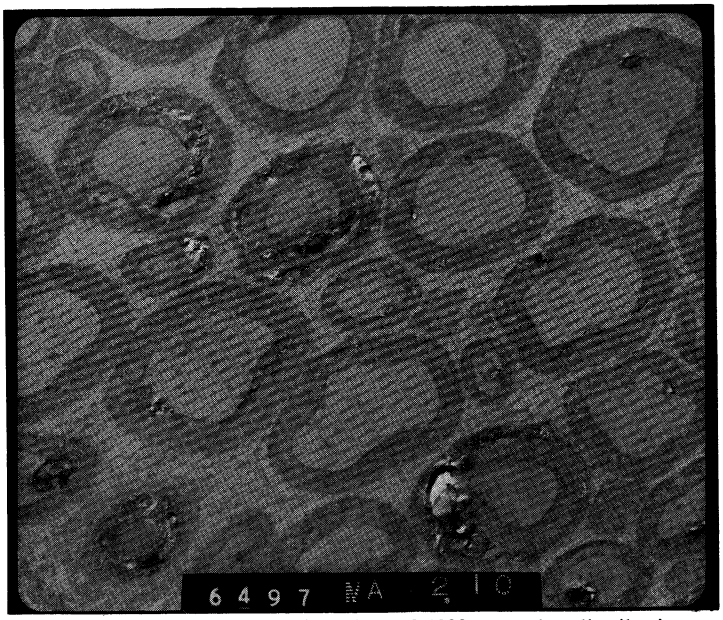
($\tilde{Z} < -t_\alpha(21) = -2.316$.) Therefore the spatial pattern of normal axons in this photo was classified as regular.

Figure 3(ii) is a photo of a regenerated ~~axon in~~ 8 weeks after graft. There were 58 myelinated fibers in the study area. Index A, the x value and the standard deviation were calculated ; $A = 1.9287$, $x = 0.6586$ and $1/2\sqrt{2n+1} = 0.0463$. The sample size n was large, so the corollary was applied and the value x was ranged over

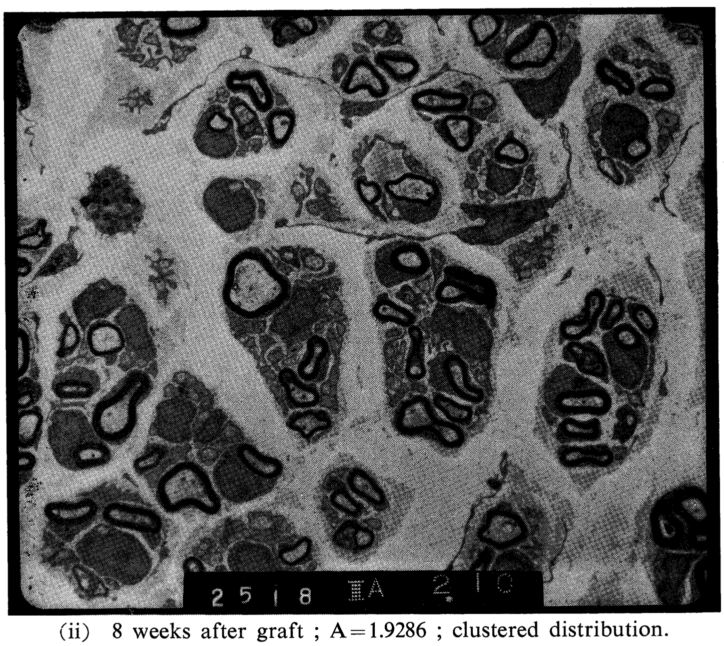
$$x > 1/2\sqrt{2n+1} \quad .$$

(The Z value also indicated $\tilde{Z} = 3.425 > Z_\alpha = 1.96$.) Thus, the spatial pattern of the regenerated axons was considered to be clustered.

Figure 3(iii) is a photo taken 12 weeks after graft for which the analysis was done. The values obtained were; $A = 0.8909$, $x = 0.4712$, $n = 59$ and $1/2\sqrt{2n+1} = 0.0459$. The corollary was applied to this data and the value x was in the interval



(i) Normal nerve ; A=0.2244, x=0.1833 ; regular distribution.

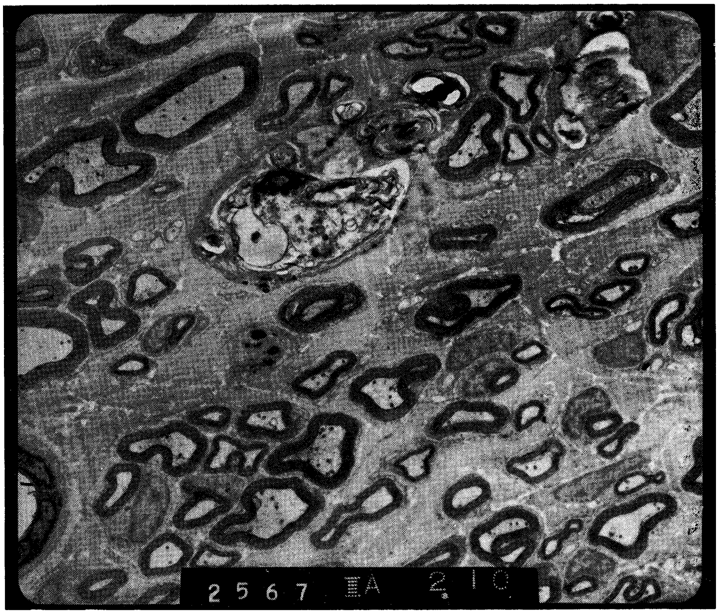


(ii) 8 weeks after graft ; A=1.9286 ; clustered distribution.

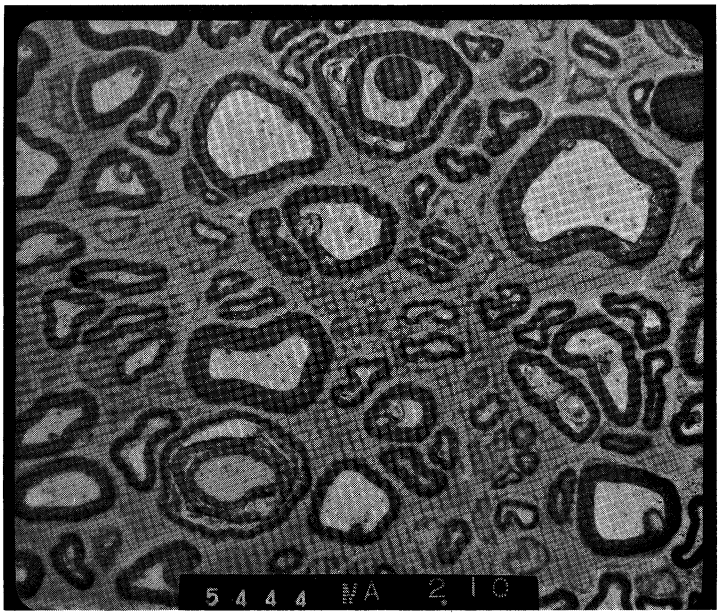
$$1/2 - 1/\sqrt{2n+1} \leq x \leq 1/2 + 1/\sqrt{2n+1} \quad .$$

(The calculated Z value of -0.6275 also showed $|\tilde{Z}| < Z\alpha = 1.96$.) As a result, this spatial pattern was judged to be random.

Figure 3(iv) is a photo taken 20 weeks after graft. The obtained values



(iii) 12 weeks after graft ; A=0.8909, x=0.4712 ; random distribution.



(iv) 20 weeks after graft ; A=0.4115, x=0.2915 ; regular distribution.

Fig. 3. Transverse sections of the peroneal nerve fibers of rats magnified 2,100×.

TABLE 1. Results of spatial pattern analysis of axons.

	Photo No.	n	A	x	$1/2\sqrt{2n+1}$	$\tilde{Z}$	Spatial Patterns
Normal (N - b)	6493	25	.2798	.2186	.0700	-4.020	regular
	6494	24	.3075	.2352	.0714	-3.707	regular
	6495	28	.4157	.2936	.0662	-3.118	regular
	6496	23	.2771	.2170	.0729	-3.882	regular
	6497	22	.2244	.1833	.0745	-4.251	regular
	6498	27	.4436	.3073	.0674	-2.859	regular
	6499	26	.2668	.2106	.0689	-4.200	regular
	6500	30	.3590	.2642	.0640	-3.684	regular
	6501	24	.3297	.2480	.0714	-3.529	regular
	6502	29	.4538	.3122	.0651	-2.885	regular
Regeneration (After 8 weeks) (8 - D - b)	2515	90	.6093	.3786	.0372	-3.263	regular
	2516	77	1.2499	.5555	.0402	1.381	random
	2517	64	.9075	.4758	.0440	-0.550	random
	2518	58	1.9287	.6586	.0462	3.433	clustered
	2519	49	3.37907	.7716	.0503	5.400	clustered
	2520	79	1.1889	.5431	.0397	1.086	random
	2521	55	.9683	.4919	.0475	-0.171	random
	2522	66	.7729	.4360	.0434	-1.475	random
	2523	60	1.9328	.6590	.0455	3.495	clustered
	2524	53	1.9659	.6628	.0483	3.371	clustered
Regeneration (After 12 weeks) (12 - D - b)	2560	61	.8102	.4476	.0451	-1.162	random
	2561	48	.6787	.4043	.0508	-1.884	random
	2562	70	.6971	.4108	.0421	-2.119	regular
	2563	66	.6056	.3772	.0434	-2.829	regular
	2564	66	.4378	.3045	.0434	-4.505	regular
	2565	66	.5766	.3657	.0434	-3.094	regular
	2566	58	.6275	.3855	.0462	-2.478	regular
	2567	59	.8909	.4712	.0458	-0.629	random
	2568	39	.6607	.3979	.0563	-1.813	random
	2569	59	.7130	.4162	.0458	-1.830	random

	Photo No.	n	A	x	$1/2\sqrt{2n+1}$	$\tilde{Z}$	Spatial Patterns
Regeneration (After 20 weeks) (20 - D - b)	5435	75	.4379	.3045	.0407	-4.803	regular
	5436	71	.5697	.3629	.0418	-3.280	regular
	5437	54	.5083	.3370	.0479	-3.403	regular
	5438	69	.4522	.3114	.0424	-4.448	regular
	5439	49	.7232	.4197	.0503	-1.596	random
	5440	73	.5679	.3622	.0412	-3.345	regular
	5441	67	.8016	.4449	.0430	-1.281	random
	5442	58	.4919	.3297	.0462	-3.686	regular
	5443	78	.3678	.2689	.0399	-5.792	regular
	5444	58	.4115	.2915	.0462	-4.513	regular

TABLE 2. The transition of spatial patterns of axons in the nerve regeneration process.

Patterns	Clustered	Random	Regular	Total
Normal	0	0	10	10
Regeneration (After 8 weeks)	4	5	1	10
Regeneration (After 12 weeks)	0	5	5	10
Regeneration (After 20 weeks)	0	2	8	10

were ; $A=0.4115$, $x = 0.2915$, $n=58$, $1/2\sqrt{2n+1} = 0.0462$ and $\tilde{Z}= - 4.512$. This spatial pattern was judged to be regular.

During the process of nerve regeneration, four stages were chosen ; the normal stage and 8 weeks, 12 weeks and 20 weeks after graft. For each stage, 10 photos were taken and employed in this analysis. The results of the analysis are shown in Table 1, and are summarized for the three stages of regeneration and the normal stage in Table 2.

DISCUSSION

As can be seen in Table 2, the spatial pattern of the 10 photos of normal axons were all classified as regular. Those during regeneration, on the other hand, differed from the normal type. During the beginning stage of regeneration, there were many clustered patterns of axons. During the middle stage, random patterns increased. During the final stage, most of the photos were occupied by the regular pattern.

From these findings, it can be seen that the spatial pattern during the regenerating process changed from the clustered to random to regular as the nerve fibers approached normal condition. The sequential changes in patterns after various types of surgery are not always the same. Some data have shown the spatial pattern to be regular during the beginning stage, while other data have shown the pattern to still be clustered during the final stage. The transition of patterns described in this paper, however, is indicative of the typical process of nerve regeneration.

This analysis classified the spatial patterns of axons into three categories based on the test for randomness, so the degree of difference from complete regularity to clustering could not be shown quantitatively. However, the results in Table 2 indicate that this classification shows clear changes in pattern according to the process of regeneration and that passage through the random pattern is important in regeneration to normal condition. Therefoe this analysis seems to be of significant value in the classification of the spatial patterns of axons.

The spatial pattern analysis in this paper was based on the point process, but the axons in the photos were actually not points. The diameters and areas of the axons and their numbers are also important indexes. Multivariate analyses are required to study the relationship between the spatial pattern and these indexes.

Acknowledgments

The author wishes to thank Professor Taichi Kariya and Professor Takuji Ohkura for their critical advices and suggestions. The author is also grateful to Dr. Yoshihiro Miyamoto to help for providing the experimental data.

REFERENCES

1) Sanders, F.K. and Young, J.Z. : Influence of peripheral connection on diameter of regenerating nerve fibers. J. Exp. Biol. **22** : 203–212, 1946
2) Nomura, S. : Functional recovery. *In* Degeneration and regeneration in nervous system. Tokyo, Igakushoin. 1975, pp. 365–386
3) Matsuda, H., Yamano, Y., Nakajima, K., Shimazu, A., Kariya, T. and Arita, S. : Stochastic model of regenerating axons at the site of nerve suture. Seikeigeka **28** : 1293–1297, 1977
4) Arita, S., Kariya, T. and Matsuda, H. : Stochastic model for the process of regeneration of nerve fibers passing through the site of nerve suture. Proc. of International Conference of Cybernetics and Society, Tokyo, 213–216, 1978
5) Arita, S., Miyamoto, Y. and Kariya, T. : On the distribution of axons. Rept. Inst. Math. Soc. Kyoto Univ. **384** : 143–160, 1980
6) Miyamoto, Y., Arita, S., Hori, Y. and Miyamoto, H. : Statistical analysis on spatial pattern to transected peripheral nerve regeneration. *In* Posttraumatic peripheral nerve regeneration. ed. by Gorio, A. New York, Raven Press. 1981, pp. 271–276
7) Hopkins, B. and Skellam, J.G. : A new method for determining the type of distribution of plant individuals. Annals of Botany **18** : 213–227, 1954
8) Clark, P.J. and Evans, F.C. : Distance to nearest neighbour as a measure of spatial relationships in population. Ecology **35** : 445–453, 1954
9) Morishita, M. : Measuring of the dispersion of individuals and analysis of the distributional patterns. Mem. Fac. Asc. Kyushu Univ., Ser. E(Biol) **2** : 215–253, 1959
10) Pielou, E.C. : The use of point-to-plant distances in the study of the patterns of populations. J. Ecol. **47** : 607–613, 1959

11) Ripley, B.D. : Modeling spatial patterns. J. Roy. Stat. Soc. Ser. **B** 39 : 172–212, 1977
12) Diggle, P.J. : On parameter estimation and goodness of fit-testing for spatial point patterns. Biometrics **35** : 87-101, 1979
13) Byth, K. and Ripley, B.D. : On sampling spatial patterns by distance methods. Biometrics **36** : 279–284, 1980
14) Ogata, Y. and Tanemura, M. : Estimation of interaction potentials of spatial point patterns through the maximum likelihood procedure. Annals Inst. Math. Ser. **B** **33** : 315–338, 1981