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Morphometric Analysis of the Scleral Bony Ring with Different Numbers of Ossicles in the Eye of *Coturnix Coturnix Japonica*

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With 3 figures and 2 tables

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Summary

The scleral bony ring (s. b. r.) of *Coturnix coturnix japonica* was examined and evaluated morphometrically in its characteristic formations (scleral ossicles, overlapping sectors). The modal class 14-14, the most representative, was compared to others having minor or major numbers of ossicles with complex or simple overlaps in the left and right eyes.

It was observed that the ossicles, numbered as in the literature, are ordered in a characteristic manner to form the s. b. r. The ossicle dimensions are different and numbers 4 and 7 are always smaller and larger. The s. b. r. with different numbers of ossicles compared to the model class 14-14 (0,0) showed an evident compensatory behaviour in all classes. In fact, when the ossicle number decreased, single ossicle area increased, retaining a constant s. b. r. area.

Introduction

The scleral bony ring (s. b. r.) is an anatomical formation present in all bird species, formed by a group of 14-15 bony plates, better known as the "scleral ossicles" (s. o.). They are nearly rectangular or trapezoid in shape, embedded in the outer margin of the sclera encircling the cornea, partly overlapping along the radial edges as seen in various ordered schemes (LEMMRICH et al, 1931; CURTIS/MILLER, 1938; COULOMBRE et al, 1962). Similar formations are also found in the eyes of other vertebrates, for example in some reptiles and *teleostii* fish (ROCHON-DUVIGNEAUD, 1954). In birds, however, the s. b. r. attains a high degree of morpho-structural perfection, as if to stress that it is not a *vestigium* but an organised formation with a precise function. In the s. b. r., several authors identify a system to protect and support the eyes: it is known that diving birds have the best s. b. r. (CURTIS/MILLER, 1938). In particular, NELSON (1942) stressed that the protection and physical support that the s. b. r. gives the ocular globe becomes very important in birds, due to the considerable size of the eye compared to the head. Moreover, during eye morphogenesis, various complex processes occur which are connected with genetic and local factors whose regularity is very important to produce a normal ocular globe arrangement (COULOMBRE/COULOMBRE, 1977). For this reason, the s. b. r.

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appears, in the vertebrates where it is present, as a complementary system; it has been observed that in chick embryos major eye malformations are associated with ring anomalies (CANAVESE/BELLARDI, 1981).

The s. o. in reptiles and birds are typical examples of membraneous bones and arise by direct intramembranous ossification in the pericorneal mesenchyma (which is derived from the neural crests) (HALL, 1978 and 1981; FYFE/HALL, 1983; JOHNSON, 1986; PINTO/HALL, 1991; HALL/MIYAKE, 1992). In fish they form indirectly by endochondral ossification of scleral cartilage, while in sharks they are permanently cartilaginous (HALL/MIYAKE, 1992). In the chick embryo, for example, the s.o. begin to be visible from the 12th day of incubation, and are all present from the 13-14th day (AMBROSI et al., 1973; COULOMBRE/COULOMBRE, 1973). Their appearance is preceded by an equal number of transitory epithelial formations, the scleral papillae, which, during the 7 to 10 day embryonic period, interact with the skeletogenic mesenchyme allowing scleral ossicles to form at 12 days of incubation (O'RAHILLY, 1960; COULOMBRE et al, 1962; STEWART/MCCALLION, 1975; BEE/THOROGOOD, 1980; FYFE et al, 1988). In fact, it has been observed that a reduced number of papillae results in a reduction in the number of ossicles, both in natural conditions, such as the *scaleless* mutant (PALMOSKY/GOETINCK, 1970) and under experimental conditions (JOHNSON, 1973).

During growth, the s. o. begin overlapping each other along the radial edges, to a greater or lesser extent producing two different overlapping modes: simplex (si. o.) and complex overlappers (c. o.) (COULOMBRE/COULOMBRE, 1973). The regularity of the overlapping is interrupted, depending on species, by one or more overplates - ossicles which overlap those adjoining with both radial edges - which, of course, correspond with one or more underplates - ossicles whose radial edges are both under those adjoining. These plates are identified and conventionally numbered (CURTIS/MILLER, 1938; COULOMBRE et al, 1962) giving positional formulae (p. f.) which, in many species, is very regular (CURTIS/MILLER, 1938; CANAVESE et al, 1986; CANAVESE et al, 1988). The number of s. o., presence or absence of c. o., and p. f. of the overlapping and underlapping plates can be studied in single or paired eye populations. In this way it is possible to obtain information about the general numerical variability and about correspondence rates in the same subject (CANAVESE, 1987).

In particular, in the domestic quail, numerical variability of the s. o. is very high: 32.6 % do not have the average value of 14 but range between 12 and 18; there are c. o. in 50 % of cases. Two overplates occupy positions 1 and 7, whereas the underplates are in positions *6, 10* and *6, 9* in 69 % and 19.5 % of cases. In this species about 14 % of the s. b. r. present morphological aspects which originate with smaller ossicles than normal (CANAVESE et al, 1987).

Considering that the s. b. r. is employed as a practical experimental model, the objective of this research was to improve knowledge of the biomorphological arrangement of the s. b. r. in quails by measuring and comparing the s. o. areas in some s. b. r.

Material and Methods

The present study determined the area of the s. o. in 1850 domestic quail (*Coturnix coturnix japonica*). The samples of quail eyes were collected from healthy adults slaughtered for the retail trade. The medial part of each ocular globe was unwrapped by a paraequatorial excision through the s. b. r. major circumference and the humoral liquids, lens and retinal pigmented epithelium were removed. The samples were fixed in 96° ethanol, cleared in 0.7 % KOH aqueous solution, stained with alizarin-red sulphate, placed into freshly prepared Mall's solution, then stored using pure glycerin (DAWSON, 1926; LORKE, 1977). Under stereomicroscopy, s. b. r. couples suitable for morphometrical analysis (574 rings) and belonging to different classes were selected. These groups contained left and right eyes with corresponding or non corresponding numbers of s. o. (from 13 to 15), and absence (0,0) or presence (≠ 0) of c. o. (Table 1).

Each s. b. r. was assembled on a microscopic slide with conventional orientation (CURTIS/MILLER, 1938; COULOMBRE et al, 1962) (Fig. 1) and drawn after projection with Leitz

Neo-Promar microscopy. From the pictures, the dimensions of the whole s. b. r., single ossicles and overplates area were recorded. Data was loaded onto a computer equipped with a graphics tablet (SCHLUMBERGER 6451) and specialised 3-D Recon software (distributed by the laboratory for High Voltage Electron Microscopy at the University of Colorado) for effecting the measurements. The values obtained were convertered to the actual dimensions after compensating for the magnification factor.

Statistical analysis. The comparisons of the means between the left and right eyes were performed, using the statistical package (SPSS^x, 1985), with a *t-test* of paired data for:

- areas of the s. b. r.
- areas of the single ossicles
- areas of the overlapping sectors
- large areas, encircling by external margin of the s. b. r.
- small areas, encircling by outer margin of the s. b. r.
- ratio of large area/small area.

The areas of the s. b. r., the single ossicles and the overlapping zones were analysed using a factorial design with the following linear model:

$$1.\ y = \mu + \alpha_i + \beta_j + \gamma_k + (\beta\gamma)_{jk} + \varepsilon_{ijk}$$

where: μ = overall mean; α = effect of the ith group (i = 1, 14-14; 2, 13-13; 3, 15-15, 4, 13-14, 5, 14-15); b = effect of the position (j = 1 left; 2 right); γ = effect of complex overlapping (k = 1 absence, 2 presence); $\beta\gamma$ = interaction; ε= residual error (experimental error) (0, σ^2). The ratio between large and small areas was analysed with a factorial design using the following linear model:

$$2.\ y = \mu + \alpha_i + \beta_j + (\alpha\beta)_{ij} + \varepsilon_{ij}$$

where: μ = overall mean; α = effect of the ith group (i = 1, ... 14); β = effect of the position (j = 1 left; 2 right); $\alpha\beta$ = interaction; ε = residual error (experimental error) (0, σ^2).

The comparisons between means of the groups were performed with the Neuman-Keuls test (SNEDECOR/COCHRAN, 1980) for both the models.

Results

The morphometric data provided true measurements of the various scleral bone formations. The s. b. r. class 14-14, which included 48 % of the pairs of rings, was assumed to be very representative; the average s. b. r. area was 33,8 mm^2, that of the ossicles 2,750 - 4,175 mm^2 and that of the overlapping zones was 0,762 - 1,347 mm^2 in 3 - 4 months old subjects of both sexes. Other values represent the sum of the areas of the non-overlapping portions of ossicles (18,685 mm^2), the area which overlaps (15,115 mm^2), and, finally the area of all the ossicles which form the ring (48,915 mm^2). The area of the s. b. r. was obtained by subtracting the small from the large area (see material and methods and Table 1) and the ratio between these two areas gave indications for the morphometric evaluation of the different s. b. r. classes (Table 2). The succession of the ossicles in the rings occurred in the following manner: on every radial edge of overplates 1 and 7, ossicles were arranged so that their areas decreased regularly until meeting ossicle 4 in the medio-nasal sector, and with ossicles 11 and 12 in the lateral sector (Fig. 2). In fact, it was in this specific sector that ossicle 4 or, alternatively, ossicles 11 and 12 were always smallest. However, this sequence was not similarly ordered in the other classes different from 14-14. Ossicle 7 was always the largest of all and number 4 the smallest in all classes. In particular, ossicle 7 attained the highest values in classes 13-13 and 13-14, while ossicle 4 attained minimum values in the modal class.

The statistical analysis of the total areas of the s. b. r. and the all ossicles, performed after grouping the classes by 5, not taking account of the lack of correspondence of overlapping and of the left and right eye, highlighted how the 14/14 class differed significantly ($P \leq 0.01$) from the others. The areas of ossicles 1, 5 and 13 were significantly different ($P \leq 0.01$) from the others in class 15-15, which was seen to be the most variable, followed by 14-14 where the ossicles 8 and 9 differed from the remaining 4 classes. The same ana-

Table 1. Average areas in all classes of left and right s. b. r.

Classes	Cases	Area s. b. r. (mm^2) left ± s. d.	Area s. b. r. (mm^2) right ± s. d.
14-14 (0,0)	60	32.73** ± 2.11	33.08** ± 2.21
14-14 (≠ 0)	30	34.87 ± 2.88	35.22 ± 3.15
13-13 (0,0)	13	34.05* ± 3.55	35.19* ± 2.67
13-13 (≠ 0)	8	35.82 ± 3.11	35.16 ± 4.10
15-15 (0,0)	25	35.82 ± 3.11	35.51 ± 2.44
15-15 (≠ 0)	28	36.19* ± 2.70	35.20* ± 2.29
13-14 (0,0)	21	35.14 ± 4.54	35.38 ± 3.99
13-14 (≠ 0)	14	35.83 ± 3.19	34.94 ± 3.65
14-13 (0,0)	16	35.49 ± 2.50	35.68 ± 2.29
14-13 (≠ 0)	12	37.90 ± 2.84	37.22 ± 3.10
14-15 (0,0)	17	34.86 ± 2.60	34.82 ± 2.80
14-15 (≠ 0)	9	35.97 ± 3.94	36.21 ± 4.39
15-14 (0,0)	13	35.79* ± 2.97	37.26* ± 2.11
15-14 (≠ 0)	21	37.44 ± 2.78	37.08 ± 3.67

** = $P \leq 0.01$
* = $P \leq 0.05$

lysis of the areas of overlap partly repeated these results, with the 1 and 5 overlaps in class 15-15 and 9 in 14-14. If the total and single areas of all the classes together were analysed, this time as a function of correspondence and non-correspondence of overlap type and left or right eyes, it was observed that significant differences appeared only for the ossicles areas or the overlapping parts (and not for the total s. b. r. areas) and there was a

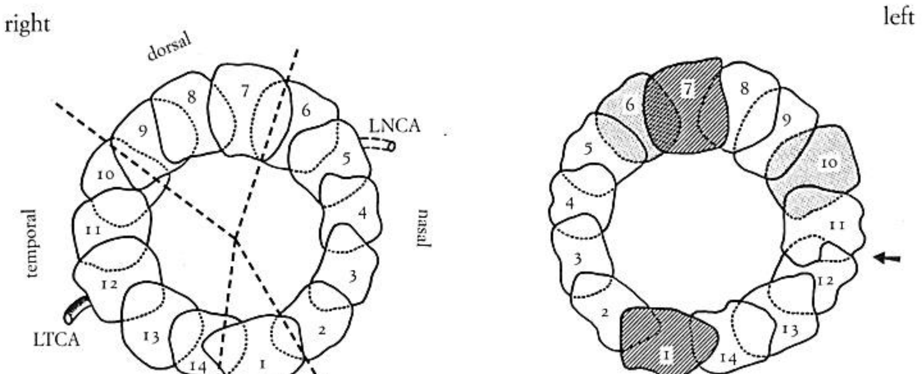
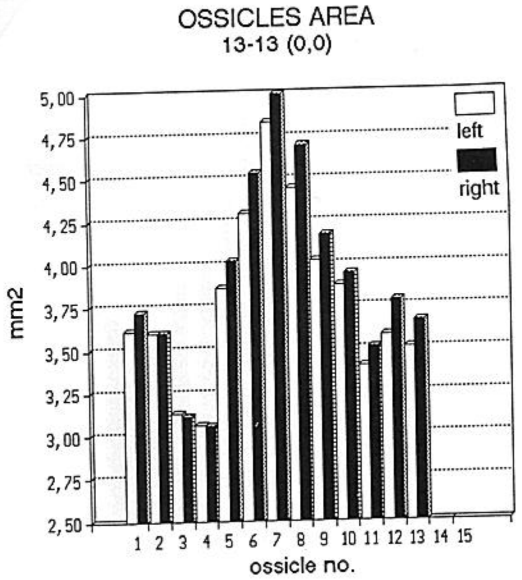


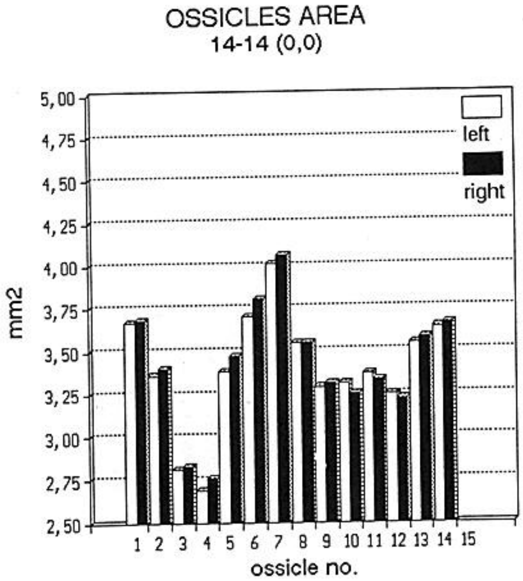
Figure 1. The s. b. r. in the left and right eyes of quail made by tile overlaps of the s. o. along their radial margins (frontal view). Conventional numbering of the ossicles according to their spatial disposition and to points of reference (CF= coroid fissure, LTCA= long temporal cilial artery, LNCA= long nasal cilial artery) in accordance with COULOMBRE et al., (1962) and AMBROSI et al., (1973). Example of complex overlap between radial margins of 11 and 12 ossicles (arrow).

Table 2. Average large and small areas in all classes of left and right s. b. r.

Classes	Cases	Large Area (mm²) left ± s. d.	c. v. %	right ± s. d.	c. v. %	Small Area (mm²) left ± s. d.	c. v. %	right ± s. d.	c. v. %
14-14 (0,0)	60	47.18** ± 2.46	5.21	47.46** ± 2.50	5.27	14.45 ± 0.95	6.55	14.49 ± 1.01	6.98
14-14 (≠ 0)	30	49.68 ± 3.64	7.33	49.49 ± 5.55	11.21	14.81 ± 1.22	8.24	14.81 ± 1.26	8.51
13-13 (0,0)	13	48.78* ± 4.35	8.92	50.51* ± 3.61	7.15	14.68 ± 0.93	6.34	15.16 ± 1.11	7.35
13-13 (≠ 0)	8	51.54 ± 3.64	7.06	50.45 ± 4.46	8.84	15.70 ± 1.30	8.28	15.29 ± 1.15	7.52
15-15 (0,0)	25	50.29 ± 3.05	6.06	50.64 ± 3.34	6.60	15.17 ± 0.95	6.28	15.13 ± 1.12	7.40
15-15 (≠ 0)	28	50.98* ± 3.65	7.16	49.66* ± 3.09	6.22	14.80* ± 1.31	8.85	14.46* ± 1.26	8.71
13-14 (0,0)	21	49.83 ± 4.80	9.63	50.22 ± 4.80	9.56	14.82 ± 1.27	8.57	15.03 ± 1.40	9.31
13-14 (≠ 0)	14	50.48 ± 3.87	7.67	49.10 ± 4.47	5.97	14.49 ± 1.03	7.11	14.32 ± 1.35	9.43
14-13 (0,0)	16	50.92 ± 3.35	6.58	50.75 ± 3.03	5.97	14.99 ± 1.20	8.01	14.83 ± 1.18	7.96
14-13 (≠ 0)	12	52.99 ± 3.36	6.34	51.89 ± 3.56	6.86	14.95 ± 1.20	8.03	14.71 ± 1.08	7.34
14-15 (0,0)	17	49.64 ± 2.95	5.94	49.85 ± 3.20	6.42	14.84 ± 1.05	7.08	15.00 ± 1.13	7.53
14-15 (≠ 0)	9	51.62 ± 4.26	8.25	51.67 ± 4.88	9.44	15.71 ± 1.59	10.12	15.45 ± 1.34	8.67
15-14 (0,0)	13	50.35** ± 3.47	6.89	52.29** ± 2.20	4.21	14.56* ± 1.29	8.86	15.03* ± 1.12	7.45
15-14 (≠ 0)	21	52.96 ± 3.44	6.50	52.50 ± 4.54	8.65	15.52 ± 1.41	9.09	15.42 ± 1.25	8.11

** = $P \leq 0.01$
* = $P \leq 0.05$





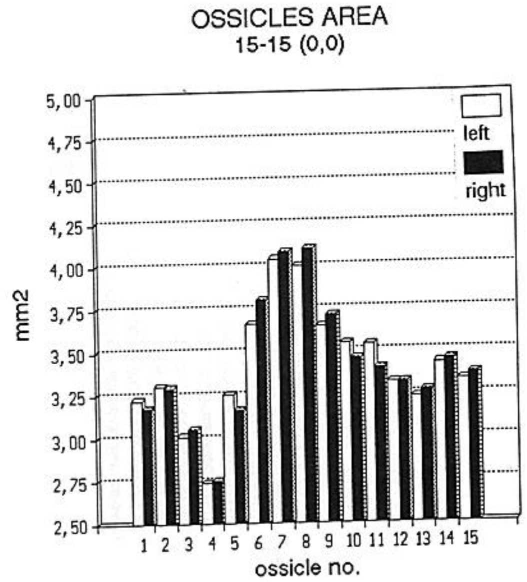


Figure 2. Histograms of the average s. b. r. areas in the left and right eyes of quail for classes with equal (0,0) numbers of ossicles. The compensating function of ossicles in s. b. r. with different number of ossicles is evident.

general tendency to diversification, particularly in the dorsal and temporal sectors, that is from ossicles 8 to 13. Figures 2 and 3 show the analytical results and it is immediately obvious where the differences are most significant and how these differences were more numerous in the classes of rings with different numbers of ossicles, in apparent contrast with the absence of significant differences between left and right s. b. r. with different number of ossicles.

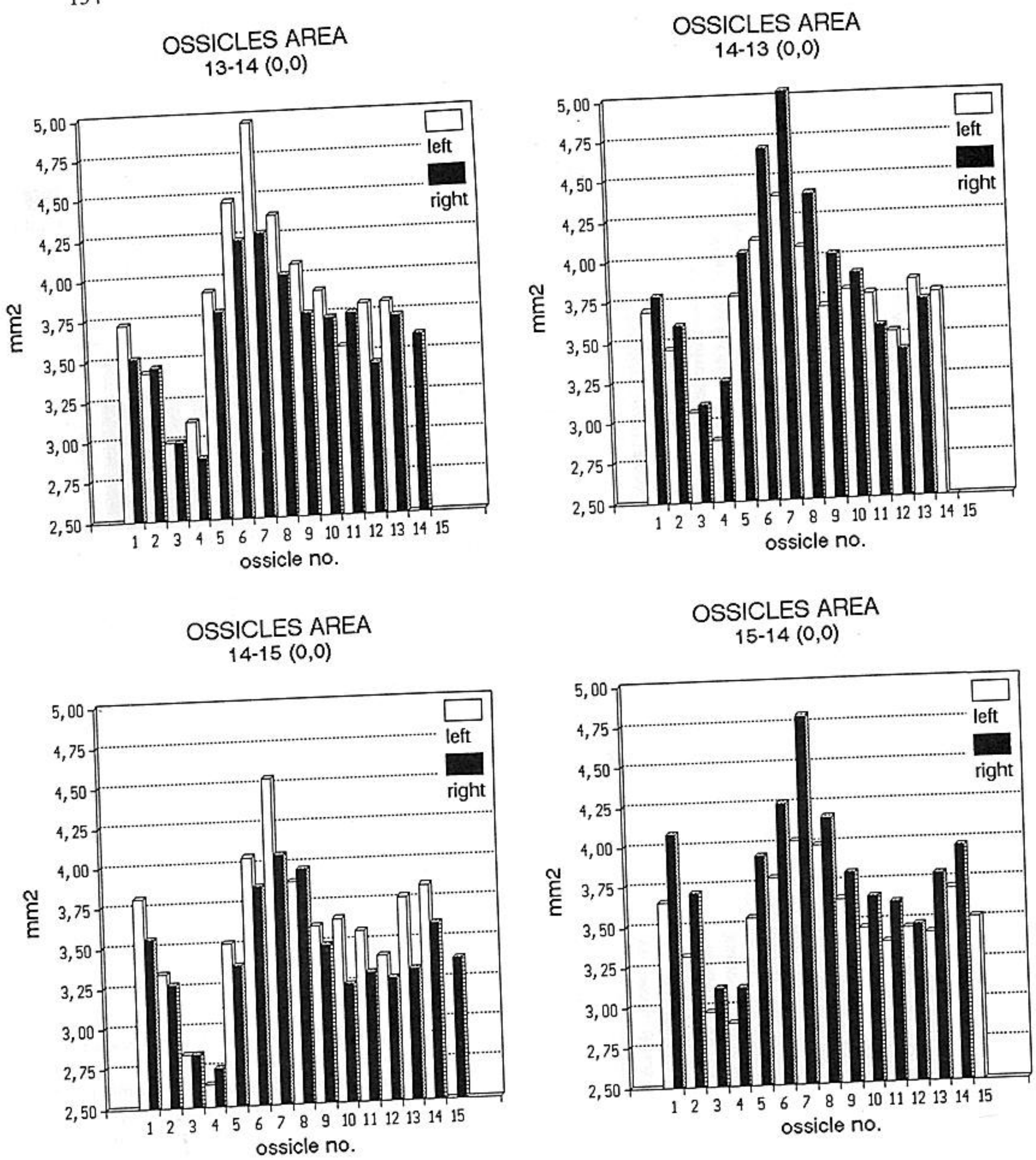


Figure 3. Histograms of the s. b. r. average areas in the left and right eyes of quail for classes with different (≠ 0) number of ossicles. The compensative function of ossicles in pairs of s. b. r. with different numbers of ossicles is evident.

Discussion

The s. b. r. class 14-14 was the most representative and this was the reason it was chosen as the model to compare and analyse the s. b. r. biomorphometric data from the other classes. The diversity in the other classes, with respect to the model, was believed to be due to the unusual situation caused by the number of ossicles differing from the modal value of 14. In fact the coefficients of variability of the model class relating to the large and small areas (as defined above) were lower than the corresponding values in the other classes. This indicated a more homogeneous value distribution in the model class.

However, ossicles 1 and 7, which were observed to have the maximum individual variations in all classes, had the minimum variation in the model class, which was quite similar to other ossicles. This could indicate that the appearance of the complete trim of the s. b. r. 14-14 (0,0) occured in a very coherent and regular manner within the limits of the established architectural scheme. A factor of change, which would be the different number of ossicles, gave autocompensating responses which were expressed by greater or smaller area variations of the s. o. trying to maintain as far as possible the general trim of the s. b. r. as a coherent function of the regular geometry of the eyeball.

Overplates 1 and 7, from which, as observed, became the regular arrangement in superficial extension of the other ossicles, constitute "fitting-units" (the parts which are included) between two distinct portions of the s. b. r. - the medio-nasal and the lateral; given the higher degree of variability demonstrated, these support a greater autocompensating workload. However, at the same time, the considerable variability in sector 8-13 indicates that this zone has greater lability which more frequently favours the appearance of s. b. r. anomalies, as it is demostrate by teratological observations. The general effort of the ossicle to occupy more developmental surface in the space liberated by the absence of the correct ossicle is well documented from the major and minor forms of microophthalmia in 18-21 day old embryos (CANAVESE/BELLARDI, 1981). In these cases, the few ossicles present increase their extent to complete the ring. In general, biomorphometric data highlights how the numerical variation of the ossicles is accompanied by a simultaneous variation of their surfaces. In other words, in s. o. formation dificiencies, an unaltered space remains available, so other factors act in a new situation to resolve the requirements which the available space produced. The small number of significant differences between the s. b. r. areas belonging to classes with differing numbers of ossicles in two eyes is not contradictory, and can thus have an explanation in that the single ossicles could be arranged in their various formations to suit the overall needs of the s. b. r.

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