EGGSHELL STRUCTURE OF LIZARDS OF TWO SUB-FAMILIES OF THE GEKKONIDAE

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ABSTRACT

The aim of this study was to describe and compare shell structure of parchment-shelled and rigid-shelled eggs of gekkonid lizards. Scanning electron microscopy was used to describe eggshells of two species of gecko of the Eublepharinae and four species of Gekkoninae. Eggshell from a lacertid lizard was also described. The crystalline nature of calcium carbonate deposits on eggshells was studied; an aragonitic chelonian eggshell was used as a control. Eublepharine eggshells consist mainly of a fibrous membrane with an external layer of calcite. They resembled lacertid and other lizard shells described previously. Gekkonine eggshells have thin, fibrous shell membranes overlain by a relatively thick layer of calcite and resemble other hard-shelled gecko eggs reported elsewhere. A layer of fibres in a matrix coats the external surface of the calcite layer of gekkonine shells. Shell structure is considered important in determining water loss from gecko eggs, nest location and embryonic development. A more detailed examination of the reproductive biology of gekkonid eggs could help in assessing the role of the shell in reptilian development.

INTRODUCTION

In recent years interest in the structure of reptilian eggshells has increased for two reasons. First, the shell has been used as a taxonomic character (Bustard, 1968) that may also shed some light on the evolutionary relationships between reptiles and birds (Erben, 1970; Packard and Packard, 1980). Second, studies on gas exchange of reptilian eggs have led to research into the role of the shell during incubation (Packard, Taigen, Packard and Shuman, 1979; Packard, Packard and Boardman, 1982a; Packard, Burns, Hirsch and Packard, 1982b).

Oviparous lizards and snakes (Squamata) typically lay soft, parchment-shelled eggs (Halliday and Adler, 1986). Gekkonid lizards are exceptional in this respect; lizards in two sub-families of the Gekkonidae (Gekkoninae and Sphaerodactylinae) lay rigid-shelled eggs (Bustard, 1968), whereas in the other sub-families (Eublepharinae and Diplodactylinae) soft-shelled eggs are laid (Kluge, 1967; Bustard, 1968; Werner, 1972). Published descriptions of eggshell structures have been limited to the Gekkoninae. Light microscopy was used to study *Lepidodactylus lugubris* eggshells (Schmidt, 1943) and scanning electron microscopy was used to examine the shell structure of *Tarentola mauritanica* (Krampitz, Erben and Kriesten, 1972; Erben and Newesley, 1972) and *Hemidactylus turcicus* (Packard, *et al.*, 1982a). This study was therefore undertaken to provide information on the eggshell structure of representatives of two sub-families of gekkonid lizards, the previously unstudied Eublepharinae with soft shells and the Gekkoninae with hard shells. It was hoped that this would provide information on the functional and taxonomic significance of shell structure in the Gekkonidae.

MATERIALS AND METHODS

Eggshells from six species of gecko were examined. For comparison, a single representative of the family Lacertidae and a single species of the Testudinidae were also studied. The details of the sources of these eggs, the species represented and the number of specimens available are shown in Table 1.

The particular crystal morph of any $CaCO_3$ present in the shell was identified by using Meigen's reaction (Deer, Howie and Zussman, 1966). This involves boiling shell fragments for two minutes in 0.5 mole.l^{-1} cobalt nitrate ($Co(NO_3)_2$). In this reagent aragonite stains a pink-purple colour whereas calcite remains colourless.

Eggshell structure was studied by immersing fragments in liquid nitrogen for a few seconds so that they could easily be fractured. Two pieces of each shell were mounted on brass stubs using a conductive silver paint and were sputter-coated *in vacuo* with gold (Edwards S150B). The shells were then examined using a JEOL T300 scanning electron microscope at an operating voltage of 25 or 30 kilovolts. The effect of acid treatment to remove any calcium carbonate was assessed by etching fragments of *Eublepharis* and *Chondrodactylus* shell in 1 mole.l^{-1} hydrochloric acid for thirty seconds prior to examination.

Family Sub-family Species	N	Embryo	Source of eggs
Gekkonidae			
Eublepharinae			
Eublepharis macularius	12	Hatched	The Zoological Society of London, England.
Hemitheconyx caudicinctus	8	Hatched	The Zoological Society of London, England.
Gekkoninae			
Chondrodactylus angulifer	6	Hatched	The Zoological Society of London, England.
Gehyra mutilata	1	Dead	The Jersey Wildlife Preservation Trust, Channel Islands, U.K.
Phelsuma agalegae	1	?	British Museum (Natural History), London. (Specimen Number BMNH 1975. 1165).
Phelsuma guentheri	1	Dead	The Jersey Wildlife Preservation Trust, Channel Islands, U.K.
Lacertidae			
Lacerta lepida	4	Hatched	The Zoological Society of London, England.
Chelonia			
Testudinidae			
Geochelone radiata	1	Infert.	The Jersey Wildlife Preservation Trust, Channel Islands, U.K.

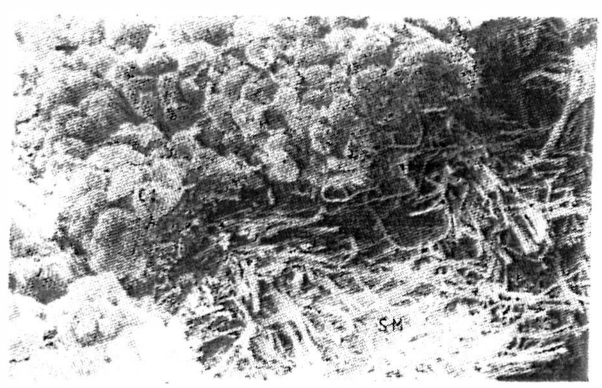
N = Number of specimens; Hatched = Embryo hatched successfully; Dead = Embryo dead in shell at late stage of incubation; Infert. = Infertile egg.

TABLE 1: Details of the numbers and sources of lizard and tortoise eggshells used for structural examination.

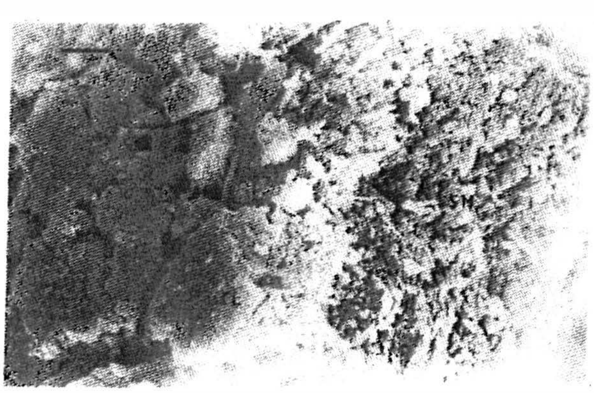
RESULTS

Staining eggshells with cobalt nitrate showed that all the shells in the study were calcitic except that of the tortoise, *Geochelone radiata*, which was aragonitic thus serving as a control for the method.

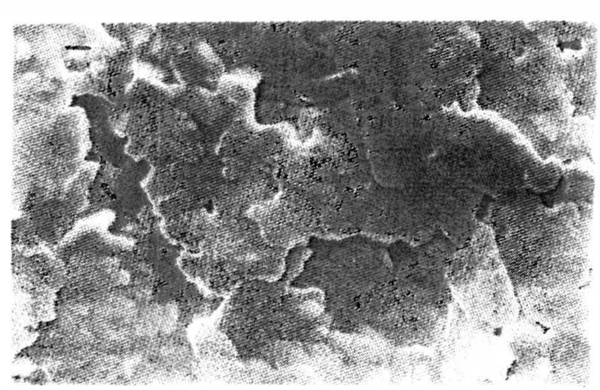
The structure of eggshells from eublepharine geckos (*Eublepharis macularius* and *Hemitheconyx caudicinatus*) were similar and resembled that of *Lacerta lepida.* The bulk of the shell (90 per cent of the total thickness) consists of fibres (Plates 1a and 1b) with a thin layer of calcite, less than $10 \mu m$ thick, on the shell's outer surface. The external appearance of the calcium carbonate differs among the three species; the calcite is nodular in *Eublepharis* and *Lacerta* shells (Plate 1a and 1b) whereas plaques of calcite are seen on *Hemitheconyx* eggshells (Plate 1c). Hydrochloric acid removed the mineralised layer from the surface of *Eublepharis* shells exposing the upper surface of the fibrous layer (Plate 1d).



1b



1a



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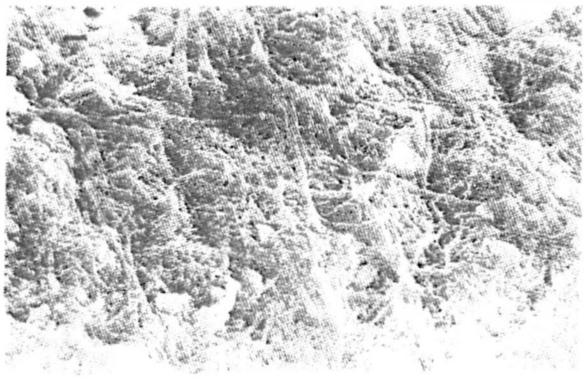

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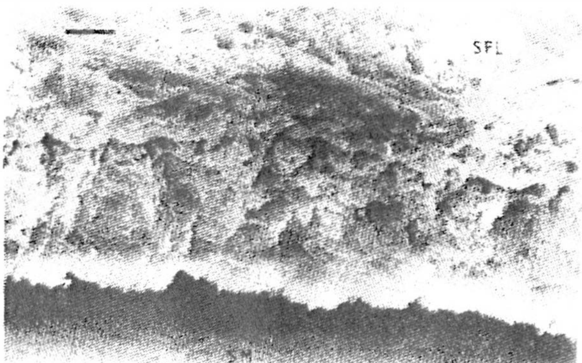
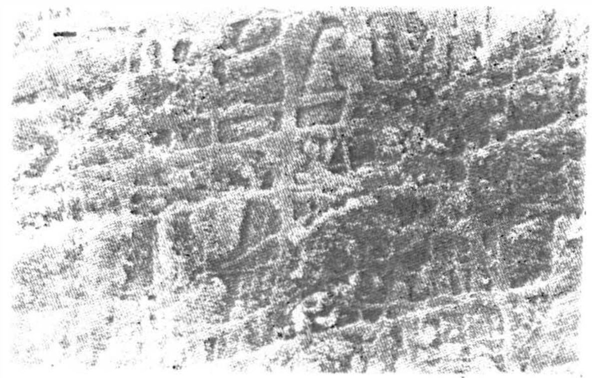


1e

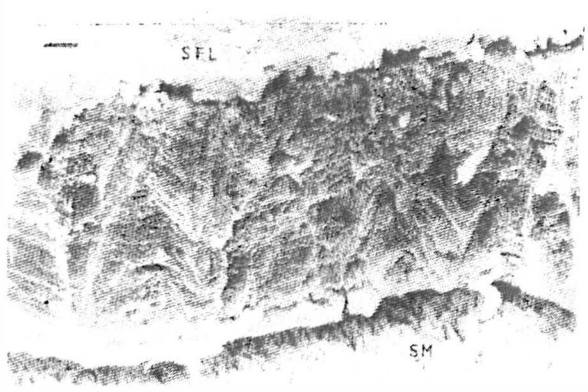
Plate 1. Scanning electron micrographs of eggshells from geckos of the Eublepharinae, a gecko of the Gekkoninae and a lizard of the Lacertidae. (a) *Eublepharis macularius*. Surface of a radial fracture showing the fibrous membrane (SM) which forms the bulk of the shell. The outer shell surface is covered by a thin layer of calcite (CL). (b) *Lacerta lepida*. Surface of a radial fracture showing the fibrous shell membrane (SM) and the thin layer of calcite (CL) covering the outer shell surface. (c) *Hemitheconyx caudicinatus*. Outer shell surface shwoing calcite deposits. (d) *Eublepharis macularius*. Outer shell surface after etching in 1 mole.l^{-1} hydrochloric acid for 30 seconds. The fibres of the shell membrane are exposed. (e) *Chondrodactylus angulifer*. Radial fracture of the eggshell showing the fibrous shell membrane (SM) which is detached from the calcite layer (CL). The arrows indicate holes in the top of the calcite layer. There is an external covering of fibres in a spherulitic matrix (SFL). Scale bars = 50μm.

The eggshell structure of the four gekkonine lizards, *Chondrodactylus angulifer*, *Phelsuma agalegae*, *P. guentheri* and *Gehyra mutilata* are shown in Plates 1e and 2. Each shell consists of a fibrous shell membrane (10 per cent of total shell thickness) covered by an outer, continuous layer of calcite. A layer of fibres in an unidentified matrix is found external to the calcite layer. The continuity of the calcite layer of *Chondrodactylus angulifer* shells is broken by series of holes near the external surface of the shell (Plate 1e). The external surface of *Chondrodactylus* eggshell is covered by numerous fibres embedded in a matrix (Plate 2a). Etching the shell with hydrochloric acid simply removed a superficial layer of the matrix exposing the surface of the fibres (Plate 2b). The surface layer of *Phelsuma agalegae* shell consists largely of the matrix (Plate 2c). Fibres appear to be more numerous in *Phelsuma guentheri* shells and can be seen protuding from the surface layer in Plate 2d. The surface layer of *Gehyra mutilata* shell is not illustrated but it has few fibres and consists mainly of matrix. The calcite layer of *Gehyra* has numerous crystals covering its internal surface (Plate 2f).



2a

2b



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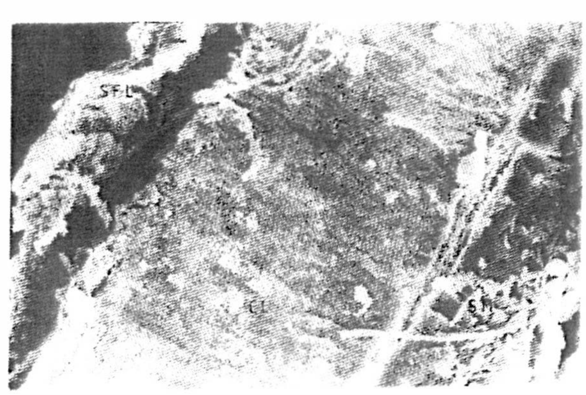
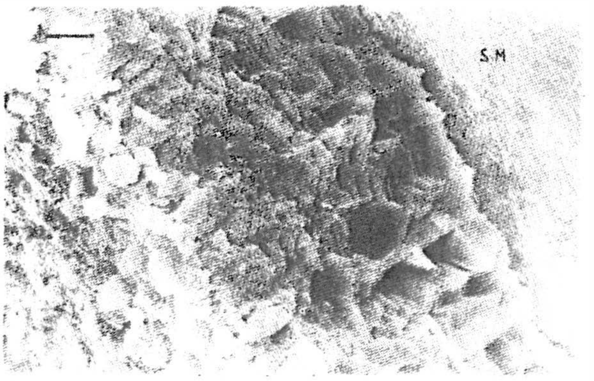


2d



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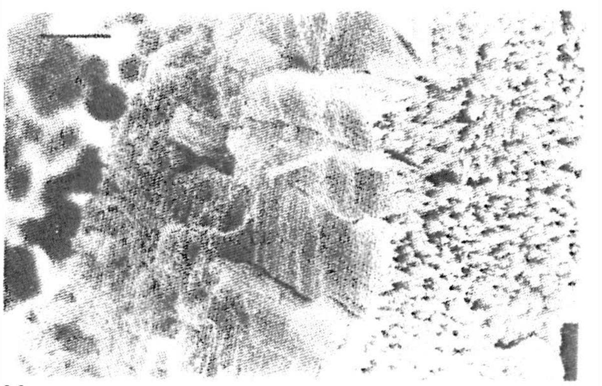

2f

Plate 2. Scanning electron micrographs of gekkonine eggshells. (a) *Chondrodactylus angulifer*. The surface of a shell showing fibres embedded in a matrix. (b) *Chondrodactylus angulifer*. Outer shell surface after etching in 1 mole.l^{-1} hydrochloric acid for 30 seconds. (c) *Phelsuma agalegae*. Radial shell fracture showing the upper surface of the shell membrane (SM). This is covered by a layer of calcite (CL) which is covered in turn by a surface fibrous layer (SFL). (d) *Phelsuma guentheri*. Radial eggshell fracture showing the fibrous shell membrane (SM) beneath the calcite layer (CL). The arrows show the fibres that protrude from the surface fibrous layer (SFL). (e) *Gehyra mutilata*. Radial fracture of the shell showing the external surface of the shell membrane (SM). The surface fibrous layer was lost in this preparation exposing the upper surface of the calcite layer (CL) which is covered with small spheres (arrowed). (f) *Gehyra mutilata*. A radial shell fracture showing the under surface of the calcite layer (CL). Numerous crystals can be seen (arrowed) on the under surface of the calcite layer. Scale bars = 50μm.

DISCUSSION

The presence of two types of eggshell in one squamate family is unique. The parchment-like eggshells of *Eublepharis* and *Hemitheconyx* consist of a fibrous shell membrane with an outer covering of calcite. This study confirms the similarities in the structure of shells of eublepharine geckos, lacertid eggshells (present study), several iguanid eggshells (*Anolis sagrei, A. limifrons, A. carolinensis, Callisaurus draconoides, Dipsosaurus dorsalis* and *Iguana iguana*; Kriesten, 1975; Sexton, Veith and Phillips, 1979; Packard and Packard, 1980; Packard *et al.*, 1982a; Packard *et al.*, 1982b), and teiid lizards (*Cnemidophorus sexlineatus*; Trauth and Fagerberg, 1984) as these shells are also fibrous membranes overlaid by a thin layer of calcite. Parchment-shelled eggs only occur in about a quarter of the total number of genera of geckos (Kluge, 1967; Bustard, 1968) and contrary to the impression given by some reports (Packard *et al.*, 1982b) calcareous, rigid-shelled eggs predominate within the Gekkonidae.

The structures for gekkonine eggshells reported in the present study (Schmidt, 1943; Krampitz *et al.*, 1972; Packard *et al.*, 1982a) are similar to those reported previously. The gekkonine shell membrane forms a thin fibrous layer outside which the calcite layer forms the bulk of the shell making it comparable to the palisade layer of the avian shell (Erben and Newesley, 1972; Krampitz *et al.*, 1972). The surface fibrous layer is unique to gekkonine eggshells (Packard *et al.*, 1982a; present study). The appearance of the outer surface of *Chondrodactylus* eggs was found to be almost identical to that reported for *Hemidactylus* (Packard *et al.*, 1982a). Similar layers of fibres in a matrix were found for all of the other species in the present study though the numbers of fibres was variable. The role of this fibrous layer is not clear. Other reports of deposits on reptilian shells are limited to a single description of a thin amorphous layer on *Chelonia mydas* eggshells (Baird and Solomon, 1979). In contrast, thin, amorphous cuticular coverings are common on avian eggshells (Tullett, 1984).

Poorly calcified eggshells are generally thought to be highly permeable. Squamate eggshells, including *Eublepharis*, have water vapour conductance values 100 times higher than equivalent avian eggs (Ackerman, Dmi'el and Ar, 1985). Pliable-shelled *Chelydra* eggs had shells that were 55 times more permeable than avian eggs of comparable size whereas rigid-shelled turtle (*Trionyx*) and *Alligator* eggs have shells five times more permeable (Packard *et al.*, 1979). In contrast, rigid-shelled eggs of sphaerodactyline geckos have rates of water loss half that of predicted rates (Dunson, 1982).

Reptilian nest sites are usually characterised by their high humidity and are often found in soil or compost (Packard and Packard, 1980). Gekkonine nest sites are unusual in that the eggs are stuck to walls and crevices (Arnold and Burton, 1978) and are exposed to low humidities during incubation. Water retention by the egg would be advantageous in such conditions and calcareous gecko eggs appear to have developed relatively impermeable shells. This may be associated with the external fibrous layer which may act in a similar way to the avian cuticle which reduces water loss from the egg (Christensen and Bagley, 1984; Peebles and Brake, 1986; Deeming, 1987).

The rigid-shelled gecko eggs are an interesting development in vertebrate eggshell structure. The eggshell can be used as a good taxonomic character for classifying different types of gekkonid lizards (Bustard, 1968). The same is not true of chelonid eggshells as structure can vary within the class and particular families (Ewert, 1979). The importance of the eggshell in gecko biology extends well beyond its taxonomic use. The similarities and differences between the rigid eggshells of chelonians, crocodilians, gekkonid lizards, dinosaurs and birds (Erben, 1970;

Erben and Newesley, 1972) may be important in determining the patterns of eggshell evolution. Further investigations may allow us to test the significance of the use of shell structure in evolutionary analyses of the major groups of reptiles (Packard and Packard, 1980).

The existence of two shell types in a group of closely related lizards provides a useful opportunity to investigate the functional significance of the eggshell. A detailed comparative study of the reproductive biology of eublepharine or diplodactyline and gekkonine or shaerodactyline geckos could provide information that would allow the relationships between eggshell structure and various aspects of incubation of the egg to be understood. In particular, initial egg composition has been shown to be related to the degree of calcification of the eggshell (Tracy and Snell, 1985) and it could be predicted that rigid-shelled eggs have a higher initial water content. Nest location and incubation conditions will differ according to shell permeability and shell structure may have some influence on the development of the embryo. A study of gas exchange across rigid and soft shells could provide useful information concerning the role of shell permeability in the physiology of the embryo.

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REFERENCES

Ackerman, R. A., Dmi'el, R. and Ar, A. (1986). Energy and water vapour exchange by parchment-shelled reptile eggs. *Physiol. Zool.*, **58**, 129-137.

Arnold, E. N. and Burton, J. A. (1978). *A Field Guide to the Reptiles and Amphibians of Britain and Europe.* Collins, London.

Baird, T. and Solomon, S. E. (1979). Calcite and aragonite in the egg shell of *Chelonia mydas* L. *J. Exp. Mar. Biol. Ecol.*, **36**, 295-303.

Bustard, H. R. (1968). The egg-shell of gekkonid lizards: A taxonomic adjunct. *Copeia*, **1968**, 162-164.

Christensen, V. L., and Bagley, R. A. (1984). Vital gas exchange and hatchability of turkey eggs at high altitude. *Poult. Sci.*, **63**, 1350-1356.

Deeming, D. C. (1987). The effect of cuticle removal on the water vapour conductance of eggshells of several species of domestic bird. *Br. Poult. Sci.*, **28**, 231-237.

Deer, W. A., Howie, R. A. and Zussman, J. (1966). *An Introduction to the Rock-forming Minerals.* Longman, London.

Dunson, W. A. (1982). Low water vapour conductance of hard-shelled eggs of the gecko lizards *Hemidactylus* and *Lepidodactylus*. *J. Exp. Zool.*, **219**, 377-379.

Erben, H. K. (1970). Ultrastrukturen und mineralisation rezenter und fossiler eischalen bei vogeln und reptilien. *Biomineralisation*, **1**, 1-66.

Erben, H. K. and Newesley, H. (1972). Kristalline bausteine und mineralbestand von kalkigen eischalen. *Biomineralisation*, **6**, 32-48.

Ewert, M. A. (1979). The embryo and its egg: development and natural history. In: *'Turtles: Perspectives and Research'*. Eds. Harless, M. and Morlock, H. John Wiley & Sons, New York.

Halliday, T. R. and Adler, K. (1986). *The Encyclopaedia of Reptiles and Amphibians.* George Allen and Unwin, London.

Kluge, A. G. (1967). Higher taxonomic categories of gekkonid lizards and their evolution. *Bull. Amer. Mus. Nat. Hist.*, **135**, 1-59.

Krampitz, G., Erben, H. K. and Kriesten, K. (1972). Uber aminosaurenzusammensetzung und struktur von eischalen. *Biomineralisation*, **4**, 87-99.

Kriesten, K. (1975). Untersuchungen uber ulrastruktur. proteinmuster und aminosaurenzusammensetzung der eischalen von *Testudo elephantopus, Caiman crocodilus* and *Iguana iguana*. *Zool. Jb. Anat.*, **94**, 101-122.

Packard, G. C., Taigen, T. L., Packard, M. J. and Shuman, R. D. (1979). Water-vapour conductance of testudinian and crocodilian eggs (Class Reptilia). *Resp. Physiol.*, **38**, 1-10.

Packard, M. J. and Packard, G. C. (1980). Evolution of the cleidoic egg among reptilian antecedents of birds. *Amer. Zool.*, **20**, 351-362.

Packard, M. J., Packard, G. C. and Boardman, T. J. (1982a). Structure of eggshells and water relations of reptilian eggs. *Herpetologica*, **38**, 136-155.

Packard, M. J., Burns, L. K., Hirsch, K. F. and Packard, G. C. (1982b). Structure of eggs of *Callisaurus draconoides* (Reptilia, Squamata, Iguanidae). *Zool. J. Linn. Soc.*, **75**, 297-316.

Peebles, E. D. and Brake, J. (1986). The role of the cuticle in water vapour conductance by the eggshell of broiler breeders. *Poult. Sci.*, **65**, 1034-1039.

Schmidt, W. J. (1943). Uber den bau der kalkigen eischale von *Lepidodactylus lugubris* D. & B. (Gekkonidae). *Zool. Anz.*, **142**, 58-61.

Sexton, O. J., Veith, G. M. and Phillips, D. M. (1979). Ultrastructure of the eggshell of two species of anoline lizards. *J. Exp. Zool.*, **207**, 227-236.

Tracy, C. R. and Snell, H. R. (1985). Interrelations among water and energy relations of reptilian eggs, embryos and hatchlings. *Amer. Zool.*, **25**, 999-1008.

Trauth, S. E. and Fagerberg, W. R. (1984). Ultrastructure and stereology of the eggshell of *Cnemidophorus sexlineatus* (Lacertilia, Teiidae). *Copeia*, **1984**, 826-832.

Tullett, S. G. (1984). The porosity of avian eggshells. *Comp. Bioch. Physiol.*, **78A**, 5-13.

Werner, Y. L. (1972). Observations on eggs of Eublepharid lizards, with comments on the evolution of the Gekkonoidea. *Zool. Meded.*, **47**, 211-224.