
Beckett, M., Baggott, G. K. and Graeme-Cook, K. (2003) Bacterial degradation of the eggshell cuticle of the mandarin duck (Aix galericulata). *Avian and Poultry Biology Reviews* 14 pp 196-197

Beckett, M., Baggott, G.K. & Graeme-Cook, K.
University of Hertfordshire & Birkbeck, University of London

Bacterial degradation of eggshell cuticle of the Mandarin Duck (*Aix galericulata*)

The Mandarin Duck is a cavity nester in the UK (Davis & Baggott, 1989). Females incubate clutches of up to 30 eggs for about 33 days. During natural incubation G_{H2O} can increase by about 70% in the first 7 days of incubation (Baggott & Graeme-Cook, 1997). The initial G_{H2O} is low, for egg weight, due to cuticle completely covering pore apertures. During the first week of natural incubation counts of surface bacteria capable of culture at incubation temperature decrease but the proportion of *Bacillus licheniformis* increases (Baggott & Graeme-Cook, 2002). As this species has the capability to digest duck cuticle (Baggott & Graeme-Cook, *op. cit*.), it is hypothesised that it may be responsible for the increase in G_{H2O} in the first week of incubation. The objective was to identify what cuticle proteins, if any, were capable of degradation by *B. licheniformis* cultured from the Mandarin eggshell surface.

Using SDS-polyacrylamide gel electrophoresis (PAGE), one major protein was identified in the cuticle of unincubated eggs of Mandarin Duck that was not detectable in the shell matrix. This protein had a molecular weight of 30 kDa as estimated using molecular weight standards and did not react with Schiff's reagent indicating that it was not a glycoprotein. Eight additional protein bands, found in the cuticular sample were also present in the shell matrix fraction. These had approximate molecular weights of
>80 kDa (x2), 79kDa, 71kDa, 60kDa, 40kDa, 15kDa,12kDa and all stained with Schiff's reagent suggesting that they contain carbohydrate moieties. In the Pekin duck, matrix proteins of 15k (lysozyme), 17k, 32k, 66k and 78kDa (ovotransferrin) have been identified (Panheleux, *et al.,* 1999); as in this species, a 45kDa matrix protein (ovalbumin) was absent from mandarin profiles. Analysis of proteins in the cuticle of commercial duck consumption eggs also showed the presence of a protein exclusive to the cuticle but in this case it had an apparent molecular weight of 40kDa.

SDS-PAGE electrophoresis of cuticular and shell fractions of Mandarin duck eggs obtained from nests after natural incubation showed the loss of all detectable cuticle proteins with the exception of the 30 kDa protein. *In vitro* at natural incubation temperatures, shell samples incubated with a strain of *Bacillus licheniformis,* originally isolated from the surface of incubated eggs, degraded all proteins of the cuticle.

In summary, only one protein exclusive to the cuticle fraction was identified. Other proteins found on the surface of the egg were also located within the shell matrix. Previously, the only protein reported to be localised in the cuticle, that of the hen, is a 32kDa protein, ovocalyxin (Gautron *et al.,* 2001). Natural incubation of eggs, and incubation *in vitro,* with a species of bacteria, *Bacillus licheniformis*, led to the loss of proteins within the cuticular layer. This suggests to us that the bacteria may be responsible for increasing the G_{H2O} off the egg during incubation by removing the cuticle layer over the pores.

Baggott, G. K., & Graeme-Cook, K. (1997). Variable shell conductance during natural incubation. *Poultry and Avian Biology Reviews*, **8,** 158.

Baggott, G.K. & Graeme-Cook, K. (2002). Microbiology of natural incubation, in *Avian Incubation, Behaviour, Environment and Evolution*, (ed. Deeming, D.C.) pp.179-191. Oxford, OUP.

Davies, A. K. and Baggott, G. K. (1989). Egg-laying, incubation and intraspecific nest parasitism by the Mandarin Duck *Aix galericulata*. *Bird Study*, **36,** 115-22.

Gautron J., Hincke, M.T., Mann, K., Panheleux, M., Bain, M., McKee, M.D., Solomon, S.E. & Nys, Y (2001) Ovocalyxin-32, a novel chicken eggshell matrix protein - Isolation, amino acid sequencing, cloning, and immunocytochemical localization. *Journal of Biological Chemistry*, **276** (42): 39243-39252.

Panheleux, M., Bain, M., Fernandez, M.S., Morales, Gautron, J., Arias, J.L., Solomon, S.E., Hincke, M. & Nys, Y. (1999) Organic matrix composition and ultrastructure of eggshell: a comparative study. *British Poultry Science* (1999) **40:** 240–252.