A comparison of the calcite/aragonite ratios found in 5 populations of *Thais lapillus*

Localities	Mean Ratio ± 1 S.D.	Mean Shell Length ±1 S.D.	*n*	Latitude (*N*)
Acadia Park (AP)	6.70 ± 1.80[a]	16.4 ± 2.7	10	44.20
Owl's Head (OH)				44.04
Young	12.79 ± 4.07	14.6 ± 1.1	10	
Adult	16.20 ± 5.10	29.8 ± 4.0	10	
Total	14.50 ± 4.82[a]	22.2 ± 8.1	20	
Pemaquid Point (PP)	5.99 ± 0.94[a]	15.9 ± 2.3	10	43.53
Cape Elizabeth (CE)	6.77 ± 1.60[a]	17.2 ± 2.6	11	43.37
Watch Hill (WH)	18.25 ± 7.22[a]	24.2 ± 2.5	9	41.18

[a] Student-Newman-Keuls Test: PP AP CE, OH WH ($p < 0.05$).

Water temperature records[11] indicated only a slight year-round difference among the 4 localities in Maine. Temperature alone was not sufficient to cause the observed difference since populations from the 2 extreme areas in Maine (Acadia Park-coldest; Cape Elizabeth – warmest) had the most comparable ratios. Comparison of Watch Hill and Owl's Head populations, where temperature differences may be more than 5 °C during the spring and summer, also supported the view that temperature was not an important factor governing the calcite/aragonite ratio in *T. lapillus*.

Discussion. The evidence presented in the Table shows that thickshelled populations are mineralogically different from thin-shelled ones. That such a phenomenon is not an artifact due simply to greater size or thickness itself, is shown by the results from thin-shelled immatures of the Owl's Head population. Maturity also had no effect on the ratio, despite contrary evidence in *Patella*[12]. Although temperature did not influence the ratios, other physicochemical factors were not determined. However, separation of *T. lapillus* populations based on calcite/aragonite ratios agrees excellently with separations based on physiological, morphological and ecological criteria[13]; and STAIGER[14] has shown that the morphological and ecological differences have a genetic basis. Thus, it seems quite possible that these mineralogical differences may have genetic basis, although the effect of other environmental factors cannot be excluded entirely.

Zusammenfassung. Auf Grund des Verhältnisses Calcit/Aragonit in den Schalen von *Thais lapillus* können die 5 Populationen aus den Gewässern von New England in 2 Gruppen (5.99–6.77 und 14.50–18.25) aufgeteilt werden, was mit den morphologischen Eigenschaften dieser Schneckenschalen korreliert ist. Das Alter des Gastropods und die Wassertemperatur scheinen keinen Einfluss auf das Verhältnis Calcit/Aragonit der vorliegeneden Art zu haben. Ob andere Umweltfaktoren dabei beteiligt sind, bedarf noch weiterer Untersuchungen.

R. A. WHARTON[15,16] and S. Y. FENG[17]

Marine Research Laboratory, University of Connecticut, Noank (Connecticut 06340, USA), 18 June 1974.

[12] G. SABATIER, Cah. Natu. *8*, 87 (1953).

[13] R. A. WHARTON, M. S. Thesis, Univ. of Connecticut (1971), p. 95.

[14] H. STAIGER, Année biol. *33*, 251 (1956).

[15] Acknowledgments. The authors wish to dedicate this work in memory of their good friend the late Dr. ANDREW J. NALWALK who made the GE XRD-5 X-ray Diffraction apparatus available to them and also provided his technical expertise in analytical procedures. R. A. WHARTON was a recipient of a NSF Graduate Fellowship.

[16] Present address: Department of Entomological Sciences, University of California, Berkeley, California 94770, USA.

[17] To whom reprint request should be addressed.

Experimental Data on the Taxonomical Value of an Electrophoretical Protein Component from the Eye-Lens of Birds

After our report on a typical song bird lens protein pattern obtained by agar electrophoresis, and especially on a particular component that also occurred in a few other avian orders[1], extensive disagreement with out entire work was expressed by SIBLEY and BRUSH[2], who used starch gel as a separation medium.

Although it is not difficult to refute their arguments one by one, we will not start a discussion on this matter here. Suffice it to mention that other authors[3] have already clearly pointed out that starch gel electrophoresis is not the appropriate method to examine the high-molecular lens proteins, the sequence of which is thoroughly changed by the molecular sieving effect. Especially HOENDERS[4] explained in detail: «Die umständliche Technik und die lange Laufzeit, vor allen Dingen aber die sehr bescheidenen Ergebnisse, machen die Stärkegel-Elektrophorese unter den angewandten, allgemein üblichen Bedingungen für die Analyse der Linsenproteinen ungeeignet».

This paper deals with the more positive criticisms put forward by some British investigators[5], which run as follows. No doubt the agar lens patterns as a whole, among Passeriformes and some non-Passeriformes, are similar. But the similarity of the individually corresponding fractions was not demonstrated, except by precise mobility measuring. For instance the typical song bird component (TSBC) was found in several orders where it had the same mobility. The possible explanations for this are: first, the animals have identical genes that code for the same protein, thus the amino acid sequences would be identical and one would conclude similarity.

[1] H. GYSELS, J. Orn. Lpz. *106*, 208 (1965).

[2] C. G. SIBLEY and A. H. BRUSH, Auk *84*, 203 (1967).

[3] W. J. VAN DOORENMAALEN, H. J. HOENDERS and J. ZWAAN, Protides biol. Fluids *11*, 499 (1963).

[4] H. J. HOENDERS, Thesis Univ. Amsterdam (1965), p. 116.

[5] D. W. SNOW, personal communication.

But it seems unlikely that selection was very intolerant of change and that the original and only gene still exists.

Second, the animals are not closely related but the proteins have similar mobilities as the result of a) coincidence in that at one pH the net charge is such that the mobilities are the same: or b) that the selection has been for some other parameter of the protein, which has, by chance alone, produced the same net charge, but different amino acid sequences.

In other words, it is possible, though unlikely that the Passeriformes, some Ciconiiformes, Gruiformes, Falconiformes, Strigiformes, Piciformes and Coraciiformes are so closely related that they have identical genes for the TSBC. It seems more likely that the requirements for an eye lens protein, e.g. transparency and elasticity, can be meet in a number of ways. Those substitutions which maintained the functional criteria were tolerated and those which decreased any one of them were eliminated. This would have allowed changes in sequence but would still explain similarities in the various taxa.

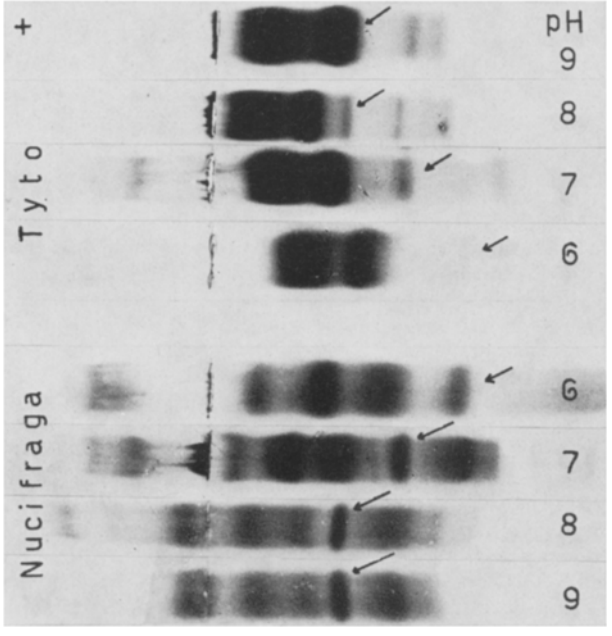

Fig. 1. Agar microelectrophoresis of lens extracts of *Nucifraga caryocatactes* (Passeriformes) and *Tyto alba* (Strigiformes), run at different pH. The mobility of the typical song bird component (also present in the owl, indicated by arrows) is varying at exactly the same rates with both birds.

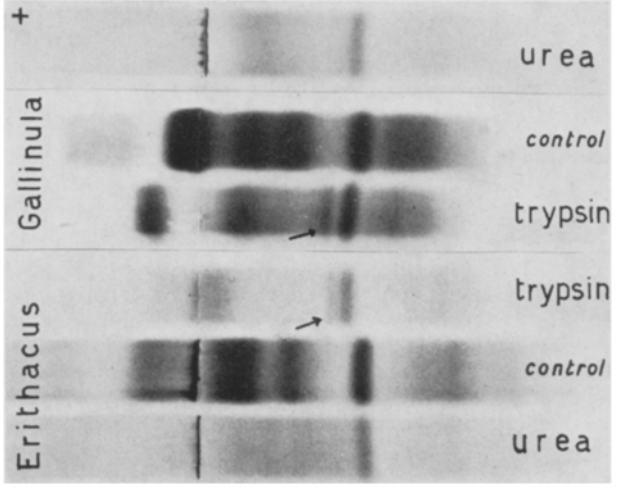

Fig. 2. Lens pherograms of *Erithacus rubecula* (Passeriformes) and *Gallinula chloropus* (Gruiformes) after treatment by protein dissociating substances. With both birds the typical song bird component shows a remarkable resistance against urea, while its mobility is slightly accelerated by trypsin; it is probably also split, while a new fraction appears at its anodal side (arrows).

Either of these suggestions regarding coincidence in mobility could be tested by running the material at different pH's. Samples of lens extracts from the Nutcracker (*Nucifraga caryocatactes*) and the Robin (*Erithacus rubecula*) were run at the same plates with extracts from the Barn Owl (*Tyto alba*, Strigiformes) and the Moorhen (*Gallinula chloropus*, Gruiformes), in a broad pH-range from 6 to 9, using barbital buffers.

Moreover the resistance of avian lens proteins, in particular the TSBC, was tested against trypsin (concentration 0.5–1‰, in barbital acetate buffer M/20, pH 8, incubation at 30 °C for 15 min). Finally a denaturation proof was added by using urea. It is well known that this substance in high concentrations (6–8 *M*) often causes molecular dissociation in proteins. All procedures were carried out according to RABAEY[6].

Electrophoresis at different pH's resulted in a completely similar migration of the TSBC with all birds examined, Passeriformes and non-Passeriformes (Figure 1). Urea activity reduced almost all lens components into 1 broad hazy spot. The TSBC, however, remained visible, and its mobility was not changed. By trypsin activity, on the other hand, its mobility was slightly accelerated. The component was possibly also split, while a newly formed fraction was recorded very close to its anodal side (Figure 2).

From these experiments, it may be concluded that the protein of which the TSBC consists is very similar, perhaps even equal in Passeriformes, Gruiformes and Strigiformes. Probably it is also similar in the other Avian orders in which it occurs. We believe that it ought to be considered seriously as a useful and important characteristic in the study of the relationships among the higher taxa of birds, as we do for other protein characters, obtained from other organs or tissues. Nevertheless, we are well aware of the danger of considering only 1 taxonomic characteristic, or of any other preliminary conclusion. We fully agree that great caution, as was urged by CRACRAFT[7] and re-urged by an 'Ibis' reviewer[8] recently, is necessary in this field.

Résumé. La fraction typique trouvée dans les phérogrammes des protéines de lentille des oiseaux chanteurs et de quelques ordres non-Passeriformes n'étant jamais identifiée que par sa mobilité constante, des expériences complémentaires ont été effectuées en variant le pH de la solution tampon. Cette procédure, ainsi que les dénaturations provoquées en utilisant la trypsine et l'urée, donnent des résultats entièrement semblables chez les Passeriformes et chez les représentants des ordres probablement apparentés.

H. GYSELS

Instituut voor Dierkunde, Rijksuniversiteit Gent, 35, K.L. Ledeganckstraat, B–9000 Gent (Belgium),
9 July 1974.

[6] M. RABAEY, Thesis University Ghent (1959).
[7] J. CRACRAFT, Bird-Banding *42*, 157 (1971).
[8] P. J. K. BURTON, Ibis *116*, 120 (1974).