

## KERATIN PROTEIN ELECTROPHORESIS AND THE IDENTIFICATION OF FEATHER REMAINS: NEW DEVELOPMENTS AND UPDATE

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### ABSTRACT

Feather identification by visual means leaves a percentage of unidentified samples at the lower taxonomic levels, genus and species, particularly single feathers or small fragments. After an initial period of technical development and research, electrophoresis of proteins extracted from feathers has been used for the last six years with a high reliability and repeatability in feather identification regardless of their origin. Protein extraction from keratin has been refined and standardised concurrently with the methods used for electroporizing protein concentrates. Current results allow accurate identifications of unknown samples to the species level provided the samples contain enough plumaceous or pennaceous feather elements to extract about 10  $\mu$ l of proteins. Comparisons of keratin profiles is now easier because the collection of gels and keratin profiles used as reference comprises over 800 North American species represented by more than one sample in most cases, for a total of nearly 3,500 keratin profiles. Results show that there is little individual variation and no sexual variation. Differences between species of a single genus can be important and can sometimes be assessed visually but gels are routinely scanned with a laser densitometer for detecting and measuring differences between curves. The values and patterns thus obtained can be used for separating closely related species. Differences between species can be small but are sufficient to identify samples. This methodology is reliable for feather samples of more than 10 mg not altered by high temperatures or by chemical products. Our results indicate a high success rate and precision in identifications, exceeding the results obtained earlier using other means of identification.

## 1. INTRODUCTION

The identification of feather remains can be a difficult task particularly when the remnants received are very small and badly mangled. When the samples are large enough, direct comparison with specimens in reference collections coupled with the macroscopic examination of visual clues is usually sufficient to insure accurate identifications. Using straight visual comparisons of the external morphology and distinctive features of feathers, with or without the use of low optical magnification, no less than 50% of the samples can be identified by an experienced ornithologist. It is necessary to employ different methods to identify the remaining 50%. A number of identification techniques have been described (Robertson *et al.* 1986) and have been used by various identifiers and the techniques of identification of unknown samples used by myself or my associates include the examination of the microscopic structure of feathers, including downy barbules (Brom 1986, 1991), using dissection, compound, and scanning electron microscopes (Chandler, 1916; Davies, 1970; Sick, 1937; Voitkevich, 1966). A critical evaluation of the results obtained using these techniques, including series of tests to verify their accuracy, has shown that the detailed and precise identification of nondescript feathers can be achieved only for an additional 25% of the samples, thus leaving about 25% of the samples submitted unidentified below the Family level. It seemed pertinent to explore other techniques to reduce the percentage of samples unidentified below the higher categories. After a series of tests and experiments in the late 1980s, electrophoresis of feather keratin proteins proved to be reliable in providing an accurate means of identifying those samples which could not be identified otherwise. The results obtained proved to be beyond our most ambitious expectations and it was decided to pursue with this technique when the other means cannot provide the information requested. Electrophoresis of feather keratin is now used when it is not possible to identify otherwise material at the Species level.

## 2. METHODS

Electrophoresis of proteins obtained from the keratin of feathers has been used in a limited way in taxonomic work and indicates that these proteins have similarities among themselves, have small molecular weights, and vary from species to species (Brush 1976; Brush and Witt 1983; Busch and Brush 1979; Knox 1980a, 1980b; O'Donnell 1973; Olsen *et al.* 1989). These results show that the technique can become a valuable alternative in the identification of feather samples that cannot be identified by other means.

## 3. PROTEIN EXTRACTION

Whole feathers, or parts of feathers, are cleaned in batches by washing in hot detergent and rinsing in hot tap water. When dry, they are rinsed in two changes of naphtha (hexanes, Fisher), and once in distilled water. Finally, they are washed again in two changes of denatured alcohol. After the final drying each sample is packaged and stored for future use (Knox 1980a). Keratin is extracted from 10 mg of finely cut feather samples from which shafts or calami have been excluded. To each of these samples 1.0 ml of the extracting solution consisting of 0.05M THAM (Fisher T-370), 8M urea (Fisher 4204-1), to which 0.05M dithiothreitol [DTT] (Pharmacia) is added at the last moment (Marshall *et al.* 1986).

The samples are stirred overnight at room temperature under an atmosphere of nitrogen. Each reaction mixture is centrifuged [12,000 rpm, 10 min; Eppendorf 5415 Microfuge] to sediment the residual feather fragments. The supernatant is removed and stored at -20 °C. To 25 µL of each extracted sample, 5 µL of 0.1M DTT is added at least 10 min before typing (Carracedo *et al.* 1986). The samples are added to Pharmacia polyacrylamide Phastgel IEF 3-9, presoaked for 15 min in a solution consisting of 1.0 µl Pharmacia Pharmalyte 3-10, 250 µl 20% NP-40, 12% sucrose, and 5.0 ml distilled H<sub>2</sub>O.

## 4. PROTEIN ELECTROPHORESIS

Isoelectric focusing proceeds at 2000V, 25mA, 4W, 20 °C for 400Vh with a prefocusing phase at

2000V, 25mA, 2W, 20° C for 50Vh using a Pharmacia Phast System Separation and Control Unit. The gels are stained in a Pharmacia Phast System Development Unit using the protocol for Phast Gel IEF silver staining techniques (Phast System Owner's Manual), modified to add an extra step, using 0.0125% DTT (Pharmacia) for 10 min at 40° C which is added between steps Nos. 8 and 9 of the protocol supplied by the manufacturer.

#### 5. GEL ANALYSIS

After drying the gels are examined visually and differences between the bands of the tracks are noted and compared. Then each track, 8 on each gel, is processed on a laser densitometer (LKB Ultro Scan XL) in order to obtain quantitative values for each of the bands shown on the tracks, as well as a graphic representation of these values. Some of the bands may go undetected through visual examination because they appear to be fused to the adjoining ones but can be separated by the beam of the densitometer. In this manner, it is possible to obtain distribution curves of the values recorded for each sample and compare these with curves of unknown samples. The curves thus obtained have been designated as "KERATIN PROFILES" and the comparison of unknown keratin profiles against profiles obtained from known samples permits the identification of unknown feathers. An exact match can be obtained in most cases although slight variations have been observed and are considered to be the result of individual variation or from variation due to the instrumentation and/or operator.

#### 6. PROTEIN VARIATION IN RELATION TO ORIGIN OF FEATHERS ON BODY

It was originally suspected that variation could occur in keratin profiles corresponding to the nature of the feathers or their origin on the body. This possibility was tested with two sets of samples from two different birds. Feathers from different parts of the body of a single individual were selected and subjected to the entire electrophoresis process and the results indicate that there is no significant difference or variation in the protein bands of the gels nor in the keratin profiles of the feathers obtained from the same individual, regardless of their origin on the body.

In a different experiment, when dealing with feathers longer than 15 to 20 mm or with a thick shaft (calamus and rachis), only the vanes are used, because the results showed meaningful differences between samples taken from the vanes and those containing only shaft material.

#### 7. INTERSEXUAL VARIATION

No intersexual differences in keratin profiles were noted between individuals of the same species upon extensive comparisons of keratin profiles of over 800 species.

#### 8. INDIVIDUAL VARIATION

The results obtained indicate that there is little individual variation in a given species. As an example, eight different individuals of the Ring-billed Gulls (*Larus delawarensis*) (Figure 1) representing different sexes and ages were compared to each other and later verified by comparing large samples of keratin profiles from several species. The tracks of the gels show a great uniformity in the location of the various bands and the keratin profiles are very similar, allowing for negligible individual variation.

#### 9. INTERSPECIFIC DIFFERENCES

Interspecific differences between closely related congeners can be important in some cases and can often be estimated visually as indicated in Figures 2a and 2b. In this case, the gel shows the protein electrophoretic patterns of seven species of gulls (*Larus delawarensis*, *L. argentatus*, *L. glaucooides* [kumlienii], *L. hyperboreus*, *L. marinus*, *L. heermanni*, *L. californicus*, *L. glaucescens*). It can be evaluated visually, without the aid of any equipment, that a number of bands on each track have a common position and that the others are situated in a different place on the track. Bands that occupy a similar position in a series of tracks can be interpreted as characteristics common to species in a same taxonomic category such as the Genus, Family, or even the Order. The other bands, located in

different places are more obvious upon examination. Differences in band positions obtained can be small but are averaged for in the keratin profiles (Figure 3.2). These differences are characteristic of different populations.

#### 10. CONCLUSIONS

The method described here for feather sampling and analysis of 10 mg of feather material by chemical and physical means with precision, especially through keratin densitometry and almost automatic computerized profiles of a

#### 11. ACKNOWLEDGMENTS

I wish to thank Dr. McKinnon for the feather samples performed for me.

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different places on the track, are considered to be Species characteristics. These differences are more obvious on the keratin profiles obtained through densitometric scanning than from the examination of the gels as shown for the eight species of gulls of Figures 2.1 to 2.9. In addition, the differences between each curve can be measured as well as any point on the curve. The values obtained can then be used for separating closely related Species or Species that have small differences such as is the case with the eight Species of gulls. Certain differences between species are small but are sufficient to distinguish between species, particularly when values are computed and averaged for the peaks or highest values of the curves. Similarly, marked differences were observed in the keratin profiles of two populations of the Gray-cheeked Thrush (*Catharus minimus*) (Figures 3.1, 3.2). These marked differences provided the basic material for an elaborate taxonomic study of these populations which proved to be two different species (Ouellet 1993).

#### 10. CONCLUSION

The methodology described above provides a technique that allows the accurate identification of feather samples even of small size. The amount of feathers available for analysis should be in excess of 10 mg and the feathers should not have been altered by excessive heat or degenerated by chemical products. For any feather meeting these basic requirements and unsuitable for identification by visual methods, our results indicate that a high success rate, combined with a high degree of precision, exceeding by far the results secured by any other identification techniques can be attained through keratin electrophoresis and subsequent analysis of the gel patterns with a scanning densitometer. At present, the data base contains more than 800 species, mainly from North America, and almost 3,500 profiles (Figures 4 and 5). It is hoped that the results of the keratin profiles can be computerized to generate rapid and accurate comparisons of unknown samples against the protein profiles of a data bank. 11.

#### 11. ACKNOWLEDGMENTS

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Figure 1.

Protein tracks on gel of eight individuals of the Ring-billed Gull (*Larus delawarensis*).



Figure 2A.

Protein tracks on gel of eight species of North American gulls (*Larus* sp.).

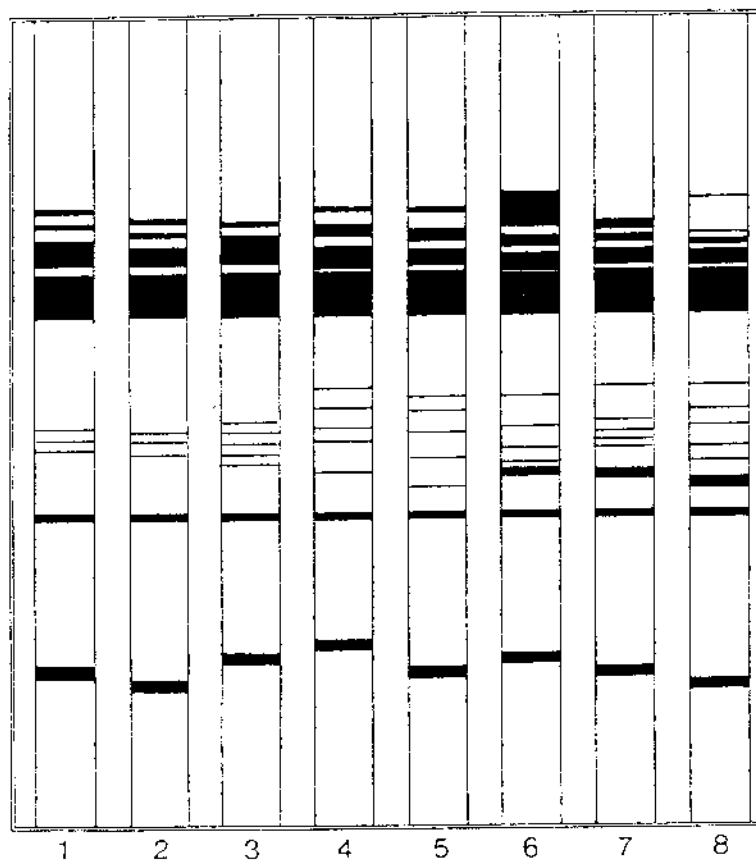
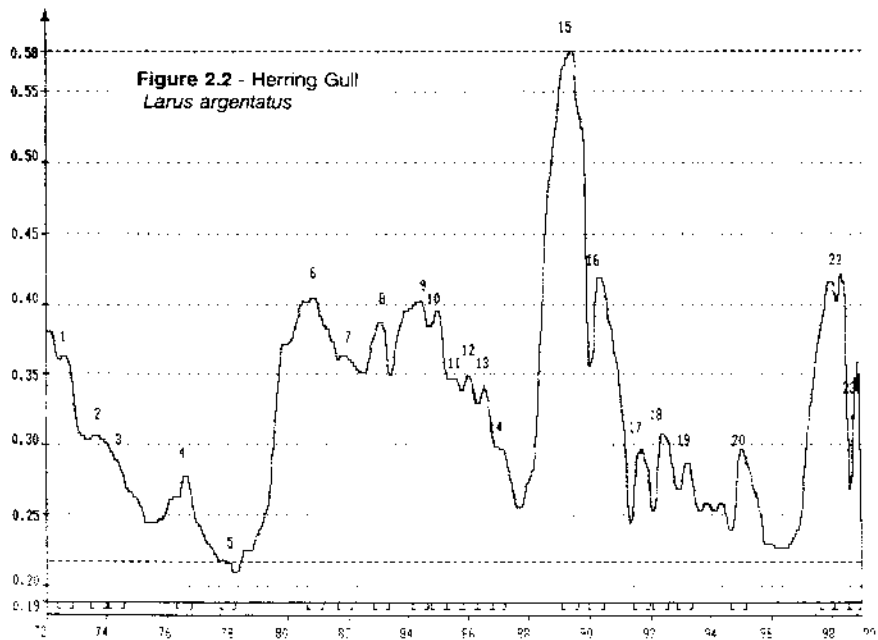
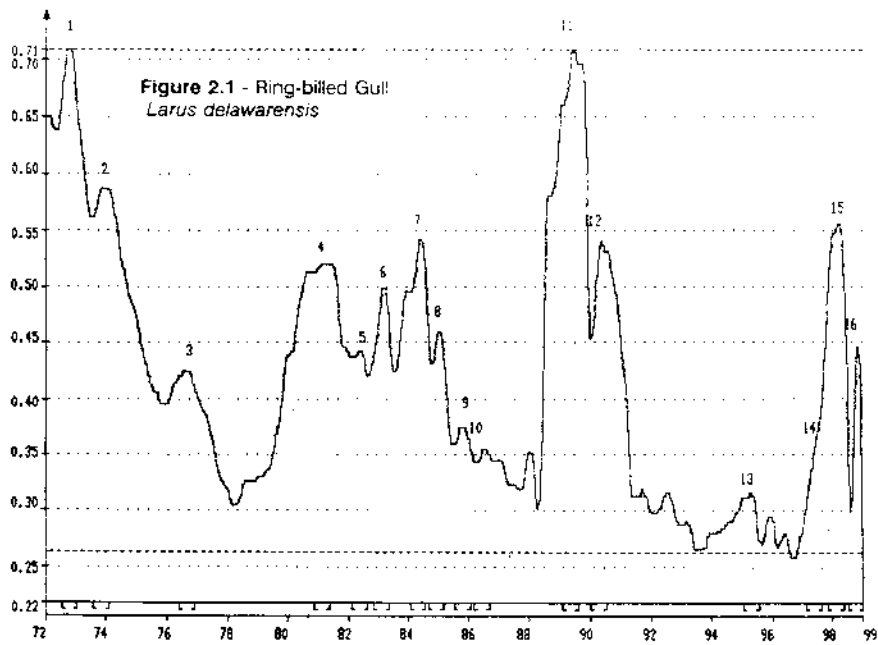
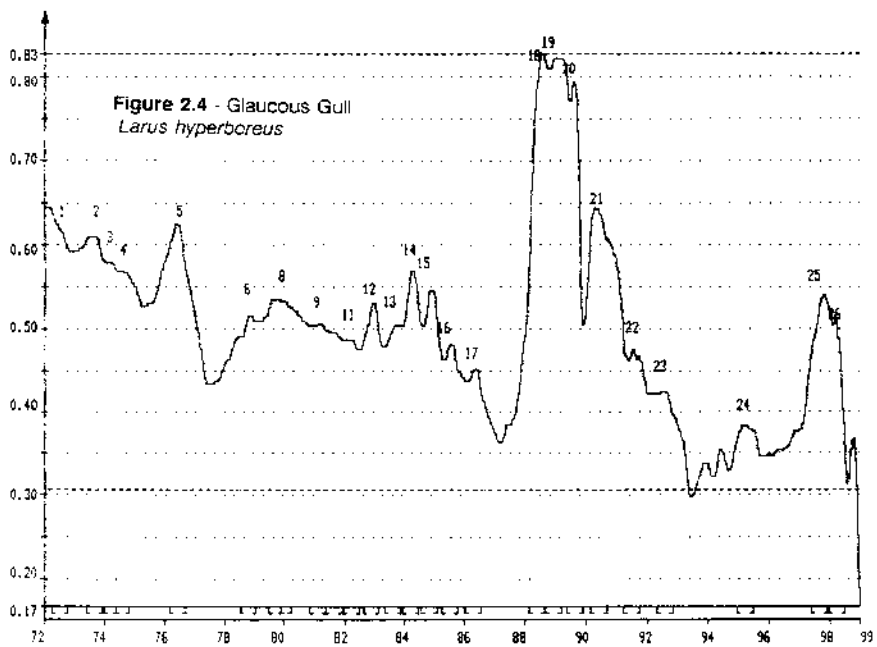
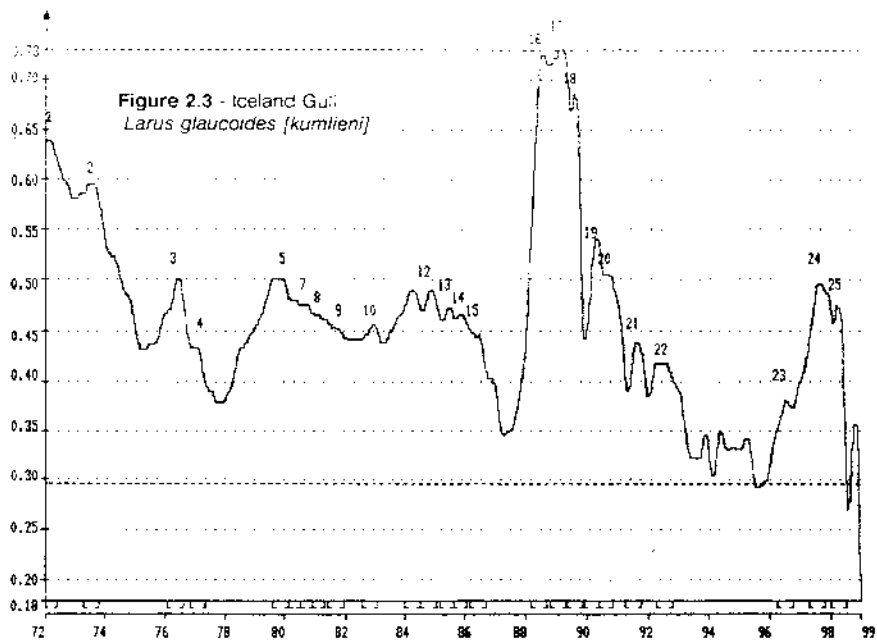


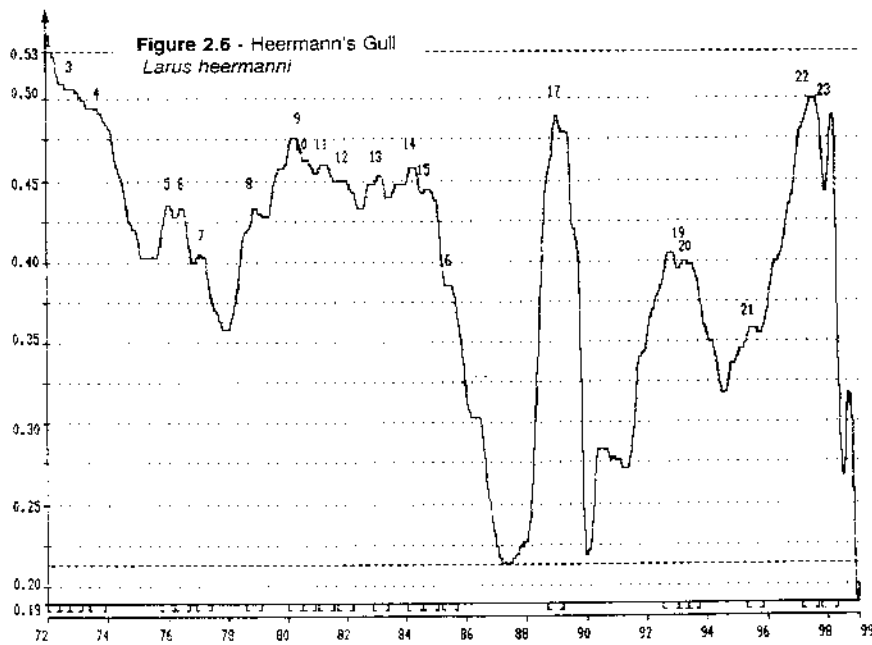
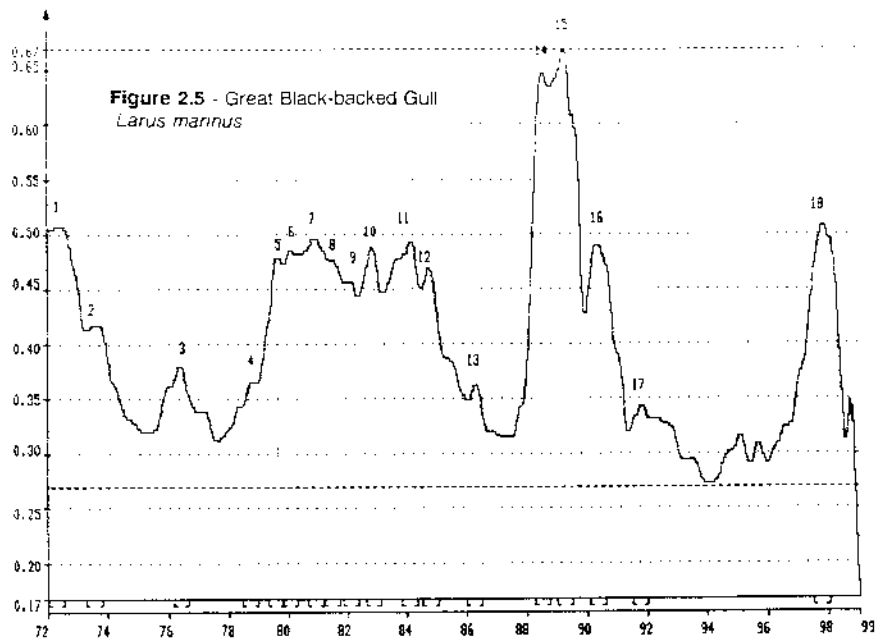
Figure 2B.

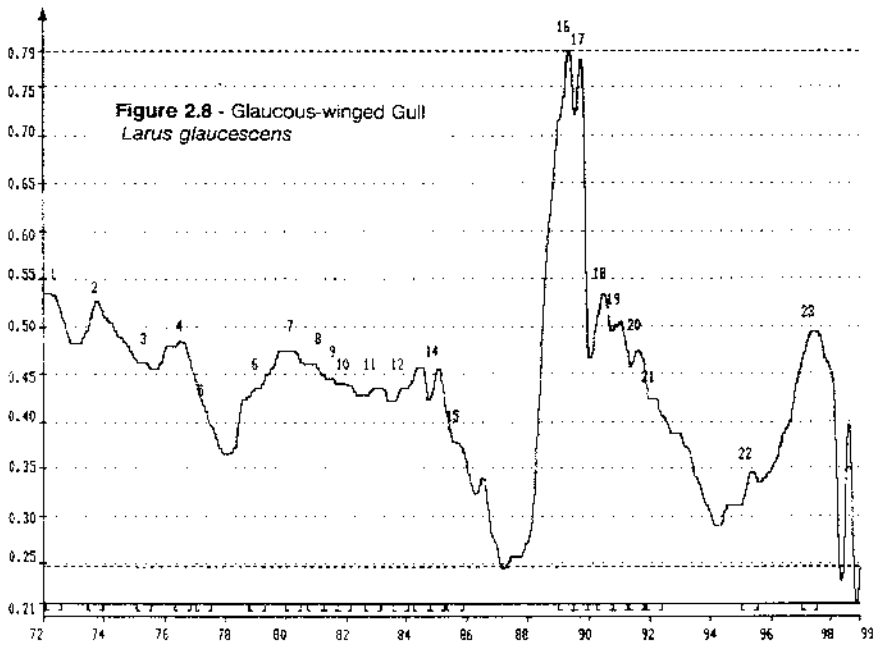
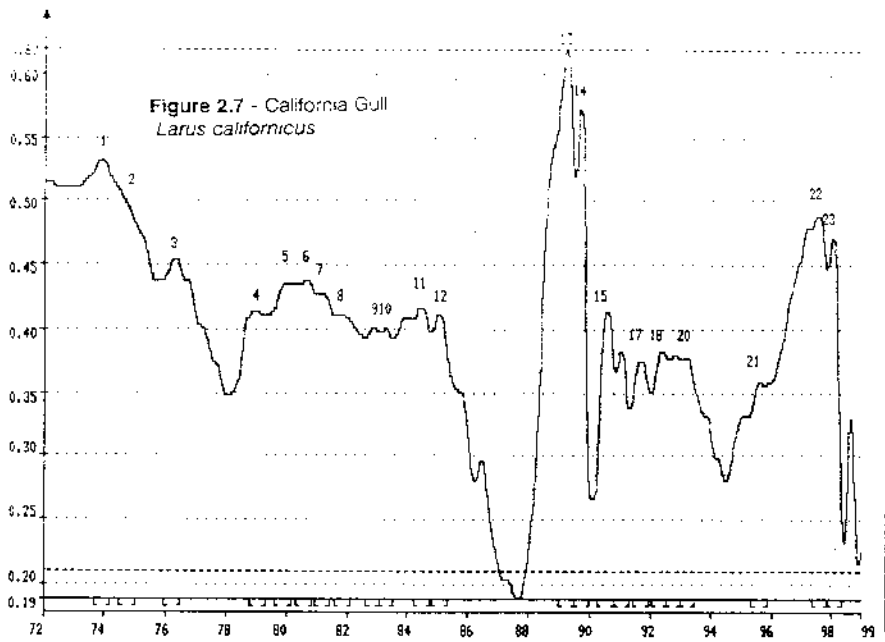
Schematized protein tracks of eight species of North American gulls (*Larus* sp.) showing locations of bands from original gel (Figure 2A).

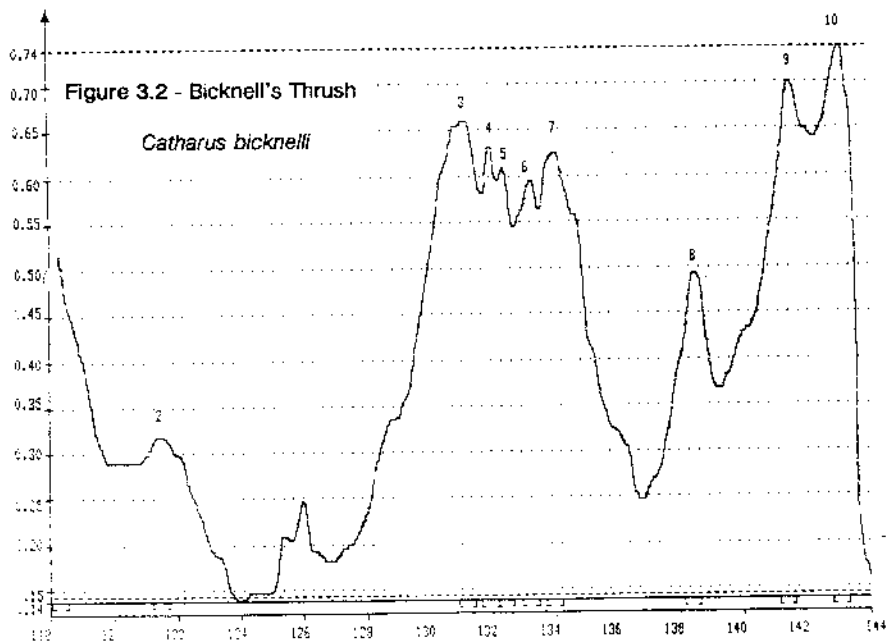
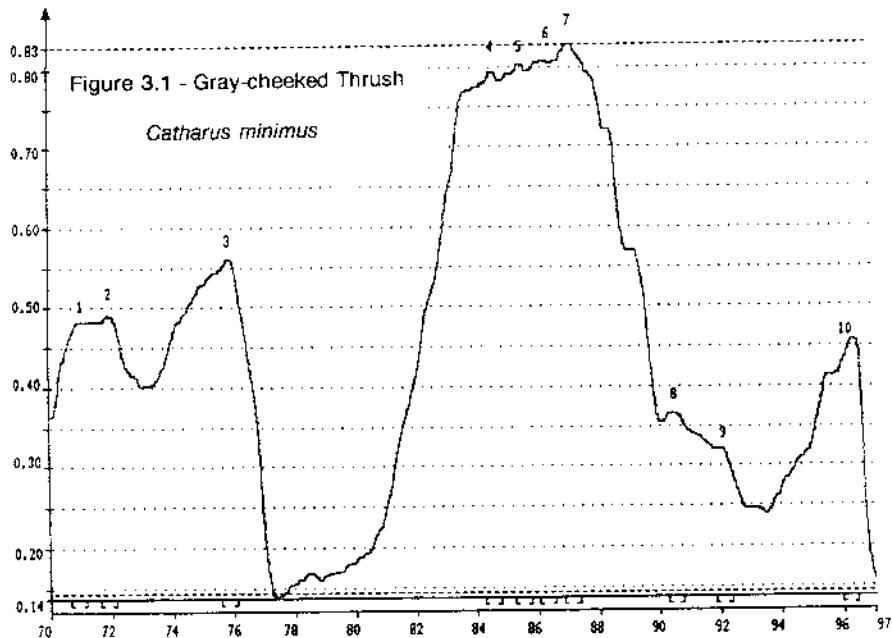


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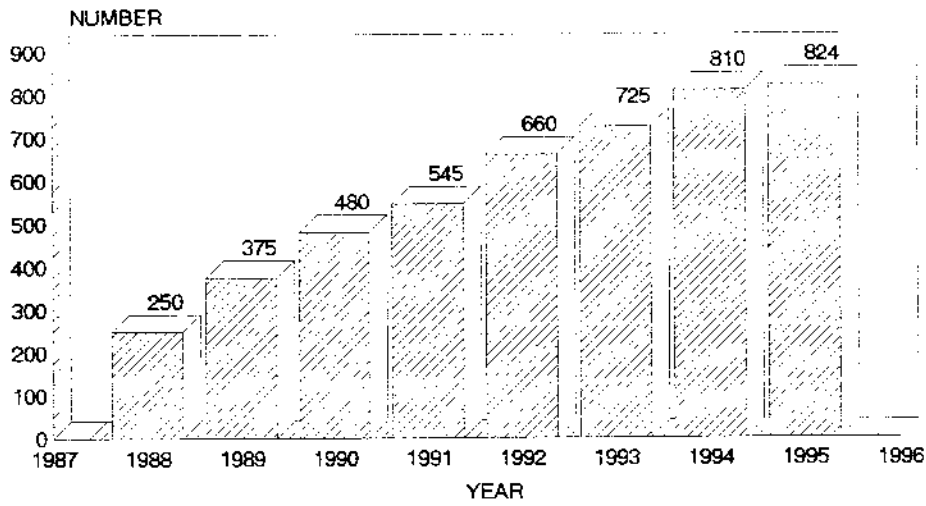






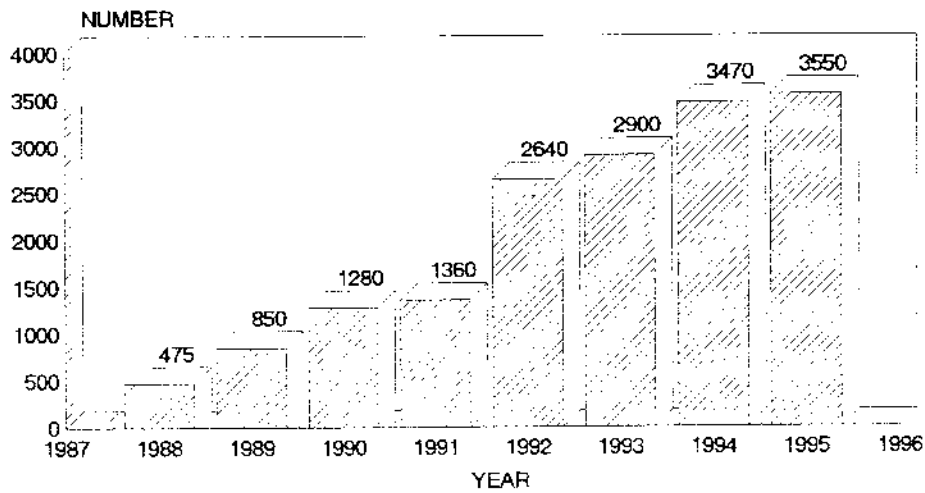


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Figure 4.



BIRD

KERATIN PROFILES  
Figure 5.



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