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THE BIOCHEMISTRY OF KERATIN IN GREEN GECKOS (*PHELSUMA*) AND ITS SYSTEMATIC VALUE

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ABSTRACT: The biochemistry of reptilian keratins was investigated using green gecko (*Phelsuma*) epidermis. The keratin components are highly heterogeneous, particularly those of the low sulphur fraction. Disc polyacrylamide gel electrophoresis indicates that these components have a range of molecular weights, but isoelectric focussing on specially formulated gels indicates that the components are far more heterogeneous with regard to their isoelectric point. In spite of some isoelectric coincidence, as revealed by two dimensional electrophoresis and a comparison of high and low sulphur fractions, isoelectric focussing of unfractionated samples is of considerable taxonomic and evolutionary value. The quantified comparison of samples based on the presence or absence of bands at a particular isoelectric point indicates that there are differences between genera, species and races and that the relative similarity in the pattern is directly related to the taxonomic/evolutionary affinity. Keratin samples are generally consistent within populations, but there is some evidence of polymorphism.

Key words: Keratin; Biochemical taxonomy; *Phelsuma*; Isoelectric focussing; Electrophoresis; Molecular evolution

BIOCHEMICAL taxonomy largely depends upon proteins extracted from living organisms. Whilst the value of biochemical systematics is evident (Ferguson, 1980), this dependency on live specimens can be a disadvantage. It can be impractical to obtain live specimens from all parts of a wide geographic range,

particularly from politically or geographically remote areas. Moreover, the need for live specimens can also exclude the study of venomous, extinct or endangered species.

Keratin monomers can be derived from dead epidermal tissue (e.g., scales, feathers). They have been used successfully in taxonomic studies utilizing feather proteins (Brush, 1976; Fry, 1980; Knox, 1980). To date, there has been no taxonomic investigation based on the keratins of reptiles, although there is some information on chemical fractionation and electrophoretic comparisons of reptile keratins. For example, Baden et al. (1974) studied two reptilian genera (*Constrictor* and *Pseudemys*) whilst Wyld and Brush (1979) published on the keratins of *Alligator, Pseudemys, Constrictor, Sphenodon* and *Tupinambis*.

Taking green geckos (*Phelsuma*) as an example, this study shows how S-carboxymethylated keratin (SCMK) components from reptilian epidermis can give useful systematic information, particularly at the interspecific level. The procedures for preparing the SCMK components and characterising them on specially formulated gels by isoelectric focussing and polyacrylamide gel electrophoresis are given in detail. The extent to which museum preservation impinges on these techniques is discussed.

Materials and Methods

Shed skin from the tail and ventral surface of various *Phelsuma* species was washed in mild detergent, rinsed thoroughly in water, ethanol and petroleum spirit, and weighed aliquots were extracted with thioglycollic acid in a moist atmosphere of nitrogen for 12 h before alkylating with sodium iodoacetate at pH 9.0 (Harrap and Woods, 1967; Knox, 1980). The S-carboxymethylated keratin (SCMK) was dialysed against distilled water and concentrated by pressure filtration using an Amicon MMC multimicro ultrafiltration system. A small quantity of urea was added before storage of samples at 5 C. Detailed information on this and the alternative extraction technique are given in Appendix I.

Both the high tyrosine and low sulphur proteins were precipitated from solution by treatment with zinc acetate leaving the high sulphur proteins in the supernatant. The precipitate contains a mixture of Type 1 and Type 2 high tyrosine proteins together with low sulphur proteins. Further fractionation of the latter, while possible, proved difficult with the small amount of protein extracted from *Phelsuma*. Routinely the low sulphur precipitate was solubilised in 0.02 M sodium citrate (Gillespie, 1972). In this paper, use of the term "low sulphur" protein includes Type 1 and 2 high tyrosine group proteins as well as the low sulphur proteins sensu stricto.

Molecular weights for components in both fractionated and unfractionated material were calculated from migration ratios during sodium dodecylsulphate (SDS) polyacrylamide disc gel electrophoresis (PAGE) using Laemmli (1970) type gels. Six standard molecular weight markers were employed: cytochrome C, 12,300; myoglobin, 17,200; chymotrypsinogen A, 25,700; ovalbumin, 45,000; albumin, 66,200; and ovotransferrin, 76,000–78,000 (BBH product no. 442642L). Migration rates of components were calculated relative to the marker dye using a Ferguson plot (Weber et al., 1972).

SCMK monomers were separated by gradient gel electrophoresis, using high purity long format Gradipore gels (Universal Scientific Ltd.) and a Tris-glycine buffer system. Gels were fixed in 7.5% TCA and stirred with 0.25% Coomassie blue. Destaining was in 10% acetic acid until all bands were resolved.

Gels for isoelectric focussing were prepared in our laboratory from 5% acrylamide, 0.15% N,N-methylenebisacrylamide, 8 M urea and 2% pH 2.5–4.0, 2% pH 4.0–6.0 and 1.7% pH 4.0–8.0 LKB ampholines. Polymerisation was initiated

with 0.2% riboflavin and the gel exposed to UV light. The resultant gel consistently yielded a pH gradient from pH 4.0–6.5. The gels were focussed using a Multiphor 2117 unit (LKB Instruments Ltd., Croydon, Surrey, England) and a Pharmacia Constant Power Supply 3000/150 set to deliver 20 W at 2000 V. Gels were focussed for 2 h at the end of which the H gradient was measured using a Probion Micro-antimony surface electrode (Probion, Leslie, Fife, Scotland). The gels were fixed in TCA and stained with Coomassie blue (Marshall and Blagrove, 1979).

Two dimensional electrophoresis employed isoelectric focussing in 10 cm × 6.7 mm tube gels for 24 h at 250 V. Each gel contained 1.2 ml of isoelectric focussing solution. Details of the gel solution and anolyte/catholytes employed are given in Appendix I. The second dimension consisted of discontinuous SDS polyacrylamide gel electrophoresis using a Protean double slab electrophoresis cell (Bio-Rad Laboratories Ltd., Watford, Herts., England). The SDS gel was based on the system first described by Laemmli (1970). The resolving gel was altered by addition of high levels of urea, cross linking agent, and Tris buffer to obtain high resolution of low molecular weight proteins. Further details are given in Appendix I.

Results

The approximate molecular weights of the SCMK components in the unfractionated, "low" and high sulphur fractions of *Phelsuma s. sundbergi* (details of *Phelsuma* nomenclature are given in Appendix II) epidermis are indicated by a diagrammatic representation of the Laemmli disc gels (Fig. 1). The relative concentration at the various molecular weights are given by Joyce Loeble Chromoscans of these gels (Fig. 2). The molecular weights fall into two broad categories. The high molecular weight category (ca. 32,000–88,000 daltons) is comprised of "low sulphur" proteins (Fig. 1B) whilst the low molecular weight category (ca. 11,000–24,000 daltons) is comprised of both high and "low sulphur" proteins (Fig. 1B,C).

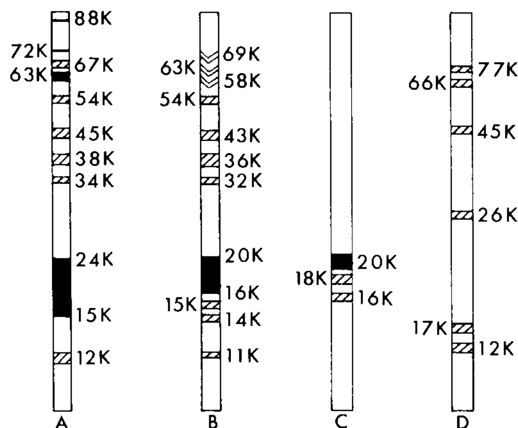


FIG. 1.—Molecular weight determination of SCMK components from fresh epidermis of *Phelsuma sundbergi sundbergi* form. The SDS gels are drawn to scale with the computed molecular weights indicated in daltons (taking into account marker dye position and gel shrinkage, etc.). (A) Unfractionated sample. (B) Low sulphur fraction. (C) High sulphur fraction. (D) Molecular weight marker proteins (control).

The "low sulphur" proteins (Fig. 1B) therefore have heterogeneous molecular weights whilst the high sulphur proteins have only a limited range of molecular weights, as indicated by the few anodal bands (Fig. 1C).

Whilst SCMK components from *Phelsuma* may be characterised by PAGE or gradient PAGE electrophoresis, we have obtained greater resolution and reproducibility by employing isoelectric focussing (IEF). IEF reveals that the high sulphur fraction of *Phelsuma s. sundbergi* is composed of three major anodal bands with very close pI values of ca. 4.7, 4.85, and 4.9, and two faint bands at pI values of 5.0 and 5.2 (Fig. 3A). The three main isoelectric components conform to the three main anodal bands observed on the SDS gel with very similar molecular weights. The high sulphur proteins are

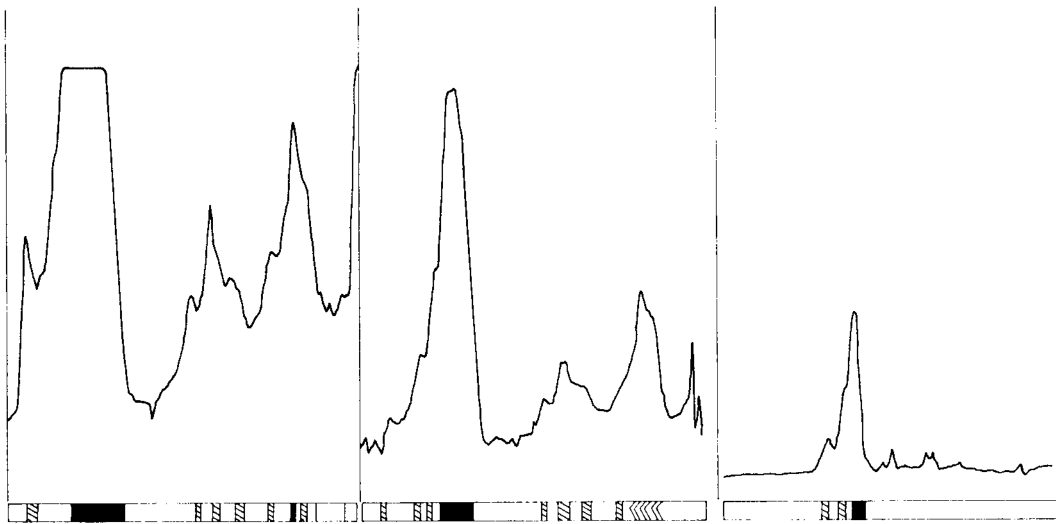


FIG. 2.—Chromoscan traces of Laemmli type gels shown alongside original gel used for molecular weight determination of SCMK components from fresh epidermis of *Phelsuma s. sundbergi*. (A) Unfractionated sample. (B) Low sulphur fraction. (C) High sulphur fraction.

therefore relatively homogeneous in their isoelectric points as well as their molecular weights.

The "low sulphur" proteins, however, have highly heterogeneous isoelectric points, in agreement with their highly variable molecular weights. There are more than 25 bands in the isoelectric focussed "low sulphur" fraction (Fig. 3B). Two of the three main high sulphur bands of *Phelsuma s. sundbergi* are isoelectrically coincident with bands in the "low sulphur" fraction (Fig. 3). Other species (e.g., *P. cepediana*) may have less isoelectric coincidence between the high and low sulphur fractions.

Extracts of keratin were prepared from the shed skin of two species, *Phelsuma s. sundbergi* and *P. cepediana*; in the case of both species, there were samples of both fresh skin and skin that had been stored for over 6 mo in 70% ethanol, in which preserved museum specimens are also normally stored. The concentrated extracts were examined by isoelectric focussing in a pH 4–6 gradient as above, in the presence of 8 M urea. Figure 3C and D shows one set of results for *Phelsuma s. sundbergi*, where it can be seen that the band patterns for fresh (Fig. 3C) and preserved (Fig. 3D) keratin are the same. In order to simulate more closely normal museum practice, we tested the effect of pretreatment of the keratin with 10% formalin for seven days prior to preservation in 70% ethanol. SCMK monomer patterns were not changed by this experimental pretreatment.

Before applying the technique to a more detailed taxonomic study of the group, an investigation was carried out to determine whether keratin taken from feet, tail, ventral and dorsal surface of the same individual would differ in band positions. It can be difficult to obtain sufficient skin from any one area of the body of some of the smaller species, and it seemed desirable to know whether banding patterns varied from one part of the body to another. Fig. 4 shows that although con-

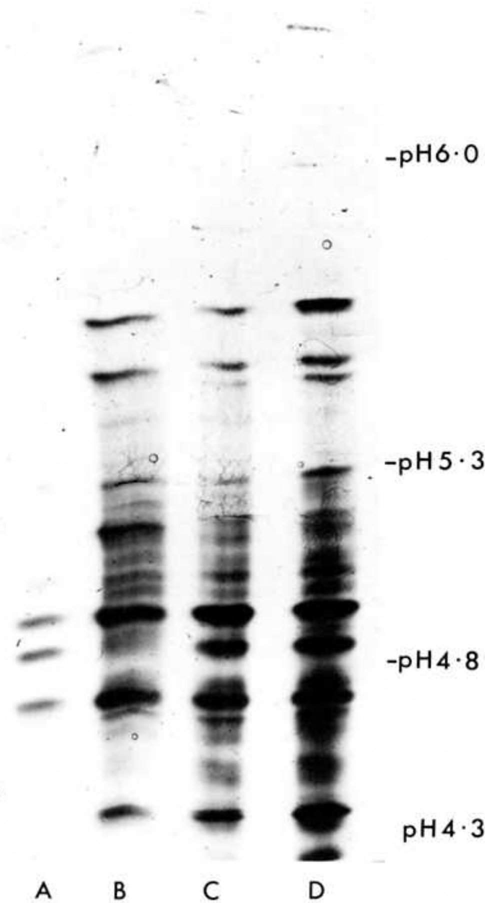


FIG. 3.—Isoelectric focussed gel of SCMK components from *Phelsuma s. sundbergi*. (A) High sulphur fraction from fresh shed skin. (B) Low sulphur fraction from fresh shed skin. (C) Unfractionated sample derived from fresh shed skin. (D) Unfractionated sample derived from shed skin preserved for over 6 mo in 70% ethanol. Note that, concentration effects aside, there is no difference between preserved and fresh keratin (D and C). The low sulphur fraction (B) has over 25 components whilst the high sulphur fraction (A) has three main components, two of which (in this species) are isoelectrically coincident with low sulphur components.

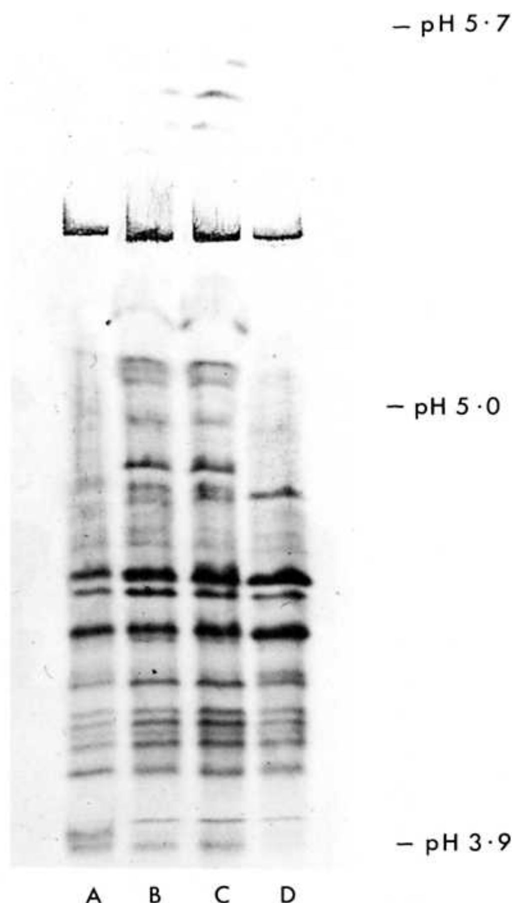


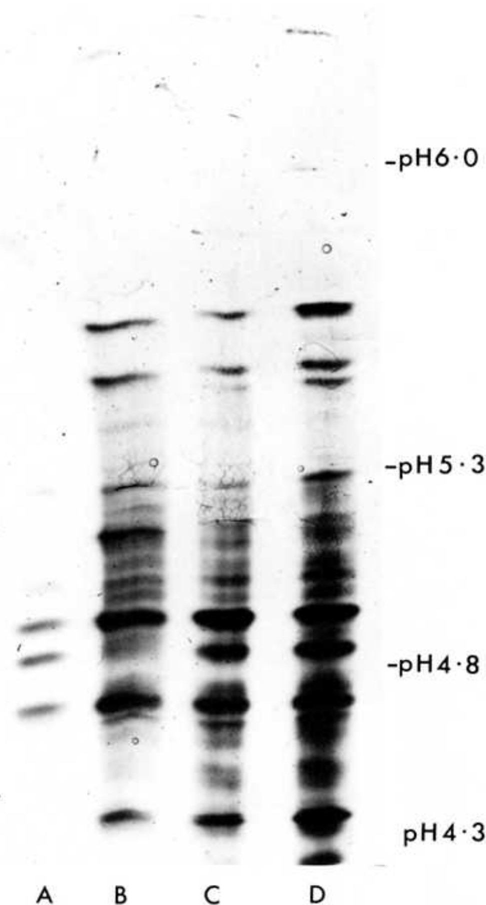
FIG. 4.—Isoelectric focussed gel of SCMK components extracted from four different sites of the same individual (*Phelsuma s. sundbergi*). (A) Feet. (B) Tail. (C) Ventral. (D) Dorsal.

centrations of individual bands vary, the basic patterns are identical.

Throughout this study, keratin was taken from the tail or ventral area, as the protein profiles of these two regions showed identical band concentration.

SCMK components from *Phelsuma laticauda*, *P. abbotti*, *P. ornata*, *P. cepediana* and *P. astriata* were isoelectrically focussed as were samples from two races of *Phelsuma sundbergi* (i.e., *P. s. sundbergi* and *P. s. longinsulae*). The nocturnal gecko *Gehyra mutilata* was used for intergeneric comparison. This gives six congeneric species of *Phelsuma*, two conspecific races and two genera; these each have a specific SCMK pattern (Fig. 5).

The similarity (I_{ij} value) in SCMK isoelectric focussing patterns between pairs of species may be quantified as:



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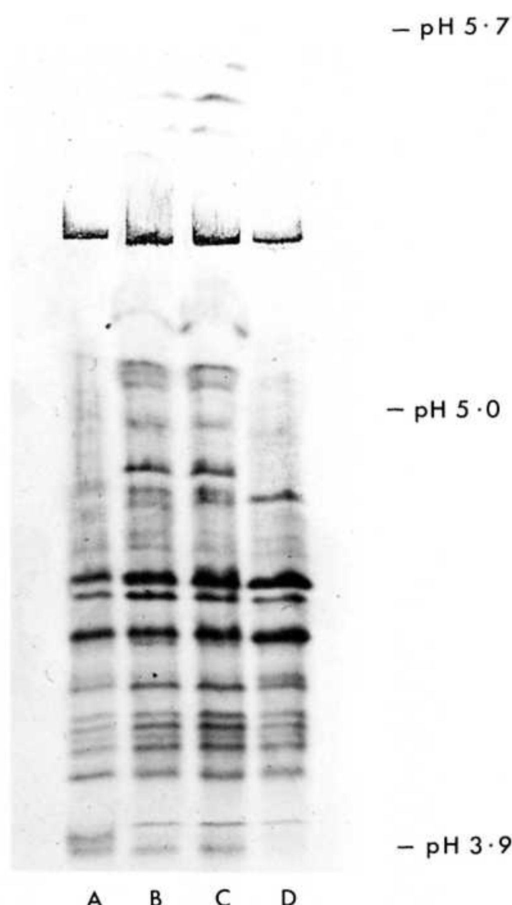

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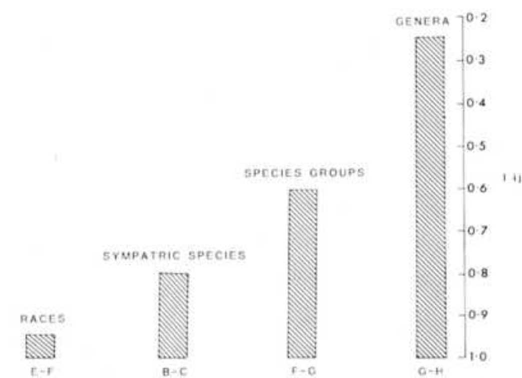

FIG. 6.—Similarity values $I_{ij} = Nm/Np$ represented in block form to show comparisons between races, sympatric species, species groups and different genera. Based on adjacent samples in Fig. 5. (B) *Phelsuma cepediana* (Mauritius); (C) *Phelsuma ornata* (Mauritius); (E) *Phelsuma sundbergi longinsulae* (Mahe); (F) *Phelsuma s. sundbergi* (Praslin); (G) *Phelsuma laticauda* (Comores); (H) *Gehyra mutilata*.

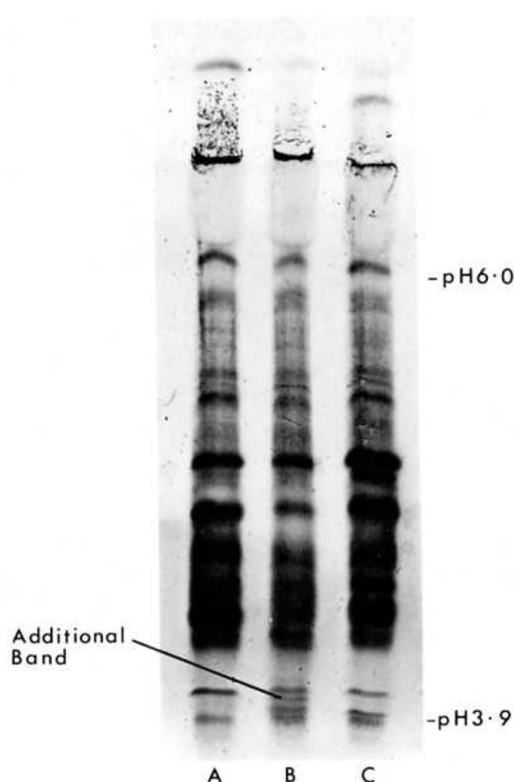

FIG. 7.—Isoelectric focussed gel showing very close similarity to SCMK components between three individuals of *Phelsuma s. sundbergi* population. Note a band with a pI of ca. 4.25 may be present or absent in the Praslin Island population.

ter-racial similarity are most conveniently and accurately portrayed by comparing the appropriate adjacent samples in Fig. 5. Fig. 6 shows that the relative similarity in IEF pattern reflects the taxonomic level of comparison. The level of similarity between races is 0.94 (*P. s. sundbergi* vs. *P. s. longinsulae*), the level of similarity between closely related sympatric species is 0.80 (*P. ornata* vs. *P. cepediana*), the level of similarity between species groups is 0.60 (*P. laticauda* vs. *P. s. sundbergi*), whilst the level of similarity between genera is 0.24 (*P. laticauda* vs. *Gehyra mutilata*).

SCMK patterns in *Phelsuma* are generally consistent within populations, although a band may be present or absent in different individuals of the same population. For example, in the Seychelles, Praslin population (*P. s. sundbergi*) a band of ca. pI 4.25 may be present or absent (Fig. 7).

DISCUSSION

It is evident that the low sulphur/high tyrosine SCMK components of reptile epidermis, as exemplified by *Phelsuma*, are highly heterogeneous. They vary considerably in their isoelectric points (as revealed by IEF) and to a much lesser extent in their molecular weights (as revealed by SDS PAGE). In the unfractionated sample, isoelectric focussing reveals ca. 35 bands, yet two dimensional electrophoresis reveals that even this is an under-estimate (Fig. 8).

SCMK components of a named form of gecko have a characteristic IEF pattern. When quantified, the relative similarity in these patterns in different forms is directly related to taxonomic similarity, even though there is some isoelectric coincidence. Patterns for races of the same species are very similar, closely related

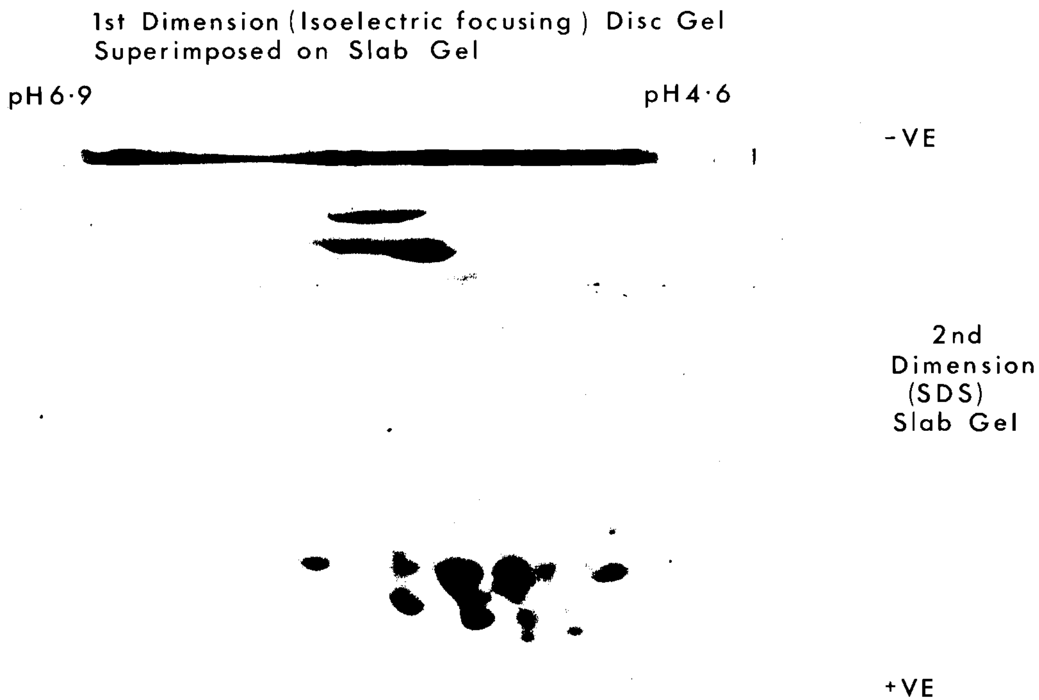

FIG. 8.—Two-dimensional gel electrophoresis of SCMK components of *Phelsuma s. sundbergi*. First dimension separation by tube isoelectric focussing, pH 4–6.5, and second dimension employed SDS/urea PAGE. Note multiple components having the same isoelectric point.

sympatric species are less similar, species of different groups have an even lower similarity, and genera have the least similarity.

It is clear that comparisons of SCMK components could have considerable taxonomic values particularly at the species level. This conclusion contradicts that of Wyld and Brush (1979) who found a high degree of intraordinal resemblance in SCMK components in reptiles and stated "the high degree of consistency of the PAGE patterns among families in these two orders made further taxonomic studies uninformative."

It is likely that the difference in conclusion between these authors and ourselves is a direct consequence of the electrophoretic technique employed. Since Wyld and Brush used SDS PAGE, they separated the SCMK components on the basis of molecular weight. They found five to seven major bands and a number of minor bands were resolved, the total number never exceeding 12, for a range of reptile species.

In fact, our SDS disc PAGE results agree with this, there being nine bands in the unfractionated protein profile of *Phelsuma*. However, for taxonomic purposes, SDS PAGE is inappropriate because intergeneric, interspecific and intraspecific differences in *Phelsuma* SCMK components are a consequence of their isoelectric point rather than their molecular weight (Fig. 5).

Brush (1976) attempted an IEF separation of SCMK components of water-fowl feather proteins but noted that this proved unsuccessful because the pI of the com-

ponents was outside the ranges of the ampholytes. However, specially formulated IEF gels of the correct pH range and containing urea are necessary for optimal focussing of SCMK components. Frenkel and Gillespie (1976) successfully focussed zinc precipitable proteins from bird beaks and Marshall and Blagrove (1979) focussed SCMK components from wool.

Using our modification of Marshall and Blagrove's procedure for preparing IEF gels, reptilian epidermal keratins can be successfully focussed. Although there is some evidence of isoelectric coincidence, both from comparing high and low sulphur fractions and from two dimensional electrophoresis, it is apparent that the degree of resolution using this IEF procedure is sufficient to distinguish between the SCMK components of races as well as species. Moreover, this can be done on the presence or absence of bands with no need to resort to considering differences in band concentration.

Previous work on bird feather keratins employing gradient PAGE (Knox, 1980) showed that protein profiles were of considerable taxonomic value but documented no clear racial differences. The interspecific differences depended both on differences in band concentration and the presence or absence of bands. However, quantification of band concentration is subjective unless one uses spectrophotometric scanning. We also found gradient PAGE to be of use with reptilian keratins but inferior to IEF. Consequently, the superior resolution and reproducibility afforded by an appropriate IEF technique may also benefit taxonomic work with bird and mammal SCMK components.

Our results show that within the Praslin population of *P. s. sundbergi*, a band may be present or absent in different individuals of the same population (Fig. 7). Whilst one cannot rule out the possibility of regulator gene effects, these do not seem likely, as the occurrence of bands is consistent between samples of keratin from various parts of the body (Fig. 4) and the specimens were of comparable age and physiological state. It is far more likely that the presence or absence of bands within a population is due to genetic polymorphism at the structural loci encoding for the particular SCMK monomer in question.

Knox (1980) suggested that in birds the evidence points to SCMK components being single gene products (Fraser et al., 1972; Kemp and Rogers, 1972; Walker, 1974). This suggestion is supported by Busch and Brush's (1979) study of tern and turkey feather keratins. There is no such evidence for reptilian SCMK components, but their inheritance is likely to be similar to that in birds. Consequently, IEF of SCMK components may conceivably be capable of indicating quite subtle genetic differences between closely related populations. However, the expense, time consuming preparation, and lack of specific stains make it inferior to conventional electrophoresis and staining of isozymes for this purpose.

The use of keratin from the epidermis of museum specimens would be a considerable practical advantage for taxonomic work. Our experiments showed that epidermis preserved in 70% alcohol (with and without prior preservation in formalin) yielded SCMK samples with IEF patterns identical to that of fresh keratin. However, attempts to extract and characterise SCMK samples from specimens of *P. edwardnewtoni* (extinct) and *P. andamanensis* that have been preserved in alcohol for a very long time (ca. 80 yr) proved unsuccessful. It is not known whether these museum specimens had been subjected to prolonged pretreatment with formalin. This would have been likely to have reduced the solubility of keratin. Moreover, prolonged exposure to alcohol might have the same effect.

The use of mercaptoethanol (see Appendix I) instead of thioglycollic acid improved the extraction of long term preserved keratin, particularly in some old

mamba (*Dendroaspis*) specimens. Whilst mercaptoethanol extraction also improved the results with old museum specimens of *Phelsuma* (yielding a few faint bands of 5.0–5.5 pI), this improvement was insufficient for taxonomic purposes.

Since the outer epidermis still maintains its identical physical appearance, superior extraction and characterization procedures may, in the future, render the epidermis from older museum specimens suitable for this technique.

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Appendix I

Extraction of Keratin—Method A

Stock reducing solution was prepared by mixing 48 g urea (8 M), 0.69 ml redistilled thioglycollic acid, 29 mg ethylenediamine tetra-acetic acid, distilled water and 1 M sodium hydroxide to give a final volume of ca. 100 ml and a pH of 9.8. Between 10–20 mg of washed keratin was extracted in an atmosphere of moist nitrogen stirring in 1 ml of reducing solution for approximately 12 h. After 12 h, 62 mg of sodium iodoacetate were added to each

vessel and the mixture was stirred. The pH was maintained as near to 9–9.5 as possible by addition of 0.1 M sodium hydroxide. Alkylation was judged complete when pH became constant. Nitroprusside tests were carried out as a further check on reaction completeness.

Extraction of Keratin—Method B

Reducing solution at pH 10.5 was prepared by mixing 48 g urea (8 M), 0.7 ml 2-mercaptoethanol (0.1 M), and 3.0 ml 2-aminoethanol (0.5 M) and adjusting the volume to 100 ml with distilled water. Approximately 10–20 mg of washed keratin was then extracted in 1 ml of the reducing solution for 2 h at 37 C using a shaking water bath. The extraction was carried out in an atmosphere of moist nitrogen. After 2 h, the material was alkylated as before for Method A. The SCMK protein profile obtained using the mercaptoethanol extraction technique gave identical results to the pattern obtained with thioglycollic acid extraction.

Molecular Weight Determination

Laemmli (1970) type gels approximately 100 mm × 5.6 mm were cast using an 11% acrylamide concentration and a 3% stacking gel to improve resolution of separated proteins. The stock electrode buffer consisted of 0.025 M Tris, 0.192 M glycine and 0.1% sodium dodecyl sulphate. It was diluted 10 times immediately before use. Samples were prepared to give the following final composition—2% SDS, 10% glycerol, 0.001% bromophenol blue, 0.062 M Tris-HCl, pH 6.8, 1% EDTA, 10% mercaptoethanol. The proteins (1 mg/ml) were completely dissociated by immersing each sealed tube in boiling water for 5 min. Samples were electrophoresed at 4 mA per gel for approximately 4 h or until the bromophenol blue tracker dye reached the end of the gel. The position of the tracker dye in each gel was measured and recorded at the end of each run as was the total length of the gel before staining. Gels were stained with 0.25% Coomassie brilliant blue R250 in 10% acetic acid, destained in 10% acetic acid and scanned with a Joyce-Loeble chromoscan (wavelength 550 nm). Migration rate of components were calculated relative to the marker dye.

Polyacrylamide Gel Electrophoresis (PAGE)

High purity long format Gradipore gradient gels (produced from recrystallised materials) size 8.3 cm × 13 cm having a continuous concave polyacrylamide gradient from 2.5% to 28%, superimposed onto which is a cross linker gradient increasing from 3.5% to 6.2% relative to monomer (Margolis and Wrigley, 1975). Prepared gels were purchased from Universal Scientific Ltd., London, which also manufactured the two cell vertical electrophoresis tank used for the separations.

Stock buffer 6 g Tris, 28.8 g glycine and distilled water to 1 l, pH 8.3 was diluted 1:10 for use. Electrophoresis was started at 2 mA for 2 min, then increased to 30 mA (constant current) and was allowed to proceed until the marker dye had reached the end of the gel. Gels were removed carefully from the cassette and fixed in 7.5% TCA for 5 min and stained in 0.25% Coomassie blue R250 in 10% acetic acid for several hours. Destaining was in 10% acetic acid until all the bands were resolved.

Isoelectric Focussing

Gels prepared as described in the Materials and Methods section were cast on Gel Bond film (FMC Corporation, Marine Colloids Division, Bio Products, Rockland, Maine) and activated before use with a 5% glycerine solution at 75 C. The anolyte was 0.03 M orthophosphoric acid containing 8 M urea and the catholyte was 0.05 M sodium hydroxide containing 8 M urea. The gels after focussing were fixed for 1 h in a solution composed of 57.5 g TCA, 17.25 g sulphosalicylic acid and 500 ml distilled water. After fixation, the gels were stained for 3 h with 0.01% Coomassie blue R250 in 25% ethanol, 10% acetic acid, and 0.1% copper sulphate. The gel was destained in a solution containing 10% ethanol and 10% acetic acid (Marshall and Blagrove, 1979).

Two-Dimensional Electrophoresis in Discontinuous Sodium Dodecyl-Sulphate Polyacrylamide Gels

1st Dimension

Gel solution was prepared from 8 M urea, 6.5% acrylamide and 2% LKB ampholyte pH 4–6.5. Polymerisation was initiated by the addition of TEMED (10 μl in 10 ml solution) and ammonium persulphate (40 μl of 6% solution in 10 μl gel solution). Gels were allowed to set for 1 h and were prefocussed at 4 C for 1 h at 110 V before sample application. The lower (anode) compartment contained 0.01 M phosphoric acid, and the upper (cathode) compartment contained 0.02 M NaOH. After prefocussing, the cathodic solution was discarded and the electrolyte was removed. The sample was layered onto the gel and carefully overlaid with 0.1 ml of a 1:1 mixture of a solution containing 0.12% Tris, 48.05% urea (8 M) (pH 8.5), and 1% ampholine in water (2.5 ml Tris/buffered urea pH 8.4 + 0.05 ml ampholine + water to 5 ml). Finally, 0.02 M sodium hydroxide was carefully overlaid onto the Tris/buffered urea to completely fill the tube and the upper electrode chamber was filled with 0.02 M sodium hydroxide. Isoelectric focussing was carried out for 24 h at 250 V at +4 C.

2nd Dimension

SDS gels were cast in cassettes employing glass plates 120 × 180 mm, and 1.5 mm PVC spacers using the cassette stand and clamps provided by BioRad. Resolving gels contained 9.4% acrylamide, 0.6% bis-acrylamide, 6.4 M urea (deionised), 0.675 M Tris-HCl, pH 8.9 and 0.39–0.5% SDS. The urea was deionised and cyanates eliminated by passing stock solutions through a mixed bed ion exchange resin (Rexyn 1–300) at 4 C immediately before casting the gels (Stinson, 1977). Polymerisation was initiated by adding 0.0035% ammonium persulphate and 0.00524% TEMED to the degassed solution.

Stacking gels contained 3% acrylamide, 0.18% bisacrylamide, 0.125 M Tris-HCl, pH 6.8 and 0.5% SDS and were polymerised with 0.008% ammonium persulphate and 0.04% TEMED. Reservoir buffer contained 0.025 M Tris base, 0.192 M glycine, and 0.1% SDS, pH 8.4 (Goldsmith et al., 1979).

The first dimension tube gel was always run in duplicate, one gel being fixed for 1 h in 11.5% TCA and 3.5% sulphosalicylic acid and was stained with 0.01% Coomassie blue R250 in 25% ethanol, 10% acetic acid and 0.1% copper sulphate. The other gel was transferred to the trough in the upper buffer chamber of the Protean cell which already contained 2 ml of molten (55 C) agarose containing a trace of bromophenol blue to act as a marker of the ion front during electrophoresis. Electrophoresis was started at 2.5 mA per gel to stack proteins before increasing to 6 mA. Electrophoresis was allowed to continue until the dye front reached the bottom of the gel (ca. 6 h).

Appendix II

Phelsuma *Nomenclature*

The taxonomic and nomenclatural status of the *Phelsuma* from the Seychelles has been confused but has recently been the subject of a comprehensive study in the Zoology Department at Aberdeen University. It emerges that some nominal species in the Central Seychelles are conspecific and that the specific name *P. sundbergi* is appropriate for all populations except those of *P. astriata*. In this paper, the Praslin population is referred to as *P. s. sundbergi* whilst the Mahe population is referred to as *P. sundbergi longinsulae* (A. Gardner, personal communication). These names are synonymous with "*Phelsuma* Seychelles α form" and "*Phelsuma* Seychelles β form" respectively, as they are referred to in Thorpe and Gidding's (1981) preliminary study of gecko keratins.