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Low fossilisation potential of keratin protein revealed by experimental taphonomy

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ABSTRACT: Recent studies have suggested the presence of keratin in Cenozoic- to Mesozoic-aged fossils. However, ultrastructural studies revealing exposed melanosomes in many fossil keratinous tissues suggest that keratin should rarely, if ever, be preserved. In this study, keratin's stability through diagenesis was tested using microbial decay and maturation experiments on various keratinous structures. The residues were analyzed by pyrolysis-gas chromatography-mass spectrometry and compared to unpublished feather and hair fossils and published fresh and fossil melanin from squid ink. Results show that highly matured feathers (200–250 °C/250 bars/24 hours) become a volatile-rich, thick fluid with semi-distinct pyrolysis compounds from those observed in less degraded keratins (i.e., fresh, decayed, moderately matured, and decayed and moderately matured), suggesting hydrolysis of peptide bonds and potential degradation of free amino acids. Neither melanization nor keratin (secondary) structure (e.g., α- vs. β-keratin) produced different pyrograms – melanin pyrolysates are largely a subset of those from proteins and proteins have characteristic pyrolysates. Analyses of fossil fur and feather lacked amides, succinimide, and piperazines (present even in highly matured keratin) and showed pyrolysis compounds more similar to fossil and fresh melanin than to non-matured or matured keratin. Although the highly matured fluid was not water soluble at room temperature, it readily dissolved at elevated temperatures easily attained during diagenesis, meaning it can leach away from the fossil. Future interpretations of fossils must consider that calcium phosphate and pigments are the only components of keratinous structures known to survive fossilisation in mature sediments.

KEY WORDS: keratin, protein, experimental taphonomy, Py-GC-MS, fossilisation

BIOMOLECULES vary in preservation potential. From least to most robust, these are roughly: nucleic acids, proteins, carbohydrates, aromatics, and lipids (Briggs and Summons 2014). The fossilization potential of these biomolecules can also be influenced by the interaction with inorganic components (Curry *et al.* 1991). Keratin is a diverse family of fibrous structural proteins and a common component of vertebrate tissues. Some keratinous structures are hardened through deposition of calcium phosphate salts (Pautard 1964) such as baleen, claws and feather rachises (Blakey *et al.* 1963). These salts are known from the fossil record (Mayr *et al.* 2016; Vinther *et al.* 2016) as thin, usually white, phosphate sheets (Benton *et al.* 2008; Bergmann *et al.* 2010). Tissues bearing melanin—a widely distributed group of pigments, also preserve well as original organic remains (Vinther *et al.* 2008; Vinther 2015). However, the survival of keratin protein itself remains controversial given that keratinous materials such as feathers or textiles are considered to have poor preservation in the archaeological record (Hargrave 1960; Messinger 1965; Brom 1986; Reinhard & Bryant 1992; Rogers *et al.* 2002; Dove *et al.* 2005). Studies have claimed to have found evidence of intact keratin based on immunohistochemistry experiments (Schweitzer *et al.* 1999*a*, 1999*b*; Moyer *et al.* 2016; Pan *et al.* 2016). These experiments indicate the preservation of tertiary protein structure, but use a method prone to false positives or statistical artefacts (Buckley *et al.* 2007; True 2008; Bern *et al.* 2009). Other studies on fossil keratinous structures have relied on infrared spectroscopy, recovering amide bands (Manning *et al.* 2009; Edwards *et al.* 2011), although pyrolysis-gas chromatography-mass spectrometry (Py-GC-MS) data did not yield any unambiguous markers for amino acids. Here we determine through taphonomic experiments what signatures degraded and intact keratin might leave in the fossil record and clarify if keratin is likely to persist in exceptionally preserved fossil material over million-year time scales.

MATERIAL AND METHODS

Various integumentary structures, such as feathers, hair, and scales, were subjected to microbial decay and maturation treatments (Table 1; extended methods in Saitta *et al.* 2017). Samples were either (a) fresh, (b) microbially decayed (with a treatment using cultured, naturally occurring feather microbes in a salt broth at ~37 °C/50 days), (c) matured and not decay treated, or (d) matured after decay treatment. These were chemically analysed using Py-GC-MS (analytical details in Saitta *et al.* 2017). Samples were sealed in noble metal (Au^{90} Pd^{10}) capsules and maturation was performed in a cold-seal, water pressurized autoclave ranging from 100–250 °C (measured with a calibrated K-type thermocouple) and 250 bars for 24 hours. The product of the highest condition maturation experiments (250 °C/250 bars/24 hours) on feather samples was subjected to solubility tests in water at room temperature for a year and then in a steam autoclave at 121 °C for 45 minutes.

Analyses were compared to previously obtained and unreported Py-GC-MS data on fossil hair from the Eocene *Palaeochiropteryx* (Messel Shale, SMF-ME 11406a) and feather material from a Lower Eocene bird head (Fur Formation, Danekræ 200, MGUH 28.929). Associated sediment was also analysed as a control and these were compared to published fossil and modern squid ink data as a reference for melanin (Glass *et al.* 2012).

Institutional abbreviations

Fossil specimens for which novel data is presented are deposited at the following institutions: Geological Museum of Copenhagen (MGUH), Copenhagen, Denmark and Naturmuseum Senckenberg (SMF), Frankfurt, Germany.

RESULTS

Very similar pyrolysis compounds were observed in the fresh, decayed, moderately matured, and decayed and moderately matured samples, regardless of keratin type or melanisation (Fig. 1; extended results in Saitta *et al.* 2017) including acetic acid, nitriles, pyridines, benzenes, toluene, amides, pyrrole-related compounds, indoles, phenols, styrene, and 2,5-diketopiperazine. Highly matured feathers (250 °C/250 bars/24 hours) lost all structural integrity and became a viscous liquid with a strong odour. Pyrolysis compounds for these highly matured samples did not differ between the white and dark feathers. Highly matured feathers shared many similar categories of compounds with the less degraded samples (i.e., nitriles, benzenes, toluene, amides, pyrrole-related compounds, phenols, styrene, and 2,5-diketopiperazine) but specific compounds showed distinct variations and their pyrograms produced noticeably different 'fingerprints' (Fig. 1). Highly matured feathers had more diverse nitriles and amides and less diverse benzenes, pyrrole-related compounds, and phenols compared to the less degraded samples. The highly matured feather fluid product showed no sign of dissolution when left in water at room temperature for over one year. However, placing this same vial in a steam autoclave (121 °C/45 minutes) resulted in rapid dissolution. It should be noted that earlier maturation runs on white feathers matured at 200 °C and 250 °C (250 bars/24 hours) in an argon gas autoclave resulted in a similar

fluid that leaked out of the unsealed sample tubes and could not be analysed, which prompted the protocol ultimately used here.

Both the fossil hair and feathers and their associated sediments yielded pyrolysates containing nitrogen or sulphur (Table 2). Slight differences were apparent between the two localities, and the sediments yielded many compounds with similar affinities to the fossils, which may be due to the presence of aromatics, such as algal porphyrins. Although some categories of compounds are shared between the fossils and experimental keratin (Table 2), fossils lacked acetic acid, pyridines, toluene, amides, indoles, styrene, succinimide, and piperazines found in the experimental keratin. Furthermore, the fossils shared many categories of compounds with the melanin of squid ink: nitriles, benzenes, pyrrole-related compounds, thiophenes, and phenols (Table 2). The fossils, compared to the fresh and treated experimental keratin and melanin, showed many unique ketones, pyrrole-related compounds, and thiophenes. Caution must be taken when comparing Py-GC-MS datasets obtained separately (Fossil data analysed at MIT, keratin data analysed at the University of Newcastle); therefore, it is important to compare more general categories of compounds rather than precise chemical species between the experiment keratin and the fossils/sediment.

DISCUSSION

The pyrolysis results from the experimental keratin are consistent with previous studies using Py-GC-MS on keratin (Brebu and Spiridon 2011) and protein in general (Bland *et al.* 1998; Reeves and Francis 1998). Py-GC-MS can detect protein or degraded protein but cannot distinguish between different keratin types nor can it distinguish between melanized and non-

136 melanized keratin. This is not entirely unexpected given that proteins appear to produce fairly
137 characteristic pyrolysates. Different keratin types are not molecularly diverse enough to result in
138 distinct pyrolysates. Also, pyrolysis compounds from melanin (Glass *et al.* 2012; Dzierżęga-
139 Lęcznar *et al.* 2012) are largely a subset of those from protein; melanin is a polymer formed from
140 oxidation products of the amino acid tyrosine. Py-GC-MS can distinguish between highly
141 matured and less degraded keratin. Succinimide was found in the highly matured feathers. It is
142 known to be linked to non-enzymatic protein degradation, acting as an intermediate during
143 deamidation, racemization, and isomerization (Geiger and Clarke 1987; Stephenson and Clarke
144 1989). However, these studies observed low temperature reactions, and the succinimide detected
145 here would be a product of pyrolysis. Combined with the observed liquefication, the results
146 suggest a breakdown of the protein structure and peptide bonds, is likely coupled with further
147 breakdown of the free amino acids in the highly matured feathers since they experienced
148 temperatures within the range of many amino acid decomposition temperatures (Dunn and
149 Brophy 1932; Lien and Nawar 1974). Diketopiperazines were found in all of the experimental
150 keratin. Although they can be byproducts of peptide terminal cleavage (Martins and Carvalho
151 2007), those identified here likely formed during pyrolysis of either peptides or free amino acids.
152 It is possible that side chains from at least one amino acid are preserved in 2,5-diketopiperazine
153 homologs. Although beyond the scope of this study, such information could provide insight into
154 the original protein amino acid composition (e.g., keratins are highly conserved with high Cys,
155 Gly, Pro, and Ser and low His, Lys, Trp, and Glu). Similar insight might be gathered from
156 detailed examination of other characteristic protein pyrolysates such as amides and succinimide.
157 Thus, if a fossil were to contain these protein pyrolysis markers, there is potential to further
158 investigate their structure and elucidate the composition of the proteins they derived from.

However, such markers were not found in the fossil samples analysed here, making this line of investigation unnecessary, and the proposed method should first be verified to determine just how much variation in pyrolysates would be expected from different proteins and whether such variation could be diagnostically useful. Similarly, methodological advances allowing for analysis of Py-GC-MS data in a statistical framework would further improve comparisons of chemical composition.

No unambiguous protein markers were found in the geologic samples, either in the sediment or the fossil material. Unambiguous protein pyrolysis markers are identified here as those categories of compounds that are present in fresh or degraded experimental keratin and absent from melanins. The similarities in pyrolysates between the sediment and the fossil from both localities, including a suite of aromatic compounds, could suggest protein or amino acid presence. However, these pyrolysates have been found in melanins and could also be products of other aromatic compounds, such as humic acids and porphyrins (Meuzelaar 1977). Aromatic compounds are also formed during pyrolysis of biomacromolecules. Regardless, the lack of amides, succinimide, and piperazines in the fossils demonstrate that proteinaceous material is lacking. Therefore, the compounds in the fossils appear overall consistent with melanin than with keratin protein. TOF SIMS analysis of the same fossil mammal hair and other feathers from the Fur Formation confirm melanin preservation in these structures (Colleary *et al.* 2015, Gren *et al.* 2016). Furthermore, both melanin and the fossils contained thiophenes, absent from the experimental keratin – although thiophenes were found in what was likely melanized keratin by Brebu and Spiridon (2011), in addition to thiazoles, which are known phaeomelanin markers (Dzierżęga-Lęcznar *et al.* 2012). Thiophenes in fossils also occur through diagenetic incorporation of sulfur in iron-poor depositional environments (Glass *et al.* 2012). Manning *et al.*

(2009) and Edwards *et al.* (2011) presented Py-GC-MS data on fossil reptile and hadrosaur skin, claiming it supported the presence of keratin. Although some of the compounds reported included nitriles, thiophenes, and benzene derivatives which are known from proteins, these are also known from fossil melanin. Similar to the fur and feather fossils examined here, the compounds are not a good match to those expected from keratin, which should present itself as a broader suite of pyrolysates. Their reports of aliphatic compounds also strongly resemble those reported through lipid *in situ* geopolymerization (Briggs 1999; Gupta *et al.* 2007). Fourier transform infrared spectroscopy has also been used to identify amides in the fossil reptile and hadrosaur skin (Manning *et al.* 2009; Edwards *et al.* 2011). However, the diverse amides present in the highly matured feather fluid prove, not surprisingly, that the presence of amides does not necessitate the presence of intact proteins, as amides can be present in simpler biomolecules. Amides were not found in pyrolysis studies of the fossil material, which raises questions as to the validity of the FTIR results and suggests the need to confirm the presence of amides detected with FTIR using other methods such as Py-GC-MS. In general, Manning *et al.* (2009) and Edwards *et al.* (2011) fail to prove the presence of intact keratin or distinguish between potential protein breakdown products from melanin or other contaminants.

Artificial maturation is known to mimic the chemical changes that occur during diagenesis. Temperatures and pressures are kept high (e.g., 200–250 °C, which corresponds to the last stages of the oil window) for short durations (e.g., days–weeks) in order to speed up chemical reactions that would occur at relatively more moderate conditions but over longer durations (e.g., millions of years). With regards to organic preservation it is important to regard both the age of the fossil and its burial history. The Messel Oil Shale, for example, is considered "thermally immature" (Ocampo *et al.* 1985) so our failure to find key protein pyrolysates in a

Messel fossil bodes poorly for keratin preservation potential in fossils of equal or greater age than the Lower Eocene and possibly even younger fossils.

Our inability to reproduce the results of McNamara *et al.* (2013, p.2) is explained by a *lapsus calami* in their methods which should state "each [maturation] experiment lasted for 1 h" not "24 h" (McNamara personal communication 2016). However, the absence of keratin proteins in the fossil record suggests the timeframe of our maturation experiments, in which original keratin protein was absent, may be a better approximation of the conditions that occur during diagenesis. Maturation experiments must be of a duration long enough to ensure accurate modeling of diagenetic chemical reactions, and the one hour experiments of McNamara *et al.* (2013) are not sufficient to draw conclusions as to the preservation potential of keratin in the fossil record.

Although the highly matured feather fluid was not water soluble at room temperature, which is expected given the relatively high abundance of hydrophobic and neutral amino acids compared to hydrophilic amino acids (at pH 7) in β-keratin (Dalla Valle *et al.* 2009), it was easily dissolved in our experiments at elevated temperatures that might be expected during diagenesis. This provides a model for how hydrolyzed and degraded proteins might aqueously dissolve and leach away from fossils.

Feathers and textiles produced from keratin are rare from archaeological sites. Toxic metals can slow keratin degradation and allow the peptides to persist in some sites, but the presence of iron only allows for the preservation of a corrosion cast. Although keratin appears robust compared to many other proteins, it is not resistant to millennia of diagenetic forces (Hargrave 1960; Messinger 1965; Brom 1986; Reinhard & Bryant 1992; Rogers *et al.* 2002; Dove *et al.* 2005). Studies of well-preserved fossil feathers have found that organic residues

consist of melanosomes, usually in alignment along barb and barbule axes (Vinther *et al.* 2008; Vinther 2015). Furthermore, colour patterns are frequently observed (Vinther *et al.* 2008; Field *et al.* 2013; Vinther *et al.* 2016), which are observed through the macroscopic presence or absence of organic material composed of melanosomes. Areas lacking melanosomes preserve nothing but feather impressions in the rock matrix (Vinther *et al.* 2008). No substance conclusively attributable to keratin has been found in any well-preserved fossil feathers, even though amorphous organic residues in more weathered specimens from localities such as the Jehol or Yanliao biota and the Green River Formation have been suggested to be keratin by some (Schweitzer *et al.* 1999*a*; McNamara *et al.* 2013). The nature of this amorphous organic residue remains to be fully described. However, Lindgren *et al.* (2015) found signatures consistent with melanin using TOF SIMS and FTIR on feathers preserved in a Late Jurassic specimen of *Anchiornis huxleyi* preserved as solid organic material along with traces of melanosomes as impressions and merged blocks of melanosomes. The only other constituent preserved in the feathers were calcium phosphate, either derived from the hardened keratin as observed elsewhere (Mayr *et al.* 2016; Vinther *et al.* 2016) or through secondary mineralisation.

CONCLUSION

The production of a viscous fluid from highly matured feathers with a characteristic Py-GC-MS signature compared to less degraded keratin suggests that keratin degrades during diagenesis and catagenesis. Py-GC-MS of fossil keratinous structures supports our experimental data, showing that non-lipid, nitrogen and sulfur containing compounds more closely match recalcitrant fossil and fresh melanin than keratin protein. Keratin can decay through microbial action and

diagenetic hydrolysis of peptide bonds. An analogous process to collagen gelatinization (Pfretzschner 2006) might occur in keratin to produce a viscous fluid. Unlike proteins encapsulated within a mineralized matrix, such as bone or shell, epidermal keratin is not stabilised nor shielded from the environment, making it more prone to microbial decay, aqueous hydrolysis, and ultimately dissolution, leaching from the fossil at higher temperatures during later diagenesis. Keratin degradation means that calcium phosphate and melanin are likely the only remaining components of keratinous structures detectable in fossils. Reports of keratin proteins in fossils, especially of Mesozoic age (Schweitzer *et al.* 1999*a*, 1999*b*; Moyer *et al.* 2016; Pan *et al.* 2016), should be reexamined. Furthermore, thermal experiments that report preserved morphological structures of feathers with weak and diffuse antibody signal persisting after a decade-long exposure at 350 °C (Moyer *et al.* 2016) not only contradict the results here, they are likely impossible given reported thermal decomposition points of amino acids (Dunn & Brophy 1932; Lien & Nawar 1974). Immunohistological keratin detection in such experimental and fossil cases are likely false positives from humic acids formed through degradation of biomolecules (Collins *et al.* 1992). This is best exemplified by the claims for immunodetection of keratin in feathers which had been converted to ash (covered in foil and placed in a 350 °C dry oven for a decade) (Moyer *et al.* 2016). Py-GC-MS, in contrast, presents itself as a useful tool for identifying protein-derived organics (i.e., those containing amino acids) in fossil samples.

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DATA ARCHIVING STATEMENT

Data for this study are available in the Dryad Digital Repository: http://datadryad.org/review?doi=doi:10.5061/dryad.h02q0

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FIGURES

433

434 **Fig. 1.** Total ion pyrograms of feathers. Subpanel shows highly matured viscous fluid from white
435 feathers (scale bar represents 500 μm). ?, tentative compound identification. Letters, compounds
436 present in less degraded samples. a, acetic acid. b, propanenitrile. c, 2-methyl propanenitrile. d,
437 2-methyl butanenitrile. e, pyridine. f, pyrrole. g, toluene. h, methyl pyridine. i, methyl pyrrole. j,
438 ethyl benzene. k, styrene. l, ethyl pyrrole. m, phenol. n, 4-methyl phenol. o, ethyl cyanobenzene.
439 p, propyl cyanobenzene. q, indole. r, methyl indole. s, 2,5-diketopiperazine. *t*, trimethyl
440 methoxyphenol. *u*, octadecanamide. Numbers, compounds exclusive to highly matured samples.
441 1, benzene. 2, acetamide. 3, methyl pentanenitrile. 4, propanamide. 5, C1-styrene. 6, methyl
442 butanamide. 7, C1-phenol. 8, hexanamide. 9, methyl pentanamide. 10, piperidinone. 11,
443 hexadecyl nitrile. 12, hexadecanamide. 13, docosenenyl nitrile. 14, eicosanamide. 15,
444 docosenamide. 16, docosanamide. 17, pyrrolidine, 1-acetyl-. 18, N-[2-hydroxyethyl]succinimide.
445 Slight retention time misalignment for the same peak between samples is a result of running
446 Py/GC/MS at different times.

447

448 **TABLES**

449

450 **Table 1.** List of maturation runs.

451

452 **Table 2.** Comparison of pyrolysates.

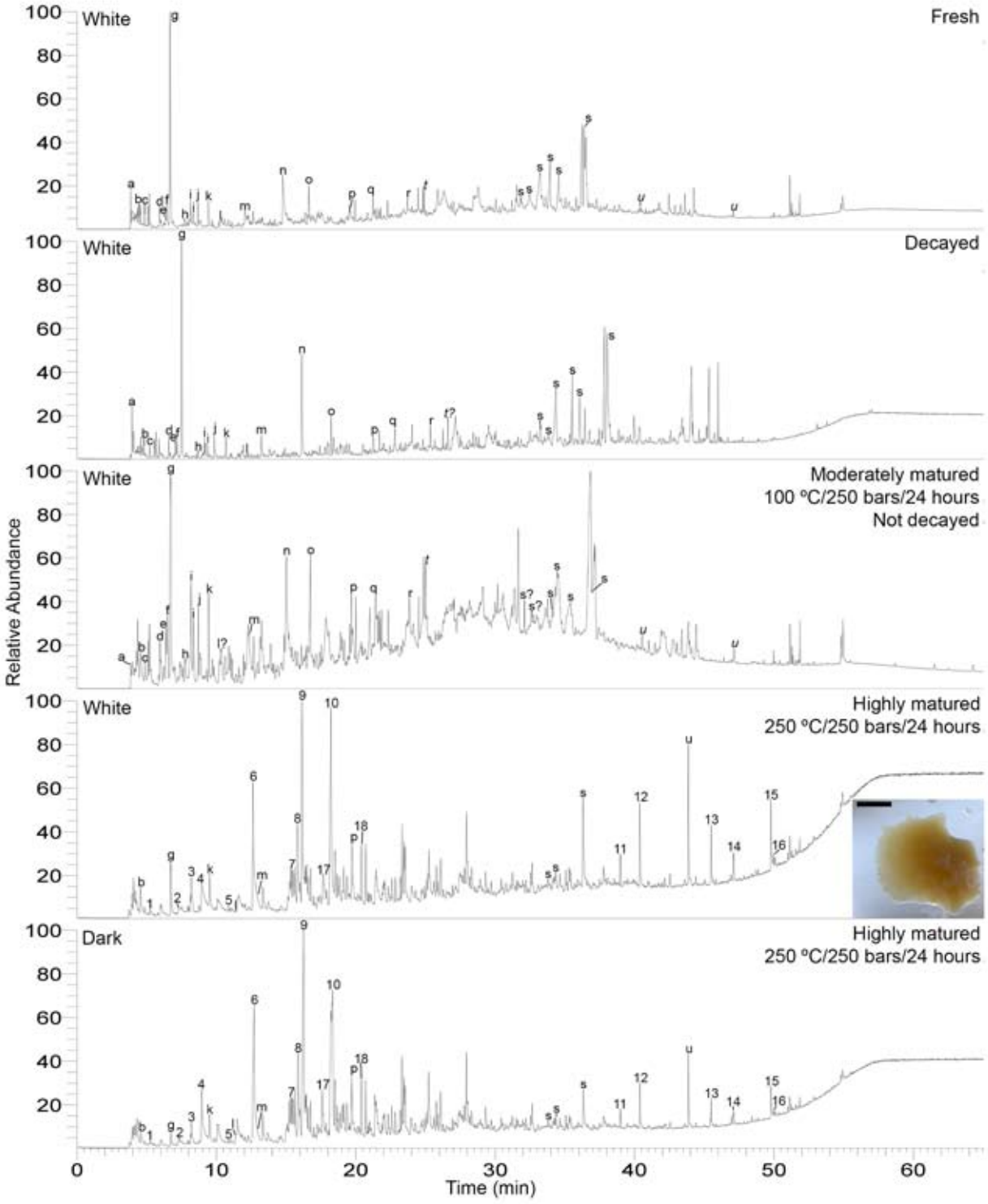
White
Fresh
White
Decayed
White
Moderately matured
100 ºC/250 bars/24 hours
Not decayed
White
Highly matured
250 ºC/250 bars/24 hours
Dark
Highly matured
250 ºC/250 bars/24 hours
Relative Abundance
Time (min)
0
10
20
30
40
50
60
100
80
60
40
20

TABLE 1. LIST OF MATURATION RUNS

Sample	Notes
Highly matured - 250°C, 250 bars, 24 hours	
White feathers*	Chicken (*Gallus gallus*); Leg region
Dark feathers*	Turkey (*Meleagris gallopavo*); Leg region; Colour gradient (from proximal to distal) of white, grey, black, and iridescent
Moderately matured - 100°C, 250 bars, 24 hours	
Feathers – iridescent*	Turkey; Wing covert
Feathers – white*	Chicken; Wing covert
Feathers – black*	Chicken; Back/neck region; White fringe on vane
Avian scutate scales†	Turkey
Avian reticulate scales§	Turkey; Multiple scales with associated epidermis
Turkey beard – adult*†	Dried samples; Small portion of epidermis still attached
Turkey beard – juvenile*†	Portion of epidermis still attached
Crocodilian scale – black§	Nile crocodile (*Crocodylus niloticus*); Flank region; Predominantly black colouration
Crocodilian scale – white§	Nile crocodile; Flank region; Predominantly white/light colouration
Mammalian hair#	Horse (*Equus ferus*) mane; Black colour; 'Outgroup'

Decayed feathers - iridescent*	Turkey; Back region
Decayed feathers - white*	Chicken; Back/neck region
Decayed feathers - black*	Chicken; Back/neck (white fringe on vane) and tail regions

Note: Samples that are not listed as decayed are fresh samples.

*Feather φ-keratin.

†Avian scale-type φ-keratin.

§Non-featherlike β-keratin.

#α-keratin.

TABLE 2. COMPARISON OF PYROLYSATES

Compound	White feather		Messel Shale		Fur Formation		Squid ink*	
	Fresh	Highly matured	Bat fur fossil	Sediment	Feather fossil	Sediment	Fossil	Modern
Acetic acid	X							
Toluene	X	X	?	?	?	?	X	X
2,3-epoxycarane					?			
N-[2-hydroxyethyl]succinimide		X						
2,5-diketopiperazine	X	X						
3,4-dimethyl benzaldehyde			X	X		?		
Dimethyl cyclopentene			?		X	?		
Dimethyl naphthalene			X	X	X	X		
Various ketones								
4-methyl-1-penten-3-one			?		?	?		
2-methyl cyclooctanone					X			
2-methyl cycloheptanone					X			
Acetophenone			?	X	?	X		
Piperidinone		X						
1-(2-vinylphenyl) ethanone					?	X		
2H-Inden-2-one, 1,3-dihydro-1-(1-oxopropoxy)-			?	?		?		
Benzophenone			X	?	X	X		
Nitriles								
Propanenitrile	X	X						
Methyl pentanenitrile		X						
Hexadecyl nitrile		X						
Docosenenyl nitrile		X						
2-methyl propanenitrile	X							
2-methyl butanenitrile	X							
Dimethyl benzonitrile			X	X				
2-phenyl acetonitrile							X	X
3-phenyl propanenitrile							X	X
Pyridines								
Pyridine	X		?	?	?	?	X	X
Methyl pyridine	X						X	X
Benzenes								
Benzene		X	?	?		?		
Propyl cyanobenzene	X	X					X	
Ethyl benzene	X		X	X	?	X	X	
Ethyl cyanobenzene	X							
Propyl benzene			?	?	X	X		
Amides								
Acetamide		X						
Propanamide		X						
Methyl butanamide		X						
Hexanamide		X						
Methyl pentanamide		X						
Hexadecanamide		X						
Octadecanamide	X	X						
Eicosanamide		X						
Docosenamide		X						
Docosanamide		X						
Pyrrole-related compounds								
Ethyl pyrrole	X	X						
Pyrrolidine, 1-acetyl-		X						
Pyrrole	X						X	X
Methyl pyrrole	X		?	X		?	X	X
Dimethyl pyrrole			X	X				
Trimethyl pyrrole			?	?				
Ethyl-methyl-1H-pyrrole			X					
Ethyl-dimethyl-1H-pyrrole				?				
Ethylmethyl-1H-pyrrole-dione			X	?				

Thiophenes								
Thiophene							X	
Methyl thiophene							X	
Ethyl thiophene							X	
Propyl thiophene			?	?	?	?	X	
Dimethyl thiophene			X	X				
Trimethyl thiophene			?			?		
Butyl thiophene			?	?	?	?		
Indoles								
Indole	X		?	?		?	X	X
Methyl indole	X		?	?		?	X	X
Phenols								
Phenol	X	X	X		X	X	X	X
Methyl phenol		X						
4-methyl phenol	X		?	?		?	X	X
5-methyl phenol							X	X
Ethyl (or dimethyl) phenol			?	?		?	X	X
Trimethyl methoxyphenol	X							
2-methyl phenol			?	?	?	?		
Di-ter-butyl phenol			?					
Styrene								
Styrene	X	X	?	?		?		
Methyl styrene		X						

*from Glass et al. (2012).