Rods and cones in an enantiornithine bird eye from the Early Cretaceous Jehol Biota

Gengo Tanaka a, Baochun Zhou b,*, Yunfei Zhang b, David J. Siveter c, Andrew R. Parker d

* Institute of Liberal Arts and Science, Kanazawa University, Kakuma-machi, Kanazawa 920-1192, Japan
b Shanghai Natural History Museum, 510 West Beijing Road, Shanghai 200041, China
c School of Geography, Geology and the Environment, University of Leicester, Leicester LE1 7RH, UK
d Green Templeton College, University of Oxford, 43 Woodstock Road, Oxford OX2 6HG, UK

* Corresponding author.
E-mail address: zhoubch@sstm.org.cn (B. Zhou).

Abstract

Extant birds have an extensive spectral range of colour vision among vertebrates, but evidence of colour vision among extinct birds has hitherto been lacking. An exceptionally well-preserved extinct enantiornithine fossil bird from the Early Cretaceous Jiufotang Formation (120 Ma) of Liaoning, China, provides the first report of mineralised soft tissue of a bird eye. Cone cells are identified, which have preserved oil droplets falling between wide ranges of size that can be compared with an extant house sparrow. The size distribution of oil droplets of extant birds demonstrates good correlation between size and the detectable wavelength range of the cone cells: UV-sensitive cones contain the smallest oil droplets, while red-sensitive cones possess the largest. The data suggests that this Early Cretaceous bird could have possessed colour vision.

Keywords: Evolution, Palaeobiology, Biological sciences

1. Introduction

Having evolved from dinosaurs during the Jurassic, birds then diversified during the Cretaceous (e.g., Xu et al., 2014). Extant diurnal birds possess colour vision,
Fig. 1. An enantiornithine bird from the Jiufotang Formation, Early Cretaceous, Liaoning, China (a–f) and retina of an extant house sparrow Passer domesticus (g,h). (a) Completely preserved specimen (SNHM: 6105). (b) Enlarged eye region of (a) showing eye orbit within the white dotted line, black material (white arrow) and sample point (yellow arrow) of the fragment of (c). (c) Digital microphotograph of an eye fragment. (d) SEM image of an eye fragment. (e) SEM of cone and rod cells with their oil droplets. (f) Labelled image of (e) showing position of oil droplets (red circles), cone (blue solid line) and rod (yellow solid line) cells. (g) SEM image of rods (yellow solid line), cones (blue solid line) and oil droplets (red circles). (h) Transmitted microphotograph of oil droplets. Scale bars: a = 2 cm; b = 5 mm; c,d = 100 μm; e–h = 10 μm.
Table 1. Area of oil droplets in an extant house sparrow *Passer domesticus*. The cone type to which each oil droplet belongs was determined based on the methods of Kolb and Jones (1982).

| No. | Cone type | Area of oil droplet (μm²) | No. | Cone type | Area of oil droplet (μm²) |
|-----|-----------|---------------------------|-----|-----------|---------------------------|
| 1   | green     | 2.9                       | 159 | red       | 2.7                       |
| 2   | green     | 2.8                       | 160 | double    | 2.6                       |
| 3   | blue      | 1.1                       | 161 | blue      | 1.8                       |
| 4   | double    | 2.8                       | 162 | double    | 2.7                       |
| 5   | red       | 3.8                       | 163 | violet    | 0.6                       |
| 6   | red       | 3.0                       | 164 | double    | 2.7                       |
| 7   | blue      | 0.9                       | 165 | red       | 3.6                       |
| 8   | red       | 3.0                       | 166 | blue      | 0.9                       |
| 9   | green     | 2.0                       | 167 | green     | 2.8                       |
| 10  | red       | 3.8                       | 168 | red       | 3.4                       |
| 11  | red       | 3.6                       | 169 | green     | 2.1                       |
| 12  | blue      | 1.4                       | 170 | violet    | 0.6                       |
| 13  | green     | 2.7                       | 171 | violet    | 0.7                       |
| 14  | red       | 3.2                       | 172 | red       | 4.3                       |
| 15  | red       | 4.2                       | 173 | double    | 3.1                       |
| 16  | double    | 3.8                       | 174 | double    | 3.0                       |
| 17  | green     | 2.3                       | 175 | double    | 2.7                       |
| 18  | double    | 2.5                       | 176 | blue      | 1.0                       |
| 19  | green     | 3.1                       | 177 | double    | 2.9                       |
| 20  | green     | 3.1                       | 178 | green     | 2.3                       |
| 21  | green     | 2.0                       | 179 | violet    | 0.4                       |
| 22  | green     | 2.3                       | 180 | violet    | 0.7                       |
| 23  | green     | 2.7                       | 181 | green     | 3.3                       |
| 24  | green     | 2.5                       | 182 | green     | 3.0                       |
| 25  | green     | 2.3                       | 183 | violet    | 0.7                       |
| 26  | green     | 2.3                       | 184 | red       | 3.9                       |
| 27  | green     | 2.5                       | 185 | violet    | 0.4                       |
| 28  | green     | 3.2                       | 186 | green     | 3.2                       |
| 29  | red       | 3.1                       | 187 | double    | 2.2                       |
| 30  | green     | 2.4                       | 188 | double    | 3.0                       |
| 31  | green     | 2.5                       | 189 | violet    | 0.9                       |
| 32  | green     | 3.0                       | 190 | green     | 2.8                       |
| 33  | green     | 2.1                       | 191 | red       | 4.1                       |
| 34  | double    | 2.5                       | 192 | double    | 3.3                       |
| 35  | green     | 2.2                       | 193 | double    | 3.2                       |

(Continued)
Table 1. (Continued)

| No. | Cone type | Area of oil droplet (μm²) | No. | Cone type | Area of oil droplet (μm²) |
|-----|-----------|---------------------------|-----|-----------|---------------------------|
| 36  | violet    | 0.6                       | 194 | double    | 2.9                       |
| 37  | double    | 2.2                       | 195 | red       | 3.9                       |
| 38  | green     | 1.7                       | 196 | green     | 3.2                       |
| 39  | double    | 2.3                       | 197 | double    | 2.7                       |
| 40  | blue      | 1.0                       | 198 | blue      | 0.9                       |
| 41  | green     | 1.9                       | 199 | green     | 3.4                       |
| 42  | double    | 3.2                       | 200 | violet    | 0.8                       |
| 43  | double    | 3.4                       | 201 | violet    | 0.9                       |
| 44  | double    | 2.5                       | 202 | violet    | 0.7                       |
| 45  | double    | 2.7                       | 203 | violet    | 0.4                       |
| 46  | double    | 3.4                       | 204 | blue      | 1.2                       |
| 47  | green     | 3.4                       | 205 | red       | 3.5                       |
| 48  | double    | 2.9                       | 206 | violet    | 1.0                       |
| 49  | double    | 3.0                       | 207 | double    | 3.0                       |
| 50  | red       | 4.2                       | 208 | red       | 3.5                       |
| 51  | double    | 2.3                       | 209 | double    | 3.1                       |
| 52  | double    | 2.6                       | 210 | green     | 3.1                       |
| 53  | green     | 2.9                       | 211 | double    | 2.6                       |
| 54  | double    | 3.0                       | 212 | green     | 2.5                       |
| 55  | red       | 4.1                       | 213 | blue      | 0.6                       |
| 56  | red       | 4.5                       | 214 | violet    | 0.6                       |
| 57  | double    | 3.0                       | 215 | violet    | 0.5                       |
| 58  | double    | 3.2                       | 216 | violet    | 0.7                       |
| 59  | double    | 2.9                       | 217 | violet    | 0.7                       |
| 60  | double    | 2.9                       | 218 | blue      | 1.1                       |
| 61  | green     | 2.4                       | 219 | violet    | 0.7                       |
| 62  | red       | 4.0                       | 220 | red       | 3.9                       |
| 63  | violet    | 1.1                       | 221 | double    | 2.8                       |
| 64  | double    | 2.8                       | 222 | double    | 2.8                       |
| 65  | double    | 3.2                       | 223 | double    | 2.5                       |
| 66  | double    | 2.5                       | 224 | double    | 3.3                       |
| 67  | violet    | 0.7                       | 225 | blue      | 0.7                       |
| 68  | violet    | 0.9                       | 226 | double    | 3.4                       |
| 69  | double    | 2.2                       | 227 | red       | 3.9                       |
| 70  | red       | 3.2                       | 228 | double    | 2.6                       |
| 71  | double    | 2.5                       | 229 | red       | 3.8                       |
| 72  | double    | 2.1                       | 230 | green     | 3.3                       |
| 73  | red       | 2.8                       | 231 | double    | 2.8                       |

(Continued)
Table 1. (Continued)

| No. | Cone type | Area of oil droplet (μm²) | No. | Cone type | Area of oil droplet (μm²) |
|-----|-----------|----------------------------|-----|-----------|----------------------------|
| 74  | blue      | 1.6                        | 232 | blue      | 1.4                        |
| 75  | double    | 2.4                        | 233 | red       | 3.2                        |
| 76  | double    | 3.2                        | 234 | blue      | 1.0                        |
| 77  | red       | 3.4                        | 235 | green     | 2.9                        |
| 78  | blue      | 1.1                        | 236 | violet    | 0.9                        |
| 79  | blue      | 1.6                        | 237 | double    | 2.3                        |
| 80  | red       | 3.5                        | 238 | violet    | 0.5                        |
| 81  | double    | 3.2                        | 239 | double    | 2.7                        |
| 82  | green     | 3.5                        | 240 | double    | 2.5                        |
| 83  | red       | 3.4                        | 241 | green     | 2.6                        |
| 84  | double    | 2.3                        | 242 | double    | 1.9                        |
| 85  | blue      | 1.4                        | 243 | double    | 2.5                        |
| 86  | blue      | 1.2                        | 244 | double    | 2.5                        |
| 87  | double    | 2.8                        | 245 | blue      | 0.4                        |
| 88  | violet    | 1.0                        | 246 | blue      | 0.8                        |
| 89  | red       | 2.9                        | 247 | double    | 2.3                        |
| 90  | green     | 1.5                        | 248 | double    | 2.6                        |
| 91  | green     | 2.1                        | 249 | violet    | 0.3                        |
| 92  | violet    | 1.2                        | 250 | violet    | 0.6                        |
| 93  | double    | 3.3                        | 251 | double    | 2.5                        |
| 94  | double    | 3.7                        | 252 | red       | 3.5                        |
| 95  | double    | 2.4                        | 253 | double    | 2.1                        |
| 96  | red       | 3.4                        | 254 | red       | 3.4                        |
| 97  | double    | 3.2                        | 255 | double    | 2.7                        |
| 98  | green     | 2.7                        | 256 | violet    | 0.6                        |
| 99  | red       | 3.4                        | 257 | double    | 3.2                        |
| 100 | double    | 2.5                        | 258 | violet    | 0.5                        |
| 101 | blue      | 1.3                        | 259 | double    | 3.1                        |
| 102 | green     | 2.8                        | 260 | double    | 2.7                        |
| 103 | blue      | 1.0                        | 261 | violet    | 0.5                        |
| 104 | red       | 3.6                        | 262 | green     | 2.8                        |
| 105 | double    | 2.6                        | 263 | violet    | 0.9                        |
| 106 | violet    | 1.1                        | 264 | double    | 2.3                        |
| 107 | double    | 2.9                        | 265 | red       | 4.0                        |
| 108 | blue      | 0.7                        | 266 | violet    | 0.8                        |
| 109 | red       | 4.3                        | 267 | violet    | 0.9                        |
| 110 | green     | 2.7                        | 268 | green     | 2.7                        |
| 111 | green     | 2.9                        | 269 | violet    | 1.1                        |
Table 1. (Continued)

| No. | Cone type | Area of oil droplet ($\mu$m$^2$) | No. | Cone type | Area of oil droplet ($\mu$m$^2$) |
|-----|-----------|-------------------------------|-----|-----------|-------------------------------|
| 112 | green     | 2.7                           | 270 | violet    | 1.0                           |
| 113 | green     | 2.5                           | 271 | green     | 2.8                           |
| 114 | green     | 2.0                           | 272 | violet    | 0.8                           |
| 115 | blue      | 0.9                           | 273 | double    | 3.0                           |
| 116 | green     | 3.8                           | 274 | blue      | 0.9                           |
| 117 | blue      | 1.7                           | 275 | double    | 3.2                           |
| 118 | violet    | 1.1                           | 276 | green     | 2.6                           |
| 119 | violet    | 1.3                           | 277 | violet    | 0.7                           |
| 120 | violet    | 1.3                           | 278 | violet    | 0.7                           |
| 121 | violet    | 1.0                           | 279 | double    | 3.5                           |
| 122 | red       | 2.9                           | 280 | double    | 3.6                           |
| 123 | double    | 2.0                           | 281 | red       | 3.9                           |
| 124 | blue      | 1.3                           | 282 | double    | 2.5                           |
| 125 | violet    | 1.1                           | 283 | green     | 2.7                           |
| 126 | violet    | 0.9                           | 284 | blue      | 0.8                           |
| 127 | double    | 2.5                           | 285 | green     | 3.1                           |
| 128 | red       | 3.3                           | 286 | blue      | 0.9                           |
| 129 | blue      | 1.4                           | 287 | violet    | 0.5                           |
| 130 | violet    | 1.0                           | 288 | green     | 2.6                           |
| 131 | violet    | 0.8                           | 289 | green     | 3.6                           |
| 132 | green     | 2.5                           | 290 | green     | 3.5                           |
| 133 | violet    | 1.0                           | 291 | double    | 2.5                           |
| 134 | red       | 2.8                           | 292 | red       | 3.9                           |
| 135 | violet    | 0.5                           | 293 | red       | 3.8                           |
| 136 | double    | 2.3                           | 294 | double    | 3.0                           |
| 137 | green     | 2.9                           | 295 | green     | 3.3                           |
| 138 | double    | 1.9                           | 296 | violet    | 1.2                           |
| 139 | red       | 3.2                           | 297 | green     | 3.2                           |
| 140 | double    | 2.6                           | 298 | red       | 3.8                           |
| 141 | double    | 2.4                           | 299 | violet    | 0.7                           |
| 142 | double    | 2.7                           | 300 | violet    | 0.7                           |
| 143 | green     | 2.3                           | 301 | double    | 3.1                           |
| 144 | red       | 2.5                           | 302 | red       | 3.6                           |
| 145 | double    | 2.2                           | 303 | green     | 2.5                           |
| 146 | violet    | 0.9                           | 304 | double    | 3.4                           |
| 147 | red       | 4.1                           | 305 | double    | 3.0                           |
| 148 | double    | 3.0                           | 306 | double    | 3.1                           |
| 149 | green     | 2.4                           | 307 | red       | 3.1                           |

(Continued)
involving five types of cone cells that together can discriminate a spectrum from ultraviolet to red (Hart, 2001a; Hart, 2004; Cuthill, 2006; Bowmaker, 2008). The discovery of fossilised melanosomes in some Mesozoic animals suggests that they were adapted to a colourful world in the geological past (Li et al., 2010; Li et al., 2012; Zhang et al., 2010; Barden et al., 2011). However, compelling evidence of colour vision in fossil birds has not been reported because photoreceptors are not usually fossilised. A fossilised visual photoreceptor (Fröhlich et al., 1992; Duncan and Briggs, 1996; Tanaka et al., 2009; Tanaka et al., 2014; Schoenemann and Clarkson, 2013; Vannier et al., 2016) or a mold equivalent (Schoenemann et al., 2012) has only been reported in a total of six fossil taxa: five arthropods and one fish. Thus, examples of fossilised visual photoreceptors from extinct birds would shed light on the retinal anatomy of fossil birds and allow an assessment of whether these taxa also had the ability to distinguish colours. The spectral sensitivity of a cone retinal photoreceptor depends upon its particular combination of an opsin photopigment and a coloured oil droplet, which is located in the distal tip of the inner segment of the cone cell and functions as a cut-off filter for incoming light (Hart, 2001a; Hart, 2004; Bowmaker, 2008; Ohtsuka, 1985; Kram et al., 2010). The filtering of the specific wavelength range is also achieved by the ellipsoid positioned in front of the oil droplet, which is located in the distal tip of the inner segment of the cone cell and functions as a cut-off filter for incoming light (Hart, 2001a; Hart, 2004; Bowmaker, 2008; Ohtsuka, 1985; Kram et al., 2010). The filtering of the specific wavelength range is also achieved by the ellipsoid positioned in front of the oil droplet, which is located in the distal tip of the inner segment of the cone cell and functions as a cut-off filter for incoming light (Hart, 2001a; Hart, 2004; Bowmaker, 2008; Ohtsuka, 1985; Kram et al., 2010). The filtering of the specific wavelength range is also achieved by the ellipsoid positioned in front of the oil droplet, which is located in the distal tip of the inner segment of the cone cell and functions as a cut-off filter for incoming light (Hart, 2001a; Hart, 2004; Bowmaker, 2008; Ohtsuka, 1985; Kram et al., 2010). The filtering of the specific wavelength range is also achieved by the ellipsoid positioned in front of the oil droplet, which is located in the distal tip of the inner segment of the cone cell and functions as a cut-off filter for incoming light (Hart, 2001a; Hart, 2004; Bowmaker, 2008; Ohtsuka, 1985; Kram et al., 2010). The filtering of the specific wavelength range is also achieved by the ellipsoid positioned in front of the oil droplet, which is located in the distal tip of the inner segment of the cone cell and functions as a cut-off filter for incoming light (Hart, 2001a; Hart, 2004; Bowmaker, 2008; Ohtsuka, 1985; Kram et al., 2010). The filtering of the specific wavelength range is also achieved by the ellipsoid positioned in front of the oil droplet, which is located in the distal tip of the inner segment of the cone cell and functions as a cut-off filter for incoming light (Hart, 2001a; Hart, 2004; Bowmaker, 2008; Ohtsuka, 1985; Kram et al., 2010).
Fig. 2. SEM images of the retinas of an Early Cretaceous enantiornithine bird and an extant house sparrow *Passer domesticus*. (a) A fragment of the retina of the fossil bird preserving many oil droplets (o) in the upper left region of the broken line, pigment epithelium (p), and rods (r) and cones (c). (b) Magnified image of (a) showing oil droplets (red circles) and pigment epithelium (p), and rods (yellow solid line) and cones (blue solid line). (c) A fragment of the fossil pigment epithelium (p) with preserved fine fibrous structures. (d) An enlargement of the fossil pigment epithelium containing elongate melanosomes (m). (e) Transverse section of the retina of an extant house sparrow. The retina is composed of pigment epithelium (p), cones (c) and rods (r) with oil droplets, an outer nuclear layer (on), and an inner nuclear layer (in) oriented in the proximal-distal plane of the eyeball. (f) A fragment of the retina of an extant house sparrow. Many oil droplets...
droplets (red circles) were removed from cones. (g) Pigment epithelium (p) of an extant house sparrow. (h) An enlargement of pigment epithelium of an extant house sparrow containing elongate melanosomes (m). Scale bars: a, e = 50 μm; b–d, f–h = 10 μm.

7.9 and 6.1 μm, yellow oil droplets between 6.4 and 3.6 μm, and colourless oil droplets between 3.5 and 2.8 μm (Kram et al., 2010).

The lower Cretaceous (120 Ma) Jehol Konservat-Lagerstätte in western Liaoning, China, yields exquisitely preserved organisms from disparate taxa including...
Fig. 4. SEM images of the retinas of an Early Cretaceous enantiornithine bird and a natural dried extant house sparrow Passer domesticus. (a) A fragment of the retina of the fossil bird preserving many oil droplets (o) in the upper left region, pigment epithelium (p), and rods (c) and cones (c). (b) Enlarged image of (a) showing oil droplets (red circles), pigment epithelium (p), and rods (yellow solid line) and cones (blue solid line). (c) A fragment of the fossil pigment epithelium (p) with preserved fine fibrous structures. (d) An enlargement of the fossil pigment epithelium containing elongate melanosomes (m). (e) The photosensitive organ of an extant house sparrow that is composed of cones (blue solid line) and rods (yellow solid line) with oil droplets (red circles). (f) An enlargement of (e). Oil droplets (red circles) and cone and rods (c & r) are rather flattened but associated with each other. (g) Pigment
feathered dinosaurs, early birds, mammals with hair, complete arthropods, and plants (Zhou et al., 2003). Fine details are preserved because the organic material was originally transported by a pyroclastic density current and sealed within the pyroclastic flow (Jiang et al., 2014). The subadult fossil bird specimen reported herein belongs to a species of Enantiornithes documented from the Early Cretaceous of Liaoning (Chiappe and Walker, 2002).

2. Material and methods

The fossil bird specimen studied herein (Fig. 1a) is a subadult stage of an unnamed enantiornithine species (personal communication with Prof. Zhonghe Zhou, Institute of Vertebrate Paleontology and Paleoanthropology, Chinese Academy of Science). A small fragment of retina measuring approximately 4 mm² was removed from the posterodorsal margin of the fossil bird eye using a craft knife (Fig. 1b–d). It was placed on a stub, viewed under a binocular optical microscope (Olympus SZH10), uncoated, and observed in a SEM (Hitachi TM-1000) under a low vacuum at the Aitsu Marine Station of Kumamoto University, Japan. The section of fossilised retinal material was observed using a confocal laser microscope (VK-9500, KEYENCE) at the Kumamoto Industrial Research Institute. An extant house sparrow, Passer domesticus (eye diameter 0.7 cm, body length 10 cm) and a Japanese quail, Coturnix japonica (eye diameter 1.1 cm, body length 15 cm), were selected for comparative purposes because they have a body length and/or eyeball size similar to the fossil bird (eye diameter 0.65 cm). Furthermore, P. domesticus and C. japonica are distantly related (Dobson, 2012), which is preferable for the purpose of excluding phylogenetic constraint.

The freshly-killed P. domesticus and C. japonica were obtained from the company Moukinya. The eyeballs of both birds were removed using a craft knife and fixed in phosphate buffered saline solution (PBS). Each retina was removed from the eyeball in the PBS and was rinsed with fresh PBS. Each retina of the two extant birds was partially freeze-dried for SEM observations.

In order to check for morphological alternations of retinal tissues, another naturally dried house sparrow eye was investigated to provide a control for early diagenesis.

To determine the precise size-colour relationship of the oil droplets of the extant quail and house sparrow, each retinal sheet was removed from the pigment epithelium in PBS by levering with a needle. The retinal fragment was then mounted on a slide glass and covered with a cover slip. The size of the same coloured oil droplets varies between different regions of the same eye (Hart, 2004),
Fig. 5. A fragment of fossil retina of an Early Cretaceous enantiornithine bird and the distribution of elements within. (a) Digital microphotographs of a retinal region. (b,c) Enlargement of an area observed by laser microscope (b) and SEM (c). (d) Backscattered electron image of (a). (e–i) Distribution of the elements P, Ca, Mn, Fe, and K.
Fig. 6. Frequency distribution of area of oil droplets of an extant house sparrow *Passer domesticus*. (a) Histogram of all oil droplet sizes. (b–f) Histogram of sizes for each type of coloured oil droplet: clear (b), light blue (c), yellow (d), dark green (e), and red (f) (these are correlated with ultraviolet sensitive single cones [UVS], short-wavelength sensitive single cones [SWS], medium-wavelength sensitive
so only the retinal sheet removed from the marginal area of the eyeball (similar to that studied in the fossil eye) was used in this study.

The oil droplets were observed under a transmitted light microscope (Olympus CH40) with a digital video camera (Nikon DS-Vi1), and images were captured using ‘Nis-Element v. 4.0’ software. The images were saved as TIFF files using graphic software (Adobe Photoshop, version 7.01). The area of each oil droplet was calculated using area-calculating software in Image J (Rasband, 1977–2012). The colour of the oil droplet was identified by eye under a transmitted light microscope. The cone type, such as ultraviolet sensitive (UVS), short-wavelength sensitive (SWS), medium-wavelength sensitive (MWS), and long-wavelength sensitive (LWS) was determined based on Table 1 of Hart (2001b). The area data was initially preserved as an .xls file. By using free statistical software (Takeyasu, 2011), a Mann-Whitney U test (Faul et al., 2007) was performed to determine whether the values of the areas between different coloured oil droplets in the extant house sparrow were statistically distinguishable. Statistical Power (1-β) was calculated with G*Power software (ver 3.1.9.2) for checking the type II error (Faul et al., 2007). By using the R packages version 3.1.3 (R Development Core Team, 2013), Akaike information criterion (AIC) analysis (Akaike, 1974) was conducted to examine the relative quality of statistical models for a given set of data.

Elemental distribution and spectral patterns were obtained using energy-dispersive X-ray fluorescence (EDXRF) microscopy (XGT-5000 V, HORIBA) at 20 kV accelerated voltage and 5.2 mA probe current, using mono-capillary primary optics to focus the X-ray beam to a diameter of 10 μm. A fragment of fossil retina was attached to an aluminum stage; its position in the vacuum chamber was adjusted using a motorized xyz platform and viewed using three integrated colour video cameras. An area of 256 μm × 200 μm was analysed under full vacuum using 50 mm steps and 200,000 frames to provide two-dimensional distribution maps and spectral patterns of three points for seven elements, Mg, Si, P, K, Ca, Mn and Fe. The EDXRF analyses were performed at the Centre of Advanced Instrumental Analysis, Kyushu University.

All illustrated specimens are deposited in Shanghai Natural History Museum, Branch of Shanghai Science and Technology Museum.

3. Results

The eye of the fossil enantiornithine specimen contains black material, like that of a fossil fish (Tanaka et al., 2014), and fills the eye orbit (Fig. 1a,b). Digital microphotographs and scanning electron micrographs (SEMs) of the black material
(Fig. 7) TDFI

- **a**
  - $n = 508$
  - mean = 2.17
  - SD = 1.48
  - CL (95%) = 0.129

- **b**
  - $n = 88$
  - mean = 0.63
  - SD = 0.23
  - CL (95%) = 0.049

- **c**
  - $n = 51$
  - mean = 0.59
  - SD = 0.25
  - CL (95%) = 0.071

- **d**
  - $n = 227$
  - mean = 1.85
  - SD = 0.43
  - CL (95%) = 0.057

- **e**
  - $n = 27$
  - mean = 2.22
  - SD = 0.48
  - CL (95%) = 0.189

- **f**
  - $n = 117$
  - mean = 4.55
  - SD = 0.66
  - CL (95%) = 0.121
and adjacent bone reveal morphological differences: the coarse surface of the black material (the area surrounded by a yellow dotted line in Fig. 1c) is clearly distinguished from that of the bone, which has a smooth surface with several Haversian canals (Fig. 1c,d). The black material contains three-dimensional, mineralized outer cones and oil droplets (Figs. 1e,f; 2a,b and 3a,b ). The shape of the cones and droplets is the same as those of the extant house sparrow (Figs. 1e–h; 2a,b,e,f; 3 and 4 ), although the oil droplets in the fossilised material (Figs. 1e,f; 2a,b; 3 and 5b ) lack colour. The diameter of the oil droplets in both the fossil bird and the extant house sparrow ranges from 2 μm to 0.9 μm (Figs. 1e–h; 2a,b,e,f; 3 and 4a,b,e,f). Fossilised pigment epithelium and its fibrous structures (Fig. 2c,d) are also preserved in the distal part of the cone and rod cells; these are comparable to those of the pigment epithelium of the extant house sparrow (Figs. 2g,h and 4g). The fossil cones, rods, and oil droplets are preserved rather flattened (Fig. 2a–d), and look like those of the natural dried extant house sparrow (Fig. 4e–h).

The house sparrow and the extant Japanese quail identifies a common tendency between the frequency distribution of area of oil droplets and their associated cone photoreceptors (cone types in Figs. 6 and 7 ; Tables 1 and 2 ); specifically, the ultraviolet (UVS) and short-wavelength sensitive cones (SWS) are smallest (Figs. 6b, c and 7b, c), the medium-wavelength sensitive cones (MWS) are mid-sized (Figs. 6d and 7d), and the long-wavelength sensitive cones (LWS) are the largest (Figs. 6f and 7f). In both samples from the extant birds, the frequency distribution of the area of the individual oil droplets also correlates with overall cone type (Figs. 6a–f and 7a–f). Further analysis was performed on the extant house sparrow, as it has a similar size range of oil droplets to the fossil bird. The result of a Mann-Whitney U test (Mann and Whitney, 1947) showed that each oil-droplet colour can be distinguished statistically (p < 0.05, 1- β > 0.8), except for the combination of the dark green/red oil droplets (Table 3). The result suggests that four morphological types of oil droplets occur in the extant house sparrow.

To determine the size (maximum projected area) differences among the fossilised oil droplets, and to assess whether the oil droplet size is comparable with that of the extant house sparrow, we examined the frequency distribution of the maximum projected area of fossil oil droplets and the size range of the oil droplets of the extant house sparrow (Fig. 8). The fossil oil droplets exhibited a broad range of sizes, correlating with the UVS to LWS (Table 4).
Table 2. Area of oil droplets in an extant Japanese quail *Coturnix japonica*. The cone type to which each oil droplet belongs was determined based on the methods of Kolb and Jones (1982).

| No. | Cone type | Area of oil droplet (μm²) | No. | Cone type | Area of oil droplet (μm²) |
|-----|-----------|---------------------------|-----|-----------|---------------------------|
| 1   | red       | 5.5                       | 255 | violet    | 0.7                       |
| 2   | violet    | 1.1                       | 256 | red       | 3.8                       |
| 3   | red       | 4.5                       | 257 | violet    | 0.5                       |
| 4   | red       | 3.8                       | 258 | violet    | 0.8                       |
| 5   | red       | 5.9                       | 259 | red       | 3.1                       |
| 6   | red       | 4.0                       | 260 | violet    | 0.5                       |
| 7   | green     | 3.2                       | 261 | green     | 1.7                       |
| 8   | red       | 5.7                       | 262 | double    | 1.7                       |
| 9   | green     | 2.4                       | 263 | double    | 2.6                       |
| 10  | green     | 3.0                       | 264 | double    | 1.7                       |
| 11  | violet    | 1.0                       | 265 | green     | 1.8                       |
| 12  | green     | 1.9                       | 266 | violet    | 0.6                       |
| 13  | red       | 4.9                       | 267 | green     | 1.8                       |
| 14  | blue      | 1.5                       | 268 | green     | 1.4                       |
| 15  | red       | 4.6                       | 269 | red       | 3.3                       |
| 16  | green     | 1.8                       | 270 | green     | 1.0                       |
| 17  | violet    | 1.0                       | 271 | green     | 1.6                       |
| 18  | red       | 5.6                       | 272 | red       | 4.6                       |
| 19  | red       | 4.2                       | 273 | green     | 1.6                       |
| 20  | red       | 6.2                       | 274 | green     | 1.2                       |
| 21  | red       | 4.3                       | 275 | red       | 4.6                       |
| 22  | red       | 4.8                       | 276 | green     | 1.7                       |
| 23  | green     | 1.9                       | 277 | green     | 1.0                       |
| 24  | green     | 2.2                       | 278 | green     | 1.1                       |
| 25  | red       | 4.7                       | 279 | blue      | 0.9                       |
| 26  | red       | 4.3                       | 280 | green     | 1.1                       |
| 27  | violet    | 1.1                       | 281 | green     | 1.7                       |
| 28  | blue      | 1.0                       | 282 | green     | 1.6                       |
| 29  | blue      | 0.9                       | 283 | red       | 4.1                       |
| 30  | double    | 3.0                       | 284 | green     | 1.4                       |
| 31  | violet    | 0.6                       | 285 | violet    | 0.6                       |
| 32  | red       | 5.1                       | 286 | violet    | 0.4                       |
| 33  | green     | 2.0                       | 287 | red       | 3.6                       |
| 34  | blue      | 1.1                       | 288 | green     | 1.5                       |
| 35  | red       | 4.6                       | 289 | violet    | 0.7                       |
| 36  | green     | 3.8                       | 290 | green     | 2.4                       |
| 37  | violet    | 0.9                       | 291 | violet    | 0.7                       |

(Continued)
Table 2. (Continued)

| No. | Cone type | Area of oil droplet (μm²) | No. | Cone type | Area of oil droplet (μm²) |
|-----|-----------|---------------------------|-----|-----------|---------------------------|
| 38  | green     | 2.3                       | 292 | green     | 1.2                       |
| 39  | violet    | 1.1                       | 293 | green     | 2.1                       |
| 40  | blue      | 0.7                       | 294 | green     | 1.6                       |
| 41  | double    | 2.8                       | 295 | green     | 1.8                       |
| 42  | red       | 4.7                       | 296 | green     | 1.1                       |
| 43  | green     | 1.1                       | 297 | green     | 1.2                       |
| 44  | green     | 1.2                       | 298 | violet    | 0.4                       |
| 45  | red       | 4.3                       | 299 | blue      | 0.7                       |
| 46  | blue      | 0.8                       | 300 | green     | 1.6                       |
| 47  | green     | 1.3                       | 301 | red       | 3.8                       |
| 48  | green     | 1.3                       | 302 | green     | 1.6                       |
| 49  | violet    | 0.7                       | 303 | green     | 1.9                       |
| 50  | violet    | 1.3                       | 304 | green     | 2.1                       |
| 51  | red       | 5.0                       | 305 | green     | 2.1                       |
| 52  | green     | 2.9                       | 306 | violet    | 0.5                       |
| 53  | double    | 2.8                       | 307 | violet    | 1.1                       |
| 54  | green     | 1.8                       | 308 | green     | 1.7                       |
| 55  | green     | 2.2                       | 309 | blue      | 0.4                       |
| 56  | violet    | 1.2                       | 310 | red       | 5.9                       |
| 57  | red       | 4.1                       | 311 | green     | 1.7                       |
| 58  | green     | 1.5                       | 312 | green     | 1.9                       |
| 59  | violet    | 1.1                       | 313 | green     | 1.8                       |
| 60  | green     | 1.7                       | 314 | green     | 1.6                       |
| 61  | green     | 2.1                       | 315 | green     | 2.2                       |
| 62  | red       | 5.7                       | 316 | red       | 4.4                       |
| 63  | blue      | 1.2                       | 317 | green     | 1.5                       |
| 64  | red       | 4.1                       | 318 | red       | 5.4                       |
| 65  | blue      | 0.8                       | 319 | green     | 1.7                       |
| 66  | green     | 1.8                       | 320 | green     | 1.4                       |
| 67  | red       | 4.0                       | 321 | green     | 1.5                       |
| 68  | double    | 2.6                       | 322 | violet    | 0.5                       |
| 69  | red       | 4.3                       | 323 | green     | 1.9                       |
| 70  | green     | 2.6                       | 324 | violet    | 0.4                       |
| 71  | green     | 2.2                       | 325 | red       | 4.7                       |
| 72  | green     | 2.2                       | 326 | blue      | 0.5                       |
| 73  | green     | 2.0                       | 327 | red       | 5.0                       |
| 74  | blue      | 0.8                       | 328 | green     | 1.8                       |
| 75  | red       | 3.5                       | 329 | red       | 5.1                       |
| 76  | violet    | 0.6                       | 330 | green     | 1.5                       |

(Continued)
Table 2. (Continued)

| No. | Cone type | Area of oil droplet (μm²) | No. | Cone type | Area of oil droplet (μm²) |
|-----|-----------|---------------------------|-----|-----------|---------------------------|
| 77  | violet    | 0.6                       | 331 | green     | 2.7                       |
| 78  | green     | 1.9                       | 332 | green     | 2.7                       |
| 79  | violet    | 1.1                       | 333 | green     | 1.7                       |
| 80  | red       | 3.9                       | 334 | green     | 1.4                       |
| 81  | blue      | 0.5                       | 335 | green     | 1.5                       |
| 82  | red       | 4.5                       | 336 | red       | 4.5                       |
| 83  | green     | 1.9                       | 337 | green     | 1.8                       |
| 84  | green     | 2.1                       | 338 | green     | 2.2                       |
| 85  | violet    | 0.8                       | 339 | green     | 2.2                       |
| 86  | red       | 5.0                       | 340 | green     | 1.5                       |
| 87  | blue      | 0.7                       | 341 | violet    | 0.8                       |
| 88  | red       | 3.7                       | 342 | green     | 2.2                       |
| 89  | green     | 1.7                       | 343 | red       | 5.6                       |
| 90  | green     | 1.8                       | 344 | violet    | 0.4                       |
| 91  | green     | 1.5                       | 345 | green     | 2.1                       |
| 92  | violet    | 0.4                       | 346 | blue      | 0.4                       |
| 93  | red       | 3.4                       | 347 | green     | 1.7                       |
| 94  | green     | 2.0                       | 348 | green     | 2.3                       |
| 95  | green     | 1.6                       | 349 | green     | 1.3                       |
| 96  | blue      | 1.2                       | 350 | violet    | 0.4                       |
| 97  | green     | 1.7                       | 351 | green     | 1.6                       |
| 98  | violet    | 0.6                       | 352 | violet    | 0.6                       |
| 99  | red       | 4.8                       | 353 | violet    | 0.5                       |
| 100 | green     | 2.2                       | 354 | green     | 1.5                       |
| 101 | green     | 1.9                       | 355 | green     | 1.5                       |
| 102 | green     | 1.4                       | 356 | violet    | 0.4                       |
| 103 | green     | 2.3                       | 357 | green     | 1.2                       |
| 104 | green     | 1.7                       | 358 | red       | 4.7                       |
| 105 | red       | 4.8                       | 359 | green     | 1.7                       |
| 106 | green     | 1.4                       | 360 | green     | 1.5                       |
| 107 | green     | 2.0                       | 361 | red       | 5.2                       |
| 108 | double    | 1.5                       | 362 | green     | 2.3                       |
| 109 | green     | 1.9                       | 363 | double    | 1.7                       |
| 110 | red       | 4.8                       | 364 | double    | 1.7                       |
| 111 | red       | 4.5                       | 365 | red       | 4.7                       |
| 112 | violet    | 0.8                       | 366 | green     | 1.2                       |
| 113 | green     | 1.6                       | 367 | green     | 1.8                       |
| 114 | green     | 2.3                       | 368 | green     | 1.8                       |
| 115 | violet    | 0.9                       | 369 | green     | 1.6                       |
Table 2. (Continued)

| No. | Cone type | Area of oil droplet (μm²) | No.  | Cone type | Area of oil droplet (μm²) |
|-----|-----------|---------------------------|-----|-----------|---------------------------|
| 116 | red       | 3.9                       | 370 | green     | 1.5                       |
| 117 | blue      | 0.9                       | 371 | blue      | 0.5                       |
| 118 | green     | 1.9                       | 372 | green     | 1.8                       |
| 119 | red       | 3.9                       | 373 | double    | 2.1                       |
| 120 | red       | 4.3                       | 374 | green     | 1.7                       |
| 121 | green     | 1.7                       | 375 | violet    | 0.5                       |
| 122 | green     | 1.4                       | 376 | red       | 3.9                       |
| 123 | violet    | 0.4                       | 377 | violet    | 0.8                       |
| 124 | green     | 1.9                       | 378 | green     | 1.2                       |
| 125 | green     | 1.6                       | 379 | green     | 1.7                       |
| 126 | violet    | 0.4                       | 380 | blue      | 0.5                       |
| 127 | red       | 4.7                       | 381 | green     | 1.7                       |
| 128 | double    | 2.7                       | 382 | violet    | 0.5                       |
| 129 | green     | 1.3                       | 383 | double    | 1.4                       |
| 130 | blue      | 0.6                       | 384 | red       | 5.2                       |
| 131 | green     | 1.5                       | 385 | green     | 1.9                       |
| 132 | blue      | 1.0                       | 386 | green     | 1.1                       |
| 133 | red       | 4.1                       | 387 | red       | 4.8                       |
| 134 | blue      | 0.9                       | 388 | green     | 1.4                       |
| 135 | green     | 1.7                       | 389 | green     | 2.2                       |
| 136 | violet    | 0.8                       | 390 | red       | 4.6                       |
| 137 | green     | 2.2                       | 391 | blue      | 0.5                       |
| 138 | red       | 5.3                       | 392 | green     | 1.8                       |
| 139 | blue      | 0.9                       | 393 | green     | 2.8                       |
| 140 | green     | 2.3                       | 394 | violet    | 0.4                       |
| 141 | red       | 4.9                       | 395 | violet    | 1.9                       |
| 142 | blue      | 0.7                       | 396 | violet    | 0.7                       |
| 143 | green     | 1.8                       | 397 | green     | 2.7                       |
| 144 | green     | 1.8                       | 398 | red       | 5.1                       |
| 145 | violet    | 0.7                       | 399 | violet    | 0.5                       |
| 146 | green     | 1.8                       | 400 | green     | 2.0                       |
| 147 | violet    | 0.6                       | 401 | green     | 1.7                       |
| 148 | blue      | 0.3                       | 402 | violet    | 0.4                       |
| 149 | violet    | 0.6                       | 403 | green     | 2.4                       |
| 150 | green     | 1.8                       | 404 | double    | 2.3                       |
| 151 | red       | 5.6                       | 405 | double    | 2.5                       |
| 152 | green     | 1.5                       | 406 | green     | 2.3                       |
| 153 | violet    | 0.7                       | 407 | violet    | 0.6                       |
| 154 | green     | 2.0                       | 408 | blue      | 0.7                       |

(Continued)
Table 2. (Continued)

| No. | Cone type | Area of oil droplet (μm²) | No. | Cone type | Area of oil droplet (μm²) |
|-----|-----------|---------------------------|-----|-----------|---------------------------|
| 155 | violet    | 0.4                       | 409 | green     | 2.3                       |
| 156 | violet    | 0.7                       | 410 | green     | 2.1                       |
| 157 | green     | 1.8                       | 411 | red       | 4.9                       |
| 158 | double    | 1.6                       | 412 | violet    | 0.4                       |
| 159 | red       | 4.0                       | 413 | blue      | 0.8                       |
| 160 | green     | 2.1                       | 414 | green     | 2.3                       |
| 161 | violet    | 0.5                       | 415 | blue      | 0.5                       |
| 162 | red       | 3.9                       | 416 | blue      | 0.5                       |
| 163 | blue      | 0.6                       | 417 | green     | 2.1                       |
| 164 | green     | 2.1                       | 418 | green     | 2.8                       |
| 165 | violet    | 0.5                       | 419 | red       | 5.4                       |
| 166 | violet    | 0.7                       | 420 | red       | 5.0                       |
| 167 | green     | 1.9                       | 421 | red       | 5.3                       |
| 168 | red       | 4.8                       | 422 | green     | 2.1                       |
| 169 | violet    | 0.6                       | 423 | violet    | 0.5                       |
| 170 | double    | 2.1                       | 424 | green     | 2.3                       |
| 171 | green     | 1.4                       | 425 | red       | 5.6                       |
| 172 | green     | 1.8                       | 426 | double    | 2.6                       |
| 173 | violet    | 0.6                       | 427 | violet    | 0.4                       |
| 174 | green     | 1.6                       | 428 | blue      | 0.6                       |
| 175 | red       | 3.9                       | 429 | green     | 2.7                       |
| 176 | green     | 2.3                       | 430 | green     | 2.4                       |
| 177 | violet    | 0.7                       | 431 | green     | 2.3                       |
| 178 | violet    | 0.8                       | 432 | green     | 1.8                       |
| 179 | green     | 1.5                       | 433 | red       | 3.9                       |
| 180 | red       | 3.9                       | 434 | violet    | 0.4                       |
| 181 | green     | 1.7                       | 435 | double    | 2.7                       |
| 182 | green     | 1.9                       | 436 | violet    | 0.3                       |
| 183 | red       | 5.8                       | 437 | green     | 1.6                       |
| 184 | green     | 1.6                       | 438 | red       | 4.0                       |
| 185 | green     | 1.7                       | 439 | red       | 4.4                       |
| 186 | green     | 1.4                       | 440 | red       | 4.8                       |
| 187 | red       | 3.6                       | 441 | violet    | 0.4                       |
| 188 | red       | 4.4                       | 442 | violet    | 0.4                       |
| 189 | green     | 1.6                       | 443 | double    | 2.8                       |
| 190 | red       | 3.9                       | 444 | green     | 1.8                       |
| 191 | blue      | 0.5                       | 445 | green     | 2.1                       |
| 192 | red       | 3.9                       | 446 | green     | 2.4                       |
| 193 | blue      | 1.1                       | 447 | red       | 4.3                       |

(Continued)
Table 2. (Continued)

| No. | Cone type | Area of oil droplet (μm²) | No. | Cone type | Area of oil droplet (μm²) |
|-----|-----------|---------------------------|-----|-----------|---------------------------|
| 194 | double    | 2.1                       | 448 | green     | 1.7                       |
| 195 | red       | 3.9                       | 449 | green     | 2.0                       |
| 196 | blue      | 0.6                       | 450 | green     | 1.7                       |
| 197 | green     | 1.9                       | 451 | violet    | 0.5                       |
| 198 | red       | 3.7                       | 452 | red       | 4.6                       |
| 199 | green     | 1.6                       | 453 | green     | 1.8                       |
| 200 | double    | 1.8                       | 454 | green     | 2.3                       |
| 201 | double    | 2.8                       | 455 | blue      | 0.4                       |
| 202 | red       | 3.9                       | 456 | green     | 2.2                       |
| 203 | green     | 1.4                       | 457 | red       | 5.5                       |
| 204 | blue      | 0.9                       | 458 | green     | 2.0                       |
| 205 | green     | 1.9                       | 459 | green     | 2.1                       |
| 206 | green     | 2.1                       | 460 | red       | 4.6                       |
| 207 | green     | 1.6                       | 461 | green     | 1.8                       |
| 208 | red       | 3.8                       | 462 | green     | 2.1                       |
| 209 | red       | 4.1                       | 463 | green     | 3.3                       |
| 210 | green     | 1.6                       | 464 | blue      | 0.6                       |
| 211 | blue      | 0.6                       | 465 | violet    | 0.5                       |
| 212 | blue      | 0.5                       | 466 | green     | 2.1                       |
| 213 | green     | 1.4                       | 467 | violet    | 0.6                       |
| 214 | green     | 1.3                       | 468 | red       | 5.3                       |
| 215 | green     | 1.7                       | 469 | double    | 2.2                       |
| 216 | red       | 3.4                       | 470 | blue      | 0.6                       |
| 217 | green     | 1.8                       | 471 | green     | 2.1                       |
| 218 | green     | 1.8                       | 472 | green     | 2.3                       |
| 219 | red       | 3.5                       | 473 | red       | 4.5                       |
| 220 | green     | 1.8                       | 474 | green     | 1.6                       |
| 221 | green     | 2.0                       | 475 | blue      | 0.5                       |
| 222 | green     | 0.5                       | 476 | green     | 2.4                       |
| 223 | green     | 1.8                       | 477 | green     | 2.2                       |
| 224 | red       | 3.7                       | 478 | green     | 2.2                       |
| 225 | green     | 1.8                       | 479 | blue      | 0.4                       |
| 226 | violet    | 0.4                       | 480 | violet    | 0.6                       |
| 227 | violet    | 0.5                       | 481 | red       | 5.3                       |
| 228 | green     | 1.5                       | 482 | double    | 1.9                       |
| 229 | red       | 4.2                       | 483 | red       | 5.2                       |
| 230 | blue      | 0.5                       | 484 | green     | 2.0                       |
| 231 | red       | 3.8                       | 485 | violet    | 0.5                       |
| 232 | red       | 4.9                       | 486 | green     | 2.0                       |

(Continued)
Table 2. (Continued)

| No. | Cone type | Area of oil droplet (μm²) | No. | Cone type | Area of oil droplet (μm²) |
|-----|-----------|---------------------------|-----|-----------|---------------------------|
| 233 | green     | 1.4                       | 487 | red       | 5.3                       |
| 234 | green     | 1.2                       | 488 | green     | 1.5                       |
| 235 | red       | 4.3                       | 489 | violet    | 0.4                       |
| 236 | green     | 1.4                       | 490 | green     | 2.6                       |
| 237 | green     | 1.4                       | 491 | double    | 2.1                       |
| 238 | violet    | 0.5                       | 492 | red       | 4.6                       |
| 239 | blue      | 0.5                       | 493 | green     | 1.5                       |
| 240 | red       | 4.5                       | 494 | green     | 2.1                       |
| 241 | green     | 1.5                       | 495 | green     | 2.5                       |
| 242 | red       | 4.8                       | 496 | red       | 5.1                       |
| 243 | blue      | 0.4                       | 497 | violet    | 0.5                       |
| 244 | red       | 4.4                       | 498 | green     | 1.7                       |
| 245 | blue      | 0.8                       | 499 | green     | 2.4                       |
| 246 | violet    | 0.6                       | 500 | red       | 4.8                       |
| 247 | blue      | 0.5                       | 501 | green     | 1.7                       |
| 248 | violet    | 0.8                       | 502 | blue      | 0.7                       |
| 249 | violet    | 0.6                       | 503 | violet    | 0.4                       |
| 250 | red       | 3.4                       | 504 | green     | 3.2                       |
| 251 | violet    | 1.0                       | 505 | blue      | 0.5                       |
| 252 | double    | 2.1                       | 506 | red       | 5.1                       |
| 253 | red       | 4.5                       | 507 | green     | 2.0                       |
| 254 | green     | 1.3                       | 508 | red       | 5.2                       |

Table 3. Result of a Mann-Whitney U test of oil droplets of an extant house sparrow *Passer domesticus*. (a) z score, (b) p-value two-tailed, (c) Statistical power (1 − β).

| a    | clear | light blue | yellow | dark green | red |
|------|-------|------------|--------|------------|-----|
| clear|       |            |        |            |     |
| light blue | 3.771 |            |        |            |     |
| yellow | 9.378 | 7.865      |        |            |     |
| dark green | 9.947 | 8.096      | 0.679  |            | z   |
| red   | 8.863 | 7.563      | 7.079  | 0.967      |     |

| b    | clear | light blue | yellow | dark green | red |
|------|-------|------------|--------|------------|-----|
| clear|       |            |        |            |     |
| light blue | 0     |            |        |            |     |
| yellow | 0     | 0          |        |            |     |

(Continued)
In order to select the relative quality of statistical models for a given set of data (Tables 1, 2 and 4), the Akaike information criterion (AIC) analysis (Akaike, 1974) was carried out (Table 5). The AIC models suggest that the size range of the fossil oil droplets forms one peak (directional asymmetry), the same as those of the extant house sparrow and Japanese quail (Table 5).

Table 3. (Continued)

| b      | clear | light blue | yellow | dark green | red |
|--------|-------|------------|--------|------------|-----|
| dark green | 0     | 0          | 0.497  | p          |     |
| red    | 0     | 0          | 0      | 0.334      |     |
| c      | clear | light blue | yellow | dark green | red |
| clear  | 0.999 |           |        |            |     |
| light blue | 1.000 | 1.000      |        |            |     |
| yellow | 1.000 | 1.000      | 0.252  |            | 1-β |
| dark green | 1.000 | 1.000      | 1.000  | 1.000      |     |
| red    | 1.000 | 1.000      | 1.000  | 1.000      |     |

Fig. 8. Frequency distribution of the maximum projected area of oil droplets of an Early Cretaceous enantiornithine bird and the range of each oil droplet size in an extant house sparrow. (a) Histogram of fossil oil droplet size based on Table 1. (b) Box-and-whisker diagrams of extant oil droplets size of house sparrow drawn from Fig. 6 and Table 2. The violet, light blue, green, gray, and red ranges correlate with ultraviolet sensitive single cones [UVS], short- wavelength sensitive single cones [SWS], medium-wavelength sensitive single cones [MWS], intermediate sensitive double cones [IWS], and long-wavelength sensitive single cones [LWS], respectively.
Table 4. Area of oil droplets in an Early Cretaceous enantiornithine bird.

| No. | Cone type | Area of oil droplet (μm²) | No. | Cone type | Area of oil droplet (μm²) |
|-----|-----------|---------------------------|-----|-----------|---------------------------|
| 1   | -         | 1.1                       | 98  | -         | 0.7                       |
| 2   | -         | 0.6                       | 99  | -         | 3.2                       |
| 3   | -         | 0.9                       | 100 | -         | 1.4                       |
| 4   | -         | 0.8                       | 101 | -         | 3.4                       |
| 5   | -         | 1.2                       | 102 | -         | 1.0                       |
| 6   | -         | 3.7                       | 103 | -         | 1.1                       |
| 7   | -         | 0.8                       | 104 | -         | 1.1                       |
| 8   | -         | 0.8                       | 105 | -         | 1.0                       |
| 9   | -         | 2.4                       | 106 | -         | 1.0                       |
| 10  | -         | 0.8                       | 107 | -         | 3.2                       |
| 11  | -         | 2.8                       | 108 | -         | 2.6                       |
| 12  | -         | 0.6                       | 109 | -         | 1.0                       |
| 13  | -         | 0.7                       | 110 | -         | 1.0                       |
| 14  | -         | 2.6                       | 111 | -         | 0.7                       |
| 15  | -         | 2.6                       | 112 | -         | 0.9                       |
| 16  | -         | 0.9                       | 113 | -         | 0.9                       |
| 17  | -         | 0.9                       | 114 | -         | 0.7                       |
| 18  | -         | 1.2                       | 115 | -         | 0.9                       |
| 19  | -         | 0.7                       | 116 | -         | 1.1                       |
| 20  | -         | 3.0                       | 117 | -         | 0.8                       |
| 21  | -         | 3.2                       | 118 | -         | 2.5                       |
| 22  | -         | 0.7                       | 119 | -         | 3.2                       |
| 23  | -         | 0.6                       | 120 | -         | 3.0                       |
| 24  | -         | 0.7                       | 121 | -         | 1.0                       |
| 25  | -         | 2.1                       | 122 | -         | 0.9                       |
| 26  | -         | 0.5                       | 123 | -         | 0.9                       |
| 27  | -         | 1.0                       | 124 | -         | 0.8                       |
| 28  | -         | 0.5                       | 125 | -         | 1.2                       |
| 29  | -         | 0.6                       | 126 | -         | 1.2                       |
| 30  | -         | 3.6                       | 127 | -         | 1.0                       |
| 31  | -         | 1.0                       | 128 | -         | 3.2                       |
| 32  | -         | 3.6                       | 129 | -         | 1.0                       |
| 33  | -         | 0.7                       | 130 | -         | 1.1                       |
| 34  | -         | 3.7                       | 131 | -         | 1.3                       |
| 35  | -         | 1.3                       | 132 | -         | 1.2                       |
| 36  | -         | 0.8                       | 133 | -         | 0.9                       |
| 37  | -         | 1.1                       | 134 | -         | 1.8                       |

(Continued)
Table 4. (Continued)

| No. | Cone type | Area of oil droplet (μm²) | No. | Cone type | Area of oil droplet (μm²) |
|-----|-----------|---------------------------|-----|-----------|---------------------------|
| 38  | -         | 0.8                       | 135 | -         | 0.8                       |
| 39  | -         | 1.1                       | 136 | -         | 1.1                       |
| 40  | -         | 3.7                       | 137 | -         | 2.7                       |
| 41  | -         | 0.7                       | 138 | -         | 1.9                       |
| 42  | -         | 0.8                       | 139 | -         | 4.1                       |
| 43  | -         | 3.1                       | 140 | -         | 1.5                       |
| 44  | -         | 4.0                       | 141 | -         | 0.8                       |
| 45  | -         | 1.0                       | 142 | -         | 0.8                       |
| 46  | -         | 0.5                       | 143 | -         | 0.9                       |
| 47  | -         | 1.0                       | 144 | -         | 1.0                       |
| 48  | -         | 0.9                       | 145 | -         | 1.1                       |
| 49  | -         | 1.2                       | 146 | -         | 2.5                       |
| 50  | -         | 2.8                       | 147 | -         | 0.9                       |
| 51  | -         | 2.8                       | 148 | -         | 0.9                       |
| 52  | -         | 3.9                       | 149 | -         | 1.3                       |
| 53  | -         | 1.7                       | 150 | -         | 4.4                       |
| 54  | -         | 1.0                       | 151 | -         | 2.0                       |
| 55  | -         | 1.1                       | 152 | -         | 0.8                       |
| 56  | -         | 0.9                       | 153 | -         | 1.9                       |
| 57  | -         | 0.9                       | 154 | -         | 2.9                       |
| 58  | -         | 0.8                       | 155 | -         | 3.2                       |
| 59  | -         | 1.6                       | 156 | -         | 1.0                       |
| 60  | -         | 1.4                       | 157 | -         | 0.9                       |
| 61  | -         | 1.1                       | 158 | -         | 4.5                       |
| 62  | -         | 4.2                       | 159 | -         | 1.2                       |
| 63  | -         | 1.3                       | 160 | -         | 1.2                       |
| 64  | -         | 4.0                       | 161 | -         | 4.2                       |
| 65  | -         | 0.9                       | 162 | -         | 4.3                       |
| 66  | -         | 1.0                       | 163 | -         | 0.7                       |
| 67  | -         | 0.9                       | 164 | -         | 3.2                       |
| 68  | -         | 1.1                       | 165 | -         | 3.2                       |
| 69  | -         | 0.9                       | 166 | -         | 1.3                       |
| 70  | -         | 0.9                       | 167 | -         | 1.1                       |
| 71  | -         | 0.9                       | 168 | -         | 0.9                       |
| 72  | -         | 0.9                       | 169 | -         | 2.9                       |
| 73  | -         | 0.9                       | 170 | -         | 0.9                       |
| 74  | -         | 0.7                       | 171 | -         | 1.0                       |
| 75  | -         | 0.5                       | 172 | -         | 1.1                       |
Table 4. (Continued)

| No. | Cone type | Area of oil droplet (μm²) |
|-----|-----------|---------------------------|
| 76  | -         | 0.7                       | 173 | -   | 1.1 |
| 77  | -         | 0.7                       | 174 | -   | 2.8 |
| 78  | -         | 0.7                       | 175 | -   | 0.9 |
| 79  | -         | 3.1                       | 176 | -   | 0.9 |
| 80  | -         | 4.2                       | 177 | -   | 1.0 |
| 81  | -         | 0.7                       | 178 | -   | 0.9 |
| 82  | -         | 0.9                       | 179 | -   | 1.0 |
| 83  | -         | 0.9                       | 180 | -   | 0.8 |
| 84  | -         | 1.3                       | 181 | -   | 0.9 |
| 85  | -         | 0.5                       | 182 | -   | 2.4 |
| 86  | -         | 3.2                       | 183 | -   | 0.9 |
| 87  | -         | 0.9                       | 184 | -   | 2.5 |
| 88  | -         | 3.4                       | 185 | -   | 1.0 |
| 89  | -         | 4.9                       | 186 | -   | 2.3 |
| 90  | -         | 3.7                       | 187 | -   | 0.6 |
| 91  | -         | 2.1                       | 188 | -   | 2.5 |
| 92  | -         | 0.6                       | 189 | -   | 1.0 |
| 93  | -         | 0.8                       | 190 | -   | 2.9 |
| 94  | -         | 0.7                       | 191 | -   | 1.1 |
| 95  | -         | 3.3                       | 192 | -   | 1.0 |
| 96  | -         | 1.3                       | 193 | -   | 2.1 |
| 97  | -         | 1.0                       |     |     |     |

Table 5. Basic statistics and AIC values for the FA, DA, AS, and Skewed AS models to discriminate the type of asymmetry for an enantiornithine fossil bird, house sparrow and Japanese quail. n = number of specimens, SD = standard deviation, FA = fluctuating asymmetry, DA = directional asymmetry, AS = Antisymmetry.

| Specimens                          | AIC for each model |
|------------------------------------|--------------------|
|                                    | n  | Mean | SD  | FA  | DA  | AS  | Skewed AS |
| Enantiornithes fossil bird         | 193| 1.59 | 1.10| 804.43| 586.50| 806.35| 588.50   |
| House sparrow (Passer domesticus)  | 316| 2.37 | 1   | 1498.4| 923.17| 1354.08| 925.17   |
| Japanese quail (Coturnix japonica) | 508| 2.16 | 1.5 | 2422.4| 1841.19| 2420.84| 1843.19  |
Elemental distribution and spectral patterns of the fossil bird sample showed that calcium and phosphate dominated both the retinal and bone areas (Fig. 5), but a higher concentration of phosphate was detected in the retinal area than in the bone area (Fig. 9).

![Fig. 9. Elemental spectral patterns of three points of a fragment of fossil retina of an Early Cretaceous enantiornithine bird and the distribution of elements. (a,b) Spectral patterns from retinal region (black coloured area). (c) Spectral patterns from bone region (light-yellow coloured area).](image)

**Fig. 9.** Elemental spectral patterns of three points of a fragment of fossil retina of an Early Cretaceous enantiornithine bird and the distribution of elements. (a,b) Spectral patterns from retinal region (black coloured area). (c) Spectral patterns from bone region (light-yellow coloured area).

**Fig. 10.** Frequency distribution of the cross-sectional area of oil droplets of a Cretaceous enantiornithine bird. (a) Histogram of oil droplet size. (b) Kernel density estimation of (a). (c) Result of number of modes ($k$) and $p$-values.

| $k$ | $p$ value |
|-----|-----------|
| 1   | 0         |
| 2   | 0.81782   |
| 3   | 0.58659   |
| 4   | 0.34735   |
| 5   | 0.07207   |

**Fig. 10.** Frequency distribution of the cross-sectional area of oil droplets of a Cretaceous enantiornithine bird. (a) Histogram of oil droplet size. (b) Kernel density estimation of (a). (c) Result of number of modes ($k$) and $p$-values.
Fig. 11. Frequency distribution of the cross-sectional area of oil droplets of an extant house sparrow *Passer domesticus*. (a) Histogram of oil droplet size. (b) Kernel density estimation of (a). (c) Result of number of modes ($k$) and $p$-values.
Fig. 12. Frequency distribution of the cross-sectional area of oil droplets of an extant Ural owl *Strix uralensis*. (a) Histogram of oil droplet size. (b) Kernel density estimation of (a). (c) Result of number of modes (k) and p-values.
Table 6. Area of oil droplets in an extant Ural owl *Strix uralensis*. The area of each oil droplet and its cone type were determined based on the literature given in Gondo and Ando (1995).

| No. | Cone type | Area of oil droplet (μm²) | No. | Cone type | Area of oil droplet (μm²) |
|-----|-----------|---------------------------|-----|-----------|---------------------------|
| 1   | pale green| 5.2                       | 69  | pale green| 6.8                       |
| 2   |           | 6.0                       | 70  |           | 5.5                       |
| 3   |           | 5.4                       | 71  |           | 6.5                       |
| 4   |           | 5.6                       | 72  |           | 5.6                       |
| 5   |           | 6.3                       | 73  |           | 7.7                       |
| 6   |           | 5.8                       | 74  |           | 6.3                       |
| 7   |           | 6.2                       | 75  |           | 5.9                       |
| 8   |           | 7.0                       | 76  |           | 7.3                       |
| 9   |           | 5.8                       | 77  |           | 6.9                       |
| 10  |           | 6.5                       | 78  |           | 5.2                       |
| 11  |           | 4.1                       | 79  |           | 7.1                       |
| 12  |           | 4.9                       | 80  |           | 6.8                       |
| 13  |           | 5.8                       | 81  |           | 6.6                       |
| 14  |           | 5.2                       | 82  |           | 6.8                       |
| 15  |           | 6.9                       | 83  |           | 5.3                       |
| 16  |           | 6.3                       | 84  |           | 4.4                       |
| 17  |           | 6.0                       | 85  |           | 4.9                       |
| 18  |           | 7.2                       | 86  |           | 6.8                       |
| 19  |           | 8.2                       | 87  |           | 6.8                       |
| 20  |           | 6.2                       | 88  |           | 6.8                       |
| 21  |           | 7.3                       | 89  |           | 3.5                       |
| 22  |           | 6.3                       | 90  |           | 8.1                       |
| 23  |           | 6.7                       | 91  |           | 6.2                       |
| 24  |           | 6.2                       | 92  |           | 5.0                       |
| 25  |           | 7.5                       | 93  |           | 6.4                       |
| 26  |           | 6.0                       | 94  |           | 6.3                       |
| 27  |           | 5.8                       | 95  |           | 6.1                       |
| 28  |           | 5.7                       | 96  |           | 5.8                       |
| 29  |           | 6.7                       | 97  |           | 6.3                       |
| 30  |           | 6.4                       | 98  |           | 6.5                       |
| 31  |           | 5.5                       | 99  |           | 6.7                       |
| 32  |           | 5.9                       | 100 |           | 6.5                       |
| 33  |           | 5.2                       | 101 |           | 8.0                       |
| 34  |           | 4.7                       | 102 |           | 6.8                       |
| 35  |           | 6.6                       | 103 |           | 7.1                       |

(Continued)
Table 6. *(Continued)*

| No. | Cone type | Area of oil droplet (μm²) | No. | Cone type | Area of oil droplet (μm²) |
|-----|-----------|--------------------------|-----|-----------|--------------------------|
| 36  | "         | 7.5                      | 104 | "         | 6.9                      |
| 37  | "         | 4.9                      | 105 | "         | 6.3                      |
| 38  | "         | 6.7                      | 106 | "         | 6.7                      |
| 39  | "         | 5.4                      | 107 | "         | 6.0                      |
| 40  | "         | 6.0                      | 108 | "         | 5.5                      |
| 41  | "         | 5.4                      | 109 | "         | 5.5                      |
| 42  | "         | 6.1                      | 110 | "         | 8.3                      |
| 43  | "         | 6.3                      | 111 | "         | 8.4                      |
| 44  | "         | 5.8                      | 112 | "         | 6.8                      |
| 45  | "         | 6.0                      | 113 | "         | 6.4                      |
| 46  | "         | 6.7                      | 114 | "         | 7.1                      |
| 47  | "         | 6.7                      | 115 | "         | 6.1                      |
| 48  | "         | 5.0                      | 116 | "         | 6.6                      |
| 49  | "         | 6.1                      | 117 | "         | 6.7                      |
| 50  | "         | 7.2                      | 118 | "         | 7.0                      |
| 51  | "         | 7.0                      | 119 | "         | 6.1                      |
| 52  | "         | 6.6                      | 120 | "         | 6.3                      |
| 53  | "         | 6.7                      | 121 | "         | 6.6                      |
| 54  | "         | 6.3                      | 122 | "         | 6.5                      |
| 55  | "         | 7.4                      | 123 | "         | 6.4                      |
| 56  | "         | 7.9                      | 124 | "         | 5.4                      |
| 57  | "         | 6.4                      | 125 | "         | 6.0                      |
| 58  | "         | 6.0                      | 126 | "         | 6.1                      |
| 59  | "         | 6.7                      | 127 | "         | 5.8                      |
| 60  | "         | 6.3                      | 128 | "         | 6.7                      |
| 61  | "         | 6.9                      | 129 | "         | 6.5                      |
| 62  | "         | 6.5                      | 130 | "         | 6.5                      |
| 63  | "         | 8.2                      | 131 | "         | 6.8                      |
| 64  | "         | 7.2                      | 132 | "         | 6.1                      |
| 65  | "         | 6.0                      | 133 | "         | 5.4                      |
| 66  | "         | 6.5                      | 134 | "         | 4.3                      |
| 67  | "         | 6.8                      | 135 | "         | 7.2                      |
| 68  | "         | 6.1                      |
4. Discussion and conclusion

This paper reports a unique case of a preserved retina in a fossil bird, including the only record of avian fossil oil droplets, cones and rods, and pigment epithelium. Furthermore, the fossil cones, rods, and oil droplets were rather flattened (Fig. 2a–d) like those of the natural dried extant house sparrow (Fig. 4e–h). This result suggests that the fossil retina was dried (thereby indicating that the bird had already died) before it was transported into water to become preserved as a fossil. Elemental spectral patterns denoted a higher concentration of phosphate in the retinal area than in the bone area (Fig. 9), indicating that soft tissues were replaced by calcium phosphate under high phosphorus levels in early diagenesis (Maeda et al., 2011; Vannier et al., 2016). These findings provide compelling evidence that the general retinal anatomy of birds was in place 120 Ma. Additionally, the presence of oil droplets of a wide range of sizes indicates that the Cretaceous bird likely possessed colour vision. Further, in terms of the bias towards the smaller end of the potential size distribution, the fossil eye oil droplets are more similar to those of the extant house sparrow than to the extant lizards (Bowmaker et al., 2005).

To extract the size (maximum projected area) differences among fossilised oil droplets, and to determine whether the oil droplet size histogram shows a single peak or not, Silverman’s test (Schwaiger and Holzmann, 2013) was carried out (Fig. 10, Table 4). The result showed that the $p$ value was $<0.05$ in $k = 1$, indicating that there were more than two types of oil droplets based on size (Fig. 10). Our examination of the extant house sparrow also indicates that there are at least two types of oil droplets based on size (Fig. 11, $p < 0.05$ in $k = 1$). Furthermore, there is a good correlation between the size of an oil droplet and its peak wavelength sensitivity (cone type in Fig. 6); specifically, the ultraviolet (UVS) and short-wavelength sensitive cones (SWS) tend to be distributed in the small size region of the histogram (Figs. 6a–c and 11). However, in a nocturnal bird, the Ural owl *Strix uralensis*, only pale green-coloured oil droplets have been found (Gondo and Ando, 1995). The result of a Silverman’s test using Fig. 7 of Gondo and Ando (1995), further suggests a single peak (Fig. 12, Table 6, $p > 0.05$ in $k = 1–5$). Therefore, the histogram of the fossil bird and extant house sparrow shows two peaks based on Silverman’s test and can be discriminated from the nocturnal bird (Ural owl).

As inferred from the opsin genes of extant species, tetrachromatic vision first evolved in jawless fish (Bowmaker, 2008). In reptiles and birds, the performance of cone cells is further enhanced by the addition of oil droplets which transmit specific wavelengths only (Stavenga and Wilts, 2014; Loew et al., 2002). Thus, the discovery of oil droplets in the fossil bird specimen here indicates that the complex optical system of cone cells had already been achieved at least by 120 Ma.
On the other hand, single-coloured oil droplets are found in the snowy owl and king penguin (Gondo and Ando, 1995). The snowy owl and king penguin inhabit snow-covered terrain, or water with a high content of blue light, and so the discrimination of multiple colours is not a selection pressure. A single oil droplet type, or filter, is as efficient at detecting objects against such a background as are multiple colour filters.

To conclude, from an examination of its retina, the Cretaceous enantiornithine bird studied here was probably a diurnal species and possessed colour vision. The frequency distribution analysis of oil droplets in the fossil bird eye appears a useful method to aid the reconstruction of its palaeoecology.

**Declarations**

**Author contribution statement**

Gengo Tanaka: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

Baochun Zhou: Performed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

Yunfei Zhang: Contributed reagents, materials, analysis tools or data; Wrote the paper.

David J. Siveter: Analyzed and interpreted the data; Wrote the paper.

Andrew R. Parker: Conceived and designed the experiments; Analyzed and interpreted the data; Wrote the paper.

**Competing interest statement**

The authors declare no conflict of interest.

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**Additional information**

No additional information is available for this paper.
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