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THE CONTROLS ON THE PRESERVATION OF STRUCTURAL COLOR IN FOSSIL INSECTS

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ABSTRACT

The colors of many animals arise from ordered nanometer-scale variations in tissue structure. Such structural colors—especially those with metallic optical effects—are widespread among modern insects but are preserved rarely in insect fossils. This suggests that a specific set of taphonomic circumstances is required for preservation of structural colors. Here we present the results of the first systematic investigation of the controls on the preservation of structurally colored tissues in fossil insects. Approximately 700 specimens of beetle taxa known to exhibit metallic structural colors were studied from seven Lagerstätten: Randecker Maar (early Miocene), Clarkia (early Miocene), Enspel (late Oligocene), Florissant (late Eocene), Eckfeld (middle Eocene), Messel (middle Eocene), and Green River (middle Eocene). The quality of preservation of metallic colors varies among, and within, these biotas; colors are well preserved in most specimens from Clarkia, Enspel, Eckfeld, and Messel, but are typically poorly preserved in specimens from Randecker Maar and absent in Florissant and Green River. These differences are independent of the age and depositional context of the biotas. Instead, variation among biotas is attributed to differences in their late diagenetic history, in particular the maximum depth to which sediments were buried and the nature of fluid flow, as well as recent weathering. Variations in the quality of structural color preservation among specimens from individual biotas are independent of precise stratigraphic and sedimentological context. These intrabiota variations usually result from differences in the extent of microbial degradation of the cuticle and of recent weathering, but also the mode of curation of specimens. The last of these has important implications for curatorial practice.

INTRODUCTION

Insects are the most numerically abundant and diverse animals on Earth and have been important members of ancient continental ecosystems since at least the Early Devonian (Grimaldi and Engel, 2005). The evolutionary success of this group stems, in part, from the multilayered chitinous cuticle, which functions in protection, structural support, homeostasis and, via its coloration, communication (Grimaldi and Engel, 2005). The striking colors of many modern insects are generated by ordered cuticular nanostructures that scatter light coherently (Vukusic and Sambles, 2003). These structural colors play important roles in sexual and social signalling (Parker, 2000; Seago et al., 2009) and can be preserved in fossil insects (Parker et al., 1998a; Parker and McKenzie, 2003; Tanaka et al., 2010; McNamara et al., 2011a, 2012a). Despite the potential of such fossils to illuminate the evolutionary history of structural colors and their functions (Parker and McKenzie, 2003), evidence of structural color in fossil insects has only recently become a focus of investigation. Insect cuticle may be fossilized with sufficient detail to allow biophotonic nanostructures to be identified and their optical properties, and visual functions, to be reconstructed (Parker et al., 1998a; McNamara et al., 2011a). Further, the suite of ultrastructural features preserved allows the former presence or absence of color-producing structures to be determined (McNamara et al., 2012a). High-fidelity preservation of cuticular ultrastructure does not, however, ensure that the original structural color survives. The original hues of structurally colored fossil beetles and moths are altered during diagenesis (McNamara et al., 2011a, 2012a). This has been attributed to a change in the refractive index of the cuticle (McNamara et al., 2012a), but the cause of such taphonomic alteration is unclear. Curiously, not all specimens of a structurally colored taxon from a single biota exhibit evidence of structural color, e.g., metallic colors (colors characterized by highly directional reflectance) (McNamara et al., 2012a). Further, such colors are notably absent in several important insect Lagerstätten, e.g., the Florissant (early Eocene, Colorado, United States) and Green River (middle Eocene, Utah and Wyoming, United States) biotas. The specific suite of taphonomic circumstances required for preservation of structural colors is unknown. In particular, the various temporal (age of the biota), sedimentological, diagenetic, and biological (taxonomy, tissue ultrastructure and composition) factors that potentially affect the preservation of structural color require investigation.

In addition to changes during fossilization, preserved structural colors can even be altered or destroyed after specimens are collected in the field. Insect specimens from the Messel (Eocene) and Enspel (Oligocene) biotas from Germany can lose their metallic colors following dehydration in air (Parker and McKenzie, 2003; Schweizer et al., 2006); loss of color is usually prevented by storing such specimens in liquid media, e.g., brine, ethanol, or glycerine. Occasionally, however, metallic colors in fossil insects alter during storage in these media (M. McNamara, personal observation, 2010; see Results, below). Understanding how such alteration originates is important to inform curation practices and may shed light on the preservation of structural colors on a geological timescale, but has not been investigated.

Here we examine the impact of temporal, sedimentological, and diagenetic factors on the preservation of structural color using a comparative analysis of fossil insects from seven lacustrine-hosted Cenozoic Lagerstätten (Fig. 1): Randecker Maar (early Miocene, 16–18 Ma, Germany; Lutz, 1997; Schweigert and Bechly, 2001) and the Clarkia (early Miocene, 17–20 Ma, Idaho, United States; Smiley et al., 1975); Enspel (late Oligocene, 25 Ma, Germany; Possmann et al., 2010); Florissant (late Eocene, 34 Ma, Colorado, United States; Meyer, 2003); Eckfeld (middle Oligocene, 44.3 Ma, Germany; Lutz et al., 2010); Messel (middle Eocene, 47.8 Ma, Germany; Schaaf and Ziegler, 1992), and Green River formations (middle Eocene, 48.5–53.5 Ma, Wyoming, United States; Smith et al., 2003). Each biota is referred to herein using the name of the locality. The ultrastructural and spectral fidelity of insect specimens with metallic colors have been investigated for each of the above biotas (McNamara et al., 2012a) except Florissant and Green River, where metallic colors have not been reported. The cuticles with metallic colors exhibit ultrastructures typical of extant insect cuticle, such as exocuticular lamination, pore canals and their filaments, fibrillar textures in the epicuticle, and extracellular membranes of the
endocuticle (McNamara et al., 2012a). Preliminary analyses have shown that these ultrastructures are poorly preserved or absent where colors are not preserved (McNamara et al., 2012a).

Beetles are the focus of this study as (1) they are abundant in each fossil biota studied, (2) they exhibit evidence of structural color more commonly than other fossil insects, and (3) the structural mechanisms that generate color in extant beetles are well understood (reviewed by Seago et al., 2009). Biophotonic nanostructures in extant beetles comprise diffraction gratings (arrays of parallel ridges or slits), three-dimensional photonic crystals (cubic, diamond, or gyroid lattices), or multilayer reflectors (alternating layers of high and low refractive index) (Vukusic, 2003; Seago et al., 2009). Multilayer reflectors are the most common structural color mechanism in extant beetles (Seago et al., 2009) and occur within the epicuticle (Schultz and Rankin, 1985; Kurachi et al., 2002), exocuticle (Neville and Caveney, 1969) or endocuticle (Hinton, 1973). The structure can be modified for diverse functions including crypsis (Parker et al., 1998b), warning coloration (Vogler and Kelley, 1998), and sexual signalling (Schultz, 1986).

Multilayer reflectors have been identified in fossil beetles from various lacustrine settings (Parker and McKenzie, 2003; Tanaka et al., 2010; McNamara et al., 2012a), including most of the Lagerstätten studied here (McNamara et al., 2012a), and in fossil moths (McNamara et al., 2011a) and flies (Parker et al., 1998a).

Here, we document the preservation of beetles in these biotas to yield the first systematic investigation of the controls on the preservation of structural color. We first assess the fidelity of color preservation in specimens from each biota, including those in which color is not preserved or has been altered during storage. We then relate these data to the sedimentological and stratigraphic context and the diagenetic history of each biota in order to identify what factors control the fossilization of structural colors.

GEOLOGICAL BACKGROUND

Each Lagerstätte studied herein is hosted in finely laminated mudstones that were deposited in the deeper, anoxic zones of a stratified lake. The paleolakes of Randecker Maar, Enspel, Eckfeld, and Messel developed within volcanic maars (Jankowski, 1981; Felder and Harms, 2003; Lutz et al., 2010; Schindler and Wuttke, 2010). The Clarkia and Florissant paleolakes, although not maar lakes, received significant inputs from volcanic sources (Smiley and Rember, 1985; Evanoff et al., 2001). The Lake Uinta sequence (Green River Formation) records a carbonate-dominated, sometimes evaporitic, lacustrine environment. Estimates of paleolake depth during deposition of the fossil-bearing intervals range from ~12 m for Clarkia (Smiley and Rember, 1985) to ~350–400 m for Messel (Felder and Harms, 2003); the other paleolakes are considered to have been between several tens of meters and 300 m deep (Jankowski, 1981; Johnson, 1981; Larsen, 2000; Pirrung et al., 2001, 2003). The fossil-bearing laminates from each locality contain abundant clay minerals, significant algal- or terrigenous-derived
organic matter and, except for Green River, diatoms. The laminites are interbedded with volcaniclastic and tuffaceous laminae at each locality except Green River; graded silt to fine sand laminae also occur at Eckfeld. Regularly alternating laminae in the Clarkia, Eckfeld, Randecker, and Florissant sediments have been interpreted as annual deposits (McLeroy and Anderson, 1966; Jankowski, 1981; Smiley and Rember, 1985; Mingram, 1998; Lenz et al., 2010).

The fossiliferous deposits from each locality vary in their burial history (Table 1). The sediments from Randecker Maar were buried to ~30 m (Krautter and Schweigert, 1991). Assuming a geothermal gradient of ~50 °C/km, an estimate within the upper part of the range typical for intraplate volcanic regions (Johnson et al., 1989), and a geobarometric gradient of ~330 bar/km (Chalokwu, 1989), this depth corresponds to temperatures of <20 °C and pressures of ~10 bar. Unlike the laminites from the other localities, those from Randecker Maar experienced extensive late-stage hydrothermal activity (Lorenz et al., 2010) that resulted in the local precipitation of siliceous cements (Jankowski, 1981) and likely exposed the laminites to higher temperatures than those estimated above. The maximum temperatures of the hydrothermal fluids, however, have not been determined.

The fossiliferous sediments at Clarkia crop out at several localities separated by <15 km and, thus, those from each locality presumably had a similar burial history. The overlying sequence has not been studied in detail (Smiley and Rember, 1981). The Clarkia sediments are considered to have been buried to ~100 m, based on stratigraphic and geomorphological evidence (William C. Rember, personal communication, 2012); this approximates to burial temperatures and pressures of <40 °C and ~33 bar. Low burial temperatures for these sediments are confirmed by the nature of the organic matter in the laminites, which is very thermally immature (Logan and Eglinton, 1994).

The Enspel and Eckfeld localities are located within the Rhenish Shield (Lutz et al., 2010; Schindler and Wuttke, 2010). The sediments at Eckfeld are considered to have been buried to ≤100 m depth based on the geomorphological evolution of this feature (Lutz et al., 2010), corresponding to burial temperatures and pressures of <40 °C and ~33 bar; sediments from Enspel presumably had a similar burial history.

The Florissant Formation is considered to have been overlain by <100 m of volcanogenic deposits prior to exhumation (Gregory and Chase, 1992), but the tectonic history of the region during the Cenozoic is not resolved completely (Meyer, 2001). An overburden thickness of 100 m would approximate to burial temperatures and pressures of <40 °C and ~33 bar. Geochemical analyses of kerogen from Messel indicate that the sediments were buried to <300 m (corresponding to maximum burial pressures of 100 bar) and experienced temperatures of ≤40 °C (Hayes et al., 1987). Geochemical analyses of kerogen from the Uinta Province of the Green River Formation indicate a maximum burial depth of 1200–6700 m (corresponding to ~400–2200 bar) and maximum burial temperatures of 65–180 °C (Nuccio and Roberts, 2003).

### MATERIAL AND METHODS

#### Fossil Material

Seven hundred and forty-one beetle specimens were studied: Randecker Maar, n = 69; Clarkia, n = 15; Enspel, n = 73; Florissant, n = 112; Eckfeld, n = 314; Messel, n = 123; and Green River, n = 35. The specimens are held by the following institutions: Generaldirektion Kulturelles Erbe, Mainz, Germany (GKE); the Museum for Comparative Zoology, Harvard University (MCZ); Naturhistorisches Museum Mainz (Germany) (NMM); Senckenberg Forschungsinstitut und Naturmuseum, Forschungsstation Grube Messel, Germany (SF-MeI); Staatliches Museum für Naturkunde Stuttgart, Germany (SMNS); and the Yale Peabody Museum of Natural History, United States (YPM). Specimens from Florissant and Green River are stored in air. Specimens from all other localities are stored in glycerine (100% or 70%–99% in water) or ethanol (70%); temporary immersion in these solutions (and water) does not alter the hue of the observed color (McNamara et al., 2012a). The specimens studied include unidentified beetles preserving metallic colors and specimens of beetle taxa known to exhibit metallic colors (even though the fossils may be black), i.e., Buprestidae, Chrysomelidae, and Tenebrionidae; most specimens are not identified to species level, but examples from a single Lagerstätte are likely to be conspecific or closely related. The Clarkia material used herein comprises unidentified Coloptera. Our dataset also includes Chrysomelidae from each of the remaining biotas, Buprestidae from each biota except for Randecker Maar, and Tenebrionidae from Eckfeld.

The quality of preservation of the cuticle can be assessed in hand specimen using such variables as cohesiveness and reflectivity and correlates with that of the multilayer reflectors (observed using electron microscopy) and the resulting structural colors (McNamara et al., 2012a). The quality of preservation of the structural color exhibited by the specimens investigated here was coded as follows: (1) well preserved if colors are bright and saturated and the cuticle is highly reflective and cohesive, (2) poorly preserved if colors are faint and the cuticle is slightly reflective, and (3) not preserved if color is absent and the cuticle is dull and friable. Some specimens could not be coded, e.g., if the cuticle was obscured by diagenetic minerals or if the plane of splitting of the slab passed through it. The percentage of specimens in each color preservation category from each biota was calculated (Fig. 2A). Differences in the abundance of specimens among the different categories and biotas were tested using the chi-squared test in PAST (PAleontological STatistics) v. 2.07 (Hammer et al., 2001). One expected value is less than five, thus reducing the accuracy of the test, and therefore an additional significance test based on a Monte Carlo randomization was used to generate a probability (p-) value; a value of p < 0.05 is considered significant.

The taxonomic distribution of specimens in each color preservation category was tested as follows. The percentage of specimens of each beetle family (i.e., Buprestidae, Chrysomelidae, and Tenebrionidae) in each preservation category was calculated (Fig. 2B); differences in the abundance of specimens of each family among the different categories were tested using the chi-squared statistic. The percentage of specimens of Buprestidae and Chrysomelidae in each color preservation category from each biota was then calculated (Figs. 2C, D). In order to facilitate statistical analysis of these data, data for specimens with poorly preserved or no color were amalgamated, yielding two categories: specimens with well-preserved color, and specimens where color is poorly preserved or absent (Figs 2C, D). Differences in the abundance of Buprestidae and Chrysomelidae in each color preservation category were tested as follows.

### TABLE 1—Summary of the diagenetic history of sediments from each biota.

<table>
<thead>
<tr>
<th>Diagenetic history</th>
<th>Fossil locality</th>
</tr>
</thead>
<tbody>
<tr>
<td>Burial depth (m)</td>
<td>Randecker Maar</td>
</tr>
<tr>
<td>30</td>
<td>16–18 Ma</td>
</tr>
<tr>
<td>Burial temperature (°C)</td>
<td>&gt; &gt; 40</td>
</tr>
<tr>
<td>Burial pressure (bar)</td>
<td>10</td>
</tr>
<tr>
<td>Hydrothermal alteration</td>
<td>yes</td>
</tr>
</tbody>
</table>

...
were tested only for biotas with a large sample size (i.e., Enspel, Eckfeld, and Messel for Buprestidae, plus Randecker Maar for Chrysomelidae) using Fisher’s exact test (which yields a $p$-value between 0 and 1) in R (Figs. 2B, C); the chi-squared test could not be used as several expected values are less than five.

Analysis of Sedimentological Context

The stratigraphic and lithological contexts of specimens are potential indicators of conditions in the lake-floor environment during deposition. Stratigraphic context can inform on temporal trends in preservation throughout a sequence and can be combined with geochemical data to inform on chemical conditions during deposition. The fossil-bearing laminae document discrete, possibly seasonal, events; centimeter-scale changes in laminite facies reflect environmental fluctuations on decadal scales. Data on precise stratigraphic position are available only for specimens from Eckfeld; these specimens were thus the focus of our sedimentological analysis. All specimens are from the uppermost 11 m of Lithozone D2 of the Eckfeld sequence (Lutz et al., 2010). The stratigraphic position of each specimen ($n = 119$) was marked on a log of the fossiliferous interval and quantified as the distance to the base of the log (Fig. 3). Due to small sample size, data were amalgamated for specimens where metallic color was poorly preserved or absent. The stratigraphic distribution of specimens in either category was tested using the Wald-Wolfowitz runs test in PAST. This is a nonparametric test for randomness in a sequence of values; it yields a $p$-value between 0 and 1 and, for $n < 20$, is corroborated with a Monte Carlo $p$-value based on 10,000 random replicates. Variations in $C_{org}$ (wt%), siderite (wt%), sulfur (wt%), and $C/N$ ratio throughout the fossiliferous interval (Mingram, 1998) were used to test the relationship between the stratigraphic position of specimens and changes in paleolake chemistry. The interpreted values for each of the above geochemical indices were noted for each insect-bearing horizon.

The laminated deposits from Eckfeld comprise predominantly gray organic-rich laminae, white clay- and quartz-rich laminae, and silty laminae (Pirrung et al., 2003). Where possible, the fossil-bearing lamina was identified for each beetle specimen. Each slab was assigned to one of the following laminated mudstone facies, based on the lamina succession exposed: Facies 1 if comprising primarily gray and white laminae, or Facies 2 if comprising primarily silty laminae. The condition of the slab was assessed visually as weathered (i.e., iron-stained) or not weathered. To test for the significance of the distribution of specimens in each color preservation category among the various facies, lamina types and slab condition, expected values were calculated as described above (Fig. 4). Differences between observed and expected values were tested using the chi-squared statistic to analyze data on distribution between facies, and Fisher’s exact test to analyze data on distribution among lamina type and slab condition.
FIGURE 3—Stratigraphic context of buprestid, chrysomelid and tenebrionid beetle specimens from Eckfeld. A) Stratigraphic location of horizons (red and black lines) with specimens used in this study. No two specimens are from the same horizon. Black lines denote horizons bearing beetle specimens with well-preserved metallic color; red lines, those where metallic color is poorly preserved or absent. Key marker horizons of the Eckfeld sequence (e.g., KaLH, HT) are indicated. B) Stratigraphic log of the section exposed at the 1993 excavation site (modified from Lutz and Kaulfuss, 2006; www.schweizerbart.de). C) Sedimentary organic matter content and C/N ratio (modified from Lutz and Kaulfuss, 2006, in turn modified from Mingram, 1998). D) Sedimentary siderite and sulfur content (modified from Lutz and Kaulfuss, 2006, in turn modified from Mingram, 1998).
Electron Microscopy

Small (2–3 mm²) samples of cuticle were removed from selected specimens from each preservation category from each biota using sterile tools. Samples from specimens in glycerine were dehydrated in the following ethanol:glycerine mixtures in sequence, each for 24 hours: 10%, 25%, 50%, 75%, 90%, 100% ethanol; samples from specimens in 70% ethanol were dehydrated in the following ethanol:water mixtures: 75%, 90%, 100%. Once in 100% ethanol, samples were prepared for scanning- and transmission electron microscopy (SEM and TEM) using the methods of McNamara et al. (2011a). In addition, one small (<0.3 mm²) sample from a specimen from Enspel with well-preserved metallic colors (Fig. 1B) was washed in water (to remove glycerine) and allowed to dry in air at room temperature and pressure. As noted previously (McNamara et al., 2012a), the hue did not change after several days (although the intensity of the color decreased). The washing and drying process was repeated several times, each resulting in a reduction in the intensity of the color, until the color was scarcely apparent; the sample was then prepared for SEM. Samples for SEM were examined using a FEI XL-30 ESEM-FEG microscope equipped with an EDAX energy dispersive X-ray spectrometer. Observations were made at an accelerating voltage of 15kV, with acquisition times of 60 seconds for electron dispersive spectra of carbon-coated samples. Samples for TEM were examined using a Zeiss EM900 TEM at 80kV with an objective aperture of 90 μm diameter.

Reflectance Spectrophotometry

Samples of cuticle for reflectance spectrophotometry were removed from the specimen in Figure 1B using sterile tools, washed in water, allowed to dry and analyzed within minutes. Spectra were collected from a 3 mm² spot on the cuticle using normal incident light at 6 mm distance and recorded in air using an Ocean Optics S2000 spectrophotometer. Recorded spectra were normalized against the spectrum of the light source from a white standard.

RESULTS

Age, Taxonomy, and Sedimentary Context

Age.—Metallic colors are well preserved in most (>92%) specimens from Clarkia, Enspel, Eckfeld, and Messel (Fig. 2A). The quality of preservation is much lower for specimens in Randecker Maar, where metallic colors are typically poorly preserved (63.4% of specimens). Metallic colors are not preserved in specimens from Florissant and Green River. These differences in the quality of color preservation among the biotas are statistically significant (511; d = 5; χ² = 20.52; p < 0.001; Monte Carlo p < 0.0001).

Taxonomy.—Differences in the abundance of buprestid, chrysomelid, and tenebrionid beetles between the different color preservation categories are not statistically significant (Fig 2B) (0.0003; d = 4; χ² = 16.27; p < 0.001). Differences in the abundance of buprestid beetles from Enspel, Eckfeld, and Messel in each color preservation category are not statistically significant (Fig. 2C) (p = 0.596). Differences in the abundance of chrysomelid beetles from these biotas and Randecker Maar in each color preservation category are statistically significant (p = 2.2e−16). This likely reflects the generally poor preservation of specimens from Randecker Maar compared with those from the other biotas used in the analysis: when data from Randecker Maar are not included in the test, the result is not significant (p = 0.768) (Fig. 2D). Preservation of metallic colors in the beetle taxa is therefore independent of taxonomy.

Stratigraphic Context.—Analysis of the relationship between the quality of color preservation and stratigraphic context was possible only for specimens from Eckfeld. The stratigraphic data reveal that specimens with well-preserved metallic colors are randomly distributed throughout the lacustrine sequence (p(rand) = 0.826) (Fig. 3). Similarly, the stratigraphic distribution of specimens where metallic colors are poorly preserved or absent is random (p(rand) = 0.797) (Fig. 3). The horizons bearing specimens with well-preserved color, and where color is poorly preserved or absent, exhibit similar variation in each of Corg (6%–19% and 4%–19%, respectively), siderite (each 0.25 wt%), sulfur (each 0.4–2.2 wt%), and C/N ratio (each 20–40) (Fig. 3). Differences in the fidelity of preservation do not, therefore, reflect variation in the hydrochemistry of the paleolake.

Lithology.—Specimens from Eckfeld occur in a variety of facies and lamina types (Fig. 4). Differences in the abundance of specimens with well- and poorly preserved colors among the different facies are not significant (0.98; d = 1; χ² = 6.64; p < 0.01) (Fig. 4A). Specimens with poorly preserved colors, however, are more common in silty laminae (p = 0.0023; Fig. 4B) and on weathered slabs (p = 1.345e−8; Fig. 4C).
Ultrastructural Basis of Color Loss

Each biota includes specimens of colored beetle taxa where metallic colors are poorly preserved or absent (Fig. 2). Analysis of cuticles from such specimens confirms previous observations (McNamara et al., 2012a) that diagnostic cuticular ultrastructures are not retained (compare Figs. 5 and 6 with Figs. 7A–C); in particular, there is little or no preservation of multilayer reflectors within any of the cuticles (Fig. 6). The mode of degradation, however, varies markedly between specimens. Cuticles from Randecker Maar that lack color are typically amorphous and gel-like with loss of all surface and internal detail (Fig. 5A). In cases where color is poorly preserved, internal details of the cuticle are poorly preserved at best (Fig. 6A) but usually absent, and diagenetic minerals may occur within the cuticle (Fig. 5B). EDS spectra of the mineral phase exhibit peaks for only Si and O; the cuticle itself reveals C, Si, and O, indicating partial replacement of the original organic material by silica. In contrast, EDS spectra of cuticles from all other localities exhibit peaks for only C and O, demonstrating that these cuticles are preserved exclusively organically.

**FIGURE 5**—Scanning electron micrographs of fossil beetle cuticles. Unless specified, images show the dorsal surface of an elytron. A) Randecker Maar, color not preserved, showing amorphous, gel-like texture. B) Randecker Maar, color poorly preserved, showing diagenetic silica precipitate (si) that forms both a quasi-planar sheet and localized rounded aggregations of crystals within the cuticle (c). C) Clarkia, color not preserved; surface texture is amorphous to porous. D) Enspel, color not preserved; surface deformed by numerous impressions of diatoms but internal structures (e.g., endocuticular chitin bundles) are visible in fractured vertical sections (inset). E, F) Florissant, color not preserved, showing shrinkage cracks (E) and amorphous texture in fractured vertical section (F). G–I) Eckfeld, color not preserved, showing amorphous texture in vertical section (G) and porous, gel-like texture on the surface (H). The porous texture is associated with recent bacteria and fungal hyphae (I). J) Eckfeld, from a coprolite; color poorly preserved. Abundant fossilized bacteria (detail in inset) are present on the surface. K, L) Messel, color poorly preserved, showing abundant fossilized bacteria (K) and fungal hyphae (L) on the surface, which is porous (K) (inset shows detail) but appears amorphous in vertical fractured section (L, inset). M) Green River, color not preserved, fractured vertical section showing amorphous texture. The arrow shows the junction between the dorsal and ventral cuticles.
Weathered cuticle from Clarkia (on an iron-stained slab) exhibits an irregular surface and a porous, granular texture (Figs. 5C, 6B). Cuticle from Enspel exhibits cracks and numerous impressions of diatoms in its surface (Fig. 5D), but retains details of endocuticular chitin bundles (inset, Fig. 5D). The cracks resemble sedimentary structures that typically form in subaerial fine-grained sediments due to shrinkage (Plummer and Gostin, 1981) and are presumably diagenetic in origin. Cuticles from Florissant also usually exhibit abundant shrinkage cracks and an amorphous surface (Fig. 5E), and lack internal detail (Figs. 5F, 6C). Similarly, cuticle from Eckfeld (on a weathered slab) lacks internal detail (Fig. 5G). The surface exhibits shrinkage cracks and an amorphous texture that appears porous at low magnifications (Fig. 5H). Closer examination of the porous regions reveals abundant ovoid (ca. 1 um long) and filamentous (>50 um long and ca. 1.5 um wide) microstructures that likely represent bacteria and fungi. The cuticle exhibits an open fibrillar texture immediately adjacent to the microbes (Fig. 5I), which is probably the result of microbial degradation. Similar porous fibrillar textures developed in the cuticle of stomatopods during degradation experiments (Hof and Briggs, 1997). The microbes associated with the cuticle from Eckfeld are noticeably flattened (Fig. 5I) following distortion under the electron beam; this instability suggests that they are recent in origin. In contrast, cuticle with poorly preserved color from a coprolite from Eckfeld is associated with abundant ovoid- to rod-shaped microstructures that are fully three-dimensional and thus likely represent fossilized bacteria (Fig. 5J); EDS analyses reveal peaks for only C and O, indicating the fossil bacteria are organically preserved. Fossil bacteria preserved organically (McNamara et al., 2009) and as organic-rich, partially mineralized remains (Toporski et al., 2002) are associated with exceptionally preserved fossils from other Cenozoic Lagerstätten.

Cuticles from Messel, as for those from Clarkia and Eckfeld, exhibit a porous texture and can be associated with three-dimensional rod-shaped (2 um long; Fig. 5K) or tubular (>100 um long and 2 um wide; Fig. 5L) microstructures interpreted herein as fossilized bacteria and fungal hyphae, respectively. Other insect and plant cuticles from Messel are associated with fossil bacteria and fungi (Richter, 1994). Internal cuticular ultrastructures are absent (Fig. 5L). The surface of cuticles from Green River is amorphous and gel-like, and no internal details are evident (Fig. 5M).

A specimen from Enspel (Fig. 1B) illustrates both ends of the preservational spectrum and demonstrates the close association between visual appearance in hand specimen and the fidelity of preservation of cuticular nanostructure. Metallic colors are well preserved in the anterior part, but poorly preserved in the posterior. Where color is well preserved, the cuticle surface is smooth and continuous (Fig. 7A) and various internal structures, including exocuticular lamination, pore canals (Fig. 7B), and an epicuticular multilayer reflector (Fig. 7C) are present. Reflectance spectra from this region of the specimen exhibit a clear unimodal peak (Fig. 8). In contrast, where color is poorly preserved, the cuticle is highly fractured (Fig. 7D), the surface is porous and amorphous to pustulose (Fig. 7E), and the epicuticle exhibits only subtle banding (Fig. 7F); ovoid microstructures associated with the porous regions (Fig. 7E) may represent recent bacteria. Reflectance spectra from this region of the specimen show no peak, and low overall reflectance (Fig. 8). A cuticle sample from the anterior part of this specimen that was allowed to dry in air exhibits significant degradation (Figs. 7G–I). Individual layers of the epicuticle remain cohesive but are delaminated locally (Fig. 7G); elsewhere, the separate epicuticular layers are difficult to differentiate and the cuticle surface exhibits a pustulose texture (Figs. 7H–I).

**Patterns in the Preservation of Structural Colors**

Patterns among Biotas

The fidelity of the fossil record varies through time, due in part to the cumulative effects of diagenesis and tectonism (Kidwell, 2001). As a result, we might predict the fidelity of preservation of structural colors to be lower in older biotas than younger examples from similar depositional settings. Our results are not consistent with this hypothesis. Most specimens from Randecker Maar exhibit poorly preserved colors, even though this biota is younger than others studied here in which the quality of color preservation is consistently high. Metallic colors are not preserved in specimens from Florissant and...
Green River, even though they are younger, or approximately coeval, respectively, with the Messel biota, in which colors are well preserved. Thus the quality of color preservation does not correlate with age. This finding parallels the demonstration that the fidelity of geochemical preservation of fossil cuticles is independent of stratigraphic age (Stankiewicz et al., 1998).

Specimens of the same beetle families were studied from each biota and their fidelity of preservation does not correlate significantly with family nor, for specific families, between biotas. Taxonomic factors (at least at the family level) do not, therefore, impact upon the fidelity of preservation of structural colors. The depositional context and host lithology of all specimens is broadly similar and there is no evidence that lithological factors are responsible for differences in color preservation among the biotas. The only variables that remain relate to the diagenetic history of the sediments hosting the fossils. This varies markedly among the different biotas; in particular, the diagenetic history of Randecker Maar, Florissant, and Green River includes unique events that are considered further below. Cuticles that lack preserved color reveal a variety of different degradation textures and features, indicating that the destruction of metallic color has multiple origins.

Unlike the other biotas, sediments from Randecker Maar experienced significant hydrothermal alteration (Lorenz et al., 1970) and are thus likely to have experienced relatively high temperatures during burial. These factors had a detrimental effect on the preservation of beetle cuticles from this biota; cuticles where colors are poorly preserved or absent exhibit amorphous textures characteristic of thermal maturation (Stankiewicz et al., 2000) and are replaced partially by silica. These diagenetic changes can alter the laminations in the cuticle (Fig. 6A), destroying the multilayer reflectors. They may also compromise color by altering the composition and refractive index of the cuticle (McNamara et al., 2012a).

The only specimens to have been buried to significant depth are those from Green River. Specimens from this biota therefore experienced higher pressures and temperatures during burial (Nuccio and Roberts, 2003). Neither cuticular ultrastructure nor metallic colors are preserved; the cuticles consistently exhibit the lowest fidelity of preservation encountered in this study. The amorphous textures of the cuticles are characteristic of thermal maturation (Stankiewicz et al., 2000).

The remaining biotas (Clarkia, Enspel, Florissant, Eckfeld, and Messel) have a broadly similar burial history incorporating relatively...
shallow burial (<300 m) without significant alteration by diagenetic fluids. Specimens from these biotas experienced low burial pressures and temperatures. The absence of metallic colors in specimens from Florissant must, therefore, reflect late diagenetic or recent events, i.e., during exhumation and exposure of the fossiliferous sediments. The absence of metallic colors in specimens from Florissant must, therefore, reflect late diagenetic or recent events, i.e., during exhumation and exposure of the fossiliferous sediments. The sediments from Clarkia, Enspel, Eckfeld, and Messel are waterlogged (the Messel oil shale has a water content of ~40% (Schaal and Ziegler, 1992)), in contrast to those from Florissant. Hydrogen isotope analysis reveals a marked difference in composition between the pore waters from the Clarkia sediments and meteoric waters at the locality, indicating the two have different origins (Yang and Huang, 2003; the pore waters have a lower oxygen content (H. Yang, personal communication, 2012) and may be ancient (Yang and Huang, 2003). The high pore water content at Clarkia, Enspel, Eckfeld, and Messel promotes preservation of cuticular nanostructures and, in particular, multilayer reflectors and the resulting metallic structural colors. Conversely, the relatively low water content at Florissant facilitates dehydration and oxidation of fossil beetle cuticles, resulting in loss of metallic colors. This hypothesis is supported by the observation that specimens from Messel and Enspel, for example, eventually lose their metallic colors upon exposure to air (Parker and McKenzie, 2003; Schweizer et al., 2006), and by our observations on the effect of dehydration on ultrastructure (see below).

Patterns within Biotas

Patterns in the preservation of structural colors occur within as well as among biotas. The relationship between the quality of preservation of metallic colors and sedimentological and stratigraphic context was analyzed at Eckfeld where data are available on the specific horizons from which specimens were collected. Our results indicate that variation in the fidelity of preservation of metallic colors is due to highly localized infiltration of the host sediments by oxygen-rich diagenetic (probably meteoric) fluids. Iron staining is usually confined to, or most extensive, in silty laminae, presumably reflecting their high permeability to diagenetic oxygen-rich fluids rather than syndepositional fluctuations in environmental conditions. The poor preservation of color in silty facies at Eckfeld can be attributed to this variation in permeability. Preservation of structural colors is, therefore, independent of precise sedimentological context and, by inference, short-term environmental variation; preservation is, however, facilitated by the broad-scale features of the depositional environment, i.e., deposition in laminated, organic-rich mudstones in profound parts of a stratified lake. Similar taphonomic controls have been identified for exceptionally preserved vertebrates from lacustrine-hosted Lagerstätten (McNamara et al., 2011b, 2012b).

Despite infiltration of the fossiliferous deposits from Randecker Maar by hydrothermal fluids, some specimens retain well-preserved colors. The sediments hosting such specimens do not exhibit evidence for recrystallization; notably, they lack the brittle, granular texture of sediments in which color is poorly preserved or absent. This supports our interpretation of variation in the fidelity of preservation of color among Randecker Maar specimens as a result of heterogenous infiltration of the deposits by silica-rich hydrothermal fluids.

The single specimen from Clarkia that lacks metallic colors (Fig. 5C) is the only example from this locality on a weathered slab. Loss of color clearly resulted from destruction of the epicuticle (and hence any multilayer reflector originally present); this, in turn, reflects oxidation during weathering. Similar textures are apparent in cuticles from Eckfeld that occur on weathered slabs, but such cuticles also show evidence of recent microbial degradation of the cuticle. Loss of color in the Eckfeld specimens may reflect a combination of weathering and microbial degradation. The timing of microbial colonization is difficult to constrain; it could have originated in the sediment or during storage: fossil insects and slabs stored in glycerine often exhibit evidence of degradation after several years.

Cuticles with poorly preserved colors from Messel exhibit porous, amorphous textures but do not occur on weathered slabs. The fossilized microorganisms associated with these specimens presumably invaded the cuticle prior to, or soon after, deposition of the specimens on the lake floor. Loss of color in these specimens reflects microbial degradation of the epicuticle.

The specimens from Enspel with poorly preserved or no color are neither from weathered intervals nor show evidence of microbial degradation. Deformation of the epicuticle by compaction on diatoms (Fig. 5D) has likely altered the structure of the multilayer reflector. Deformation due to compaction on diatoms also impacts the preservation of surface features in leaves from Enspel (see fig. 2C in Gupta et al., 2007) and Florissant (Harding and Chant, 2000). Metallic colors are well preserved in only the anterior part of the specimen investigated from Enspel in Figure 1B. The posterior, where metallic colors are poorly preserved, lies adjacent, and at an oblique angle, to the edge of the slab in a thin (ca. 10 mm thick) oxidized zone. Specimens from Enspel are routinely placed in brine immediately after collection to avoid dehydration and microbial contamination (Toporski et al., 2002) and are stored in this solution for several months until they are prepared, after which they are transferred to glycerine for curation. Metallic colors were well preserved over the entire cuticle surface of the specimen in Figure 1B immediately after collection. The oxidized zone developed during the four months it was stored in brine prior to our study (Petra Schaefers, personal communication, 2010). The porous texture of the cuticle from the altered zone of the slab resembles that in specimens from weathered slabs from Clarkia and Eckfeld. This suggests that the observed degradation of the cuticle of the Enspel specimen is due (at least in part) to oxidation. The association of bacteria with the porous regions of the cuticle suggests that microbial degradation is a contributing factor and indicates that storage of specimens in brine inhibits but does not preclude microbial degradation.

The sample of cuticle with well-preserved metallic colors from Enspel that was allowed to dry in air until the colors faded (Figs. 7G–I) exhibits delamination of the epicuticle and loss of its internal structure; loss of metallic colors is clearly due to alteration of the structure of the multilayer reflector. The pustulose texture may result from rapid changes in surface tension, which can cause significant structural deformation in tissues (Hayat, 1989), and/or oxidative reactions during...
drying. Delamination was not observed in the epicuticle of any other fossil specimen but is likely to have been prevented by the pressure of the overlying sediment.

CONCLUSIONS

The fidelity of preservation of metallic structural colors in fossil beetles from Cenozoic lacustrine-hosted settings is influenced by various diagenetic factors that operate at the level of individual specimens as well as the level of entire biotas. These factors are the extent of thermal alteration, microbial degradation, physical deformation, dehydration, and oxidation; all can alter or destroy epicuticular multilayer reflectors. These processes can occur at different stages in the taphonomic history of specimens, i.e., prior to deposition, during burial and exhumation, and postcollection. Preservation of metallic structural colors in fossil beetles is promoted by shallow burial and saturation of sediments by oxygen-poor fluids, and by the resistance of the cuticle to degradation: cuticles from beetles retain ultrastructural and chemical information when those from other taxa do not (Stankiewicz et al., 1997; Gupta et al., 2007). The extent to which these results apply to other insect taxa and to specimens from other depositional and diagenetic regimes could be assessed through detailed studies of structurally colored insects from other Cenozoic Lagerstätten. The effect of age on the fidelity of preservation of cuticles is insignificant over the time scale studied herein but may be exacerbated in Mesozoic and Paleozoic material; polymerization of molecular material in cuticles to form resistant macromolecules is, in part, time dependent (Stankiewicz et al., 2000). Our results also highlight a number of important variables that are amenable to investigation via controlled taphonomic experiments. Of particular interest are the oxidative processes that may result in loss of structural color, and the various roles of decay, pressure, and temperature in the preservation of metallic structural colors. Experiments could also shed light on the extent to which similar taphonomic controls apply to structural colors generated by other types of biophotonic nanostructures (e.g., diffraction gratings and 3D photonic crystals). The geochemical basis of structural color loss has not been investigated and thus it is not yet clear whether preservation of structural colors is coupled with a high fidelity of biochemical preservation in addition to preservation of cuticle ultrastructures. Finally, certain phenomena were identified in the course of this study that can cause or contribute to loss of preserved structural colors after collection. This has significant implications for curatorial practice. In particular, the storage of structurally colored specimens in brine, even on a temporary basis, has significant implications for curatorial practice. In particular, the storage of structurally colored specimens in brine, even on a temporary basis, has significant implications for curatorial practice. In particular, the storage of structurally colored specimens in brine, even on a temporary basis, has significant implications for curatorial practice. In particular, the storage of structurally colored specimens in brine, even on a temporary basis, has significant implications for curatorial practice. In particular, the storage of structurally colored specimens in brine, even on a temporary basis, has significant implications for curatorial practice.


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