Schoenemann B, Clarkson ENK, Horváth G. 2015. Why did the UV-A-induced photoluminescent blue–green glow in trilobite eyes and exoskeletons not cause problems for trilobites? PeerJ 3:e1492 https://doi.org/10.7717/peerj.1492

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Trilobites are extinct marine arthropods that dominated the faunas of the Palaeozoic. They were equipped with highly differentiated compound eyes. By contrast with all other arthropods, the lenses of these compound eyes were of pure calcite. Calcite shows photoluminescence under short-waved light. Here we show the phenomenon that in trilobite eyes the lenses glow under black-light illumination (UVA 365nm). Any inhomogenous distribution of light patterns across the lattice of facets in their compound eye, which is caused by an non homogenous UV-pattern inside the environment, would give orientational information to the trilobite. While many modern arthropods developed specialised UV-photoreceptor cells, the blue greenish light of the UV-induced fluorescence in the optical systems of the trilobites works without such modifications. We propose a new specialised optical system, ~400 million years old which is unique in the animal realm, and may be a role model for present technical applications.

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The Glow in Trilobite Eyes

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Key Words: Vision, Cambrian explosion, Trilobite, UV-radiation, Palaeozoic, Compound Eye,

Arthropod, Luminescene, Optics, Calcite

ABSTRACT Trilobites are extinct marine arthropods that dominated the faunas of the Palaeozoic. They were equipped with highly differentiated compound eyes (Whittington 1997, Clarkson 1998, Clarkson, Levi-Setti & Horváth 2006). By contrast with all other arthropods, the lenses of these compound eyes were of pure calcite (Towe 1973, Lee, Torney & Owen 2007). Calcite shows photoluminescence under short-wavelength light. Here we show the phenomenon that in trilobite eyes the lenses glow under black-light illumination (UVA 365nm). Any inhomogenous distribution of light patterns across the lattice of facets in their compound eye, which is caused by a non homogenous UV-pattern inside the environment, would give orientational information to the trilobite. While many modern arthropods developed specialised UV-photoreceptor cells, the blue greenish light of the UV-induced fluorescence in the optical systems of the trilobites would work without such modifications. We propose a new specialised optical system, ~400 million years old, which is unique in the animal realm, and may be a role model for present technical applications.

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INTRODUCTION The most prevalent mobile invertebrates of the Palaeozoic seas were trilobites, arthropods equipped with a thick shell and highly differentiated compound eyes from the very beginning of their appearance in the fossil record, some 522 million years ago. By contrast with all other arthropods, including those living today, the lenses of these compound eyes were of pure, orientated calcite (Towe 1973, Lee, Torney & Owen 2007). This raises questions about the specificity of this unique system, which persisted successfully for more than 250 million years, but was never reinvented again after trilobites became extinct. Calcite has the advantage of transparency and a high refractive index which allows efficient focusing even under water. Another important property of calcite is to show luminescence when illuminated with UV-light. Many calcites shine with a blueish colour during fluorescence. If trilobite eyes were able to convert UV-light into perceptible light of longer wavelengths, they would have evolved an optical system able to perceive UV-light for vision, without a need for UV-sensitive photoreceptors, as many modern arthropods possess. Here we show that the lenses in trilobite compound eyes glow, showing blueish fluorescence, when illuminated with light of 365nm. Because these lenses lie directly above the receptive system this UVA-light is available to the visual process, as is light of longer wavelengths. Thus we are here considering an ancient visual system, which was able to use a unique, and never repeated technique which widened its visual spectrum by means of a sophisticated lens.

It is well known that due to the ozone layer being deficient or absent during the Archean, high energy radiation was able to penetrate more deeply into oceans than it does at present, and thus the potential damage rates to DNA were magnitudes higher than today. DNA-damage must have been the principal factor for UV-induced mortality in the Archean oceans (Cockell 1998, 2000 a,b). Thus at 5m depth the potential DNA-damage rate may have been 2 orders of magnitude higher than today, and still one order at 15m depth [Cockell 2000a]. A quite rapid change started probably ~800 million years ago [Ma] (Qiu 2014), and by at least 700 Ma oxygen levels might have been sufficient for respiration in metazoans (Bekker et al. 2004, Hessen 2008, Margulis, Walker & Ramblerer 1976). Having just achieved an almost modern atmosphere ~520 Ma, and probably by the availability of certain minerals to establish modern shells, the 'Cambrian explosion' became possible, an era when most modern clades appeared (Hessen 2008, Margulis, Walker & Ramblerer 1976, Cowen 2005). As for many of the complex organisms of this era, the origin of trilobites probably lies before the 'Cambrian explosion' further back in the Proterozoic, though without any fossil record.

Most of the early arthropods, such as trilobites, were bottom dwellers, able to escape the potential harmful radiation to safe depths of the ancient oceans. Many forms were living in environments poor in oxygen, which may indicate a legacy of conditions of low oxygen concentrations in their habitats during the beginning of their evolution. This context may also indicate that the evolution of their compound eyes started under corresponding conditions.

MATERIALS AND METHODS

Uniquely in the animal realm trilobites developed compound eyes with lenses of oriented calcite rather than of organic material (Towe 1973, Clarkson, Levi-Setti & Horváth 2006). The use of calcite brings an evident advantage, especially for aquatic organisms. The high refractive index of calcite (~590 nm: nω=1,640 -1,660 ; 1,658 nε=1,486) by contrast with that of chitin, the lens material of arthropods (1.46, rarely up to 1.56 (Land & Nilsson 2012)) increases the difference in optical densities between the visual system of the arthropod and water (n=1.334) (seawater), and thus facilitates focusing. Of special interest has been the visual system of a suborder of trilobites, the Phacopina, because their large lenses have an elegant internal substructure probably correcting lens aberrations, which would otherwise be produced in the thick lenses that phacopid trilobites possess (Clarkson & Levi-Setti 1975). Although nothing is usually preserved below the level of the lenses, the first known sublensar sensory structures, at a cellular level, have been described in these trilobites very recently (Schoenemann & Clarkson 2013). The birefringence of calcite may not have been a problem for trilobite vision, because the difference of paths in the ordinary and extraordinary ray in relation to the size of the lenses is smaller than the diameter of the receptor cells, and thus may be irrelevant (Schoenemann & Clarkson 2011).

Because most trilobite exoskeletons fossilised in limestones are largely composed of calcite, experiments for investigating the photoluminescence of calcite lenses were performed on a species which normally fossilises in a somewhat different way. In the Bundenbachschiefer from the Lower Devonian of the Hunsrück region, Germany, the sulfur released from proteins, together with iron from the ancient mud formed pyrite (Kühl et al. 2012), while the lenses of pure calcite stayed as they were. The phacopid trilobite *Chotecops ferdinandi* (Kayser, 1880) (Fig. 1a) is very abundant at this location and possesses large compound eyes. Lens preservation, however, is extremely rare, because the lenses normally fall out of the fossil, and cavities remain where the lenses had been. Even so, very occasional examples are found such as the two isolated eyes of moulted specimens used here, each showing the phenomenon independently (Fig. 1c-f). The specimens are housed in the collection of the Geological Institute of the University of Cologne (now Institute of Geology and Mineralogy). The museum numbers are GIK 2118 and GIK 2119. They were illuminated with 365nm (UVA) from a source of low energy (6V, 4W, 40mA, ETT Comp. Braunschweig, Germany) and photographed (Panasonic DMC-TZ10). The energy of irradiation corresponds to $1.65 \cdot 10^{14}$ photons/s upon a lens with a diamter of 300µm (see supplement). The result shows that just the lenses glow blueish by fluorescence, as typical for many calcites.

At this stage it may be noted that a more quantitative spectrometric analysis of this phenomenon, rather than the present more qualitative analysis is not appropriate, because during the almost 390 million years since these phacopid trilobites lived the content of magnesium, iron and other ions that influence the spectral composition of fluorescence may have changed during the long processes of fossilisation and diagenesis, thus such an analysis would not reflect the original situation as it was in life. How strong the intensity of UV light may have been when this system developed, may remain also unknown.

RESULTS

Figure 1. The glow in the calcitic lenses of a phacopid trilobite´s eye.

(a) *Chotecops ferdinandi* (Kayser, 1880), Bundenbachschiefer, Lower Devonian, Location: Grube Eschenbach, Hunsrück, Germany, scale bar ~1cm. (housed in the collection of Steinmann Istitute, University of Bonn [ ] (b) 1. Calcite crystal (~3cm), 2. Fluorescent when illuminated with 365nm under water. (c) Isolated moult of a *Chotecops* compound eye with lenses preserved [GIK 2118]. (d) The same showing fluorescence in the clacitic lenses of the trilobite compound eye when illuminated with UVA-light (365nm). (e) Isolated moult of a *Chotecops* compound eye with lenses preserved [GIK 2119]. (d) The same showing fluorescence in the calcitic lenses of the trilobite compound eye when illuminated with UVA-light (365nm). b-f)D scale bar ~1mm.

When illuminated with UV-light (365nm) the remains of the calcitic lenses glow in a blue-greenish light as long as they are illuminated, while those parts, which are not of lens material, remain dark. Both of the extremely rare specimens show the phenomenon in the same way and independently.

DISCUSSION Trilobites are extinct arthropods that dominated the faunas of the Palaeozoic. They appeared in the fossil record during the early Cambrian, as a major component of the 'Cambrian explosion', some 522 million years ago, and the last trilobites vanished during the mass extinction of the Upper Permian ~255million years later (Clarkson, Levi-Setti & Horváth 2006). From the very beginning they were equipped with highly differentiated compound eyes, not dissimilar to those of insects and crustaceans living today, and a hard protecting shell.

There is a remarkable difference, however, to the compound eyes of their nowadays relatives – their lenses consisted of pure primary oriented calcilte.

Calcite, however, has another property also - it shows photoluminescence. Naturally occurring calcite contains different minerals such as magnesium, manganese, iron etc., and so do the lenses of phacopid trilobites (Lee, Torney & Owen 2007). As is the case for many minerals, natural calcite fluoresces when it is illuminated with light of certain wavelengths, for example of UV-light. The energy of the incident light is able to excite susceptible electrons within the atomic structure of the mineral. They leave their position and jump to higher orbits of the atomic structure. Falling back they release a small amout of energy visible as light, and producing a kind of 'glow'. The colour of this 'glow' often is different from the colour of the incident light, and depends on the composition of the mineral, while the 'glow' continues as long as the mineral is illuminated. During phosphorescence, the light is 'stored' for a while inside the atomic structure, the system becomes 'charged', and releases the energy more slowly than during the fluorescent process. The excited electron also returns to its position inside the atomic structure but it undergoes certain intersystem levels, while its state of spin turns to a higher spin multiplicity, normally a triplet state. These transitions take time in order of milliseconds, but can also persist in some materials for minutes or even hours. Here in our probe, the phosphorescence, seen in a biological time scale [milliseconds], disappears as soon as the light vanishes.

As calcite is a typical photoluminescent mineral, it is not unreasonable to assume that the lenses of phacopid trilobites may show such a 'glow' as well when illuminated with UV light. Trilobites were marine bottom dwellers, and light is absorbed quickly when penetrating into sea water. Due mainly to Rayleigh scattering but also simply absorbtion by water hardly any colours are retained at the seafloor, if this lies deep enough, because both - Rayleigh scattering and absorption [attenuation] in water depend on the wavelengths (Kullenberg 1974, Morel 1974, 24 p. 19)

$$Q = \frac{1}{\alpha^2} \int_0^\pi (i_1 + i_2) \sin \Theta \, d\Theta, \ \alpha = \pi d/\lambda.$$

Where Q is the efficiency factor of Rayleigh scattering, representing the ratio between the scattered intensity and the incident intensity, d is the diameter of the particle scattering (spherical particles assumed), i_1 and i_2 are the intensities scattered in the direction Θ (Jerlov 1968).

The absorbtion is defined by the Lambert Beer Law:

$$E_\lambda = \lg\left(\frac{I0}{I1}\right) = \varepsilon_\lambda \cdot c \cdot d \ ,$$

with E_λ extinction coefficient, I_0 and I_1 are the intensity power (power per unit area) of the incident radiation and the transmitted radiation, ε_λ absorbtivity of the material (here sea water) depending on the wavelength, c the concentratopn of attenuating species in the material (here sea water), d the distance the light travels through the material (i.e. the path length).

As a result, red light is maintained in clear waters up to 5 meters, yellow up to 30m, green to 50m, and blue to 60m, while UV, invisible for human eyes, penetrates to depths even greater than 100m (Jerlov 1968).

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Atmospheres long before the Cambrian and thus the 'Cambrian explosion', due to a composition of the atmosphere different from that of today, transmitted a higher content of short wavelength light than at the present time (Berkner & Marshall 2003, Cartling, Zahnle & McKay 2001, Cockell & Horneck 2001), which as mentioned, changed with the content of oxygen. UVB and UVC then was shielded almost completely, while UVA was able to penetrate before this change as it still does today. Short wavelength light is less scattered and absorbed in water than light with longer wavelengths (Jerlov 1068, Kullenberg 1974, Hecht 2014). If the calcitic eyes of trilobites were to glow by photoluminescence when illuminated by UV light, this would be a useful precondition, enabling such benthic animals to invade deeper areas of the oceans using high energy radiation for vision. Because compound eyes, at least their basic type, the apposition eye, produce a mosaic-like image (each facet averages the inputs inside its individual visual field to one 'pixel'), any inhomogenous distribution of light patterns over the lattice of lenses reflecting conditions of the environment would give orientational information to the trilobite. It also may represent a visual system at the beginning of the evolution of vision, exploiting sources of radiation in an archaic mode of context, and may also indicate that the origin of development of (trilobite-)compound eyes goes far back into the Proterozoic. Here we show that trilobite eyes actually can glow when illuminated with UV light.

How strong this glow may have been during the life-time of the trilobites we shall never know, because the exact composition of the lenses remains unknown, as does the intensity of the UV-light at the depth at which the trilobites lived and during the questionable period where these eyes developed. We know, however, from many crepuscular, nocturnal or even deep sea arthropods, that the sensory systems can be very sensitive to low light intensities (for an overview see Land 1981). Any inhomogenous illumination of the lattice of lenses in the trilobite eye would allow a very rough orientation to dark shelters in the environment for example, or towards moving objects passing by. A system like this would have worked even without specialised UV-sensitive photreceptor cells, because the colour of luminescence here is blue. Specialised UV-sensitive photoreceptor cells are known from modern systems such as in living bees and many other insects or crustaceans of today (Land 1981), but may not have been present in the ancient trilobites. The properties of this system allow us to speculate that at this time it may have been adapted to higher levels of UV-radiation during the rapid change early at the beginning of a photobiologically clement atmosphere, in this early stage of the evolution of metazoan vision. This system might have allowed the bottom dwelling trilobites furthermore, to invade deeper areas (Bundenbach environment $\gtrsim$200m (Kühl et al. 2012)), while using vision. The photoluminescence of their calcitic lenses in trilobites may have enhanced the width of the exploitable spectrum of vision of their bearers. By physical reasons mentioned before it may be assumed that the early photoreceptors were sensitive to blue light, as are most photoreceptors of aquatic animals still today. Transforming UV by photoluminescence to it, it might have been possible to 'catch' these wavelengths for the use of vision additionally, which are normally out of view. Whether the potential that the calcitic lenses in trilobites actually offered was realised and used cannot be known. We shall always have to remain in the dark.

AUTHOR CONTRIBUTIONS B.S. did the experiments, B.S. and E.C. wrote the manuskript.

ACKNOWLEDGEMENTS We thank Wouter Südkamp, Bundenbach, for the specimen,

… referees

There are no conflicts of interests of the authors.

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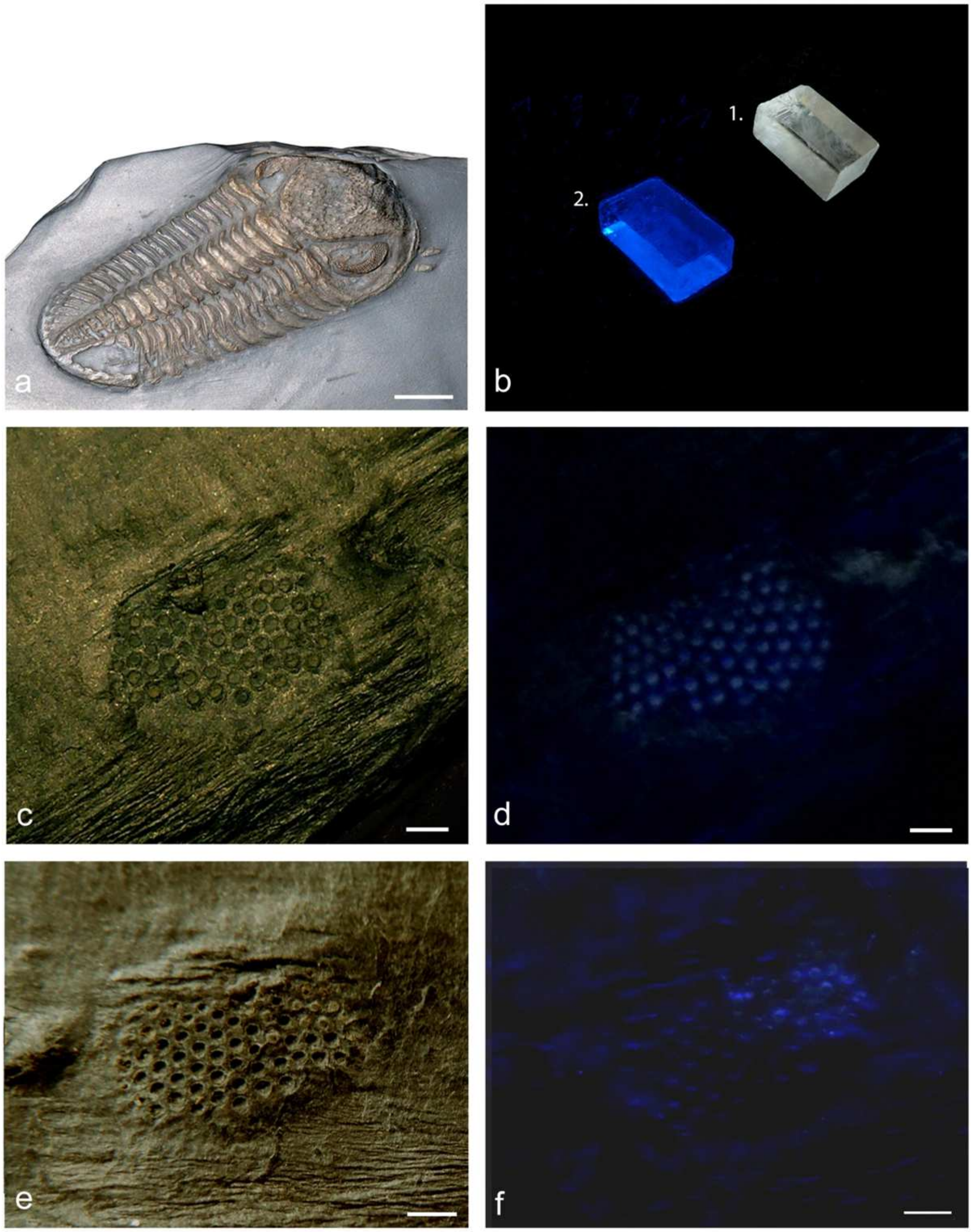
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