RESEARCH ARTICLE

How does the ‘ancient’ asexual Philodina roseola (Rotifera: Bdelloidea) handle potential UVB-induced mutations?

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SUMMARY

Like other obligate asexuals, bdelloid rotifers are expected to suffer from degradation of their genomes through processes including the accumulation of deleterious mutations. However, sequence-based analyses in this regard remain inconclusive. Instead of looking for historical footprints of mutations in these ancient asexuals, we directly examined the susceptibility and ability to repair point mutations by the bdelloid Philodina roseola by inducing cyclobutane-pyrimidine dimers (CPDs) via exposure to UVB radiation (280–320 nm). For comparison, we performed analogous experiments with the facultative asexual monogonont rotifer Brachionus rubens. Different strategies were found for the two species. Philodina roseola appeared to shield itself from CPD induction through uncharacterized UV-absorbing compounds and, except for the genome reconstruction that occurs after desiccation, was largely unable to repair UVB-induced damage. By contrast, B. rubens was more susceptible to UVB irradiation, but could repair all induced damage in ~2h. In addition, whereas UV irradiation had a significant negative impact on the reproductive output of P. roseola, and especially so after desiccation, that of B. rubens was unaffected. Although the strategy of P. roseola might suffice under natural conditions where UVB irradiation is less intense, the lack of any immediate CPD repair mechanisms in this species remains perplexing. It remains to be investigated how typical these results are for bdelloids as a group and therefore how reliant these animals are on desiccation-dependent genome repair to correct potential DNA damage given their obligate asexual lifestyle.

Key words: genome degradation, asexual reproduction, cyclobutane-pyrimidine dimers, genome repair, UV damage, bdelloid rotifer, desiccation tolerance, anhydrobiosis, deleterious mutations.

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INTRODUCTION

Bdelloid rotifers comprise a clade of small, aquatic invertebrates renowned for their paradoxical status as a successful and ancient asexual clade (Ricci and Fontaneto, 2009). Although the prevalence of sexual reproduction among metazoans is still not understood given its greater costs compared with asexual reproduction (Lehtonen et al., 2012), a purely asexual lifestyle also entails distinct disadvantages, making the long-term success of the bdelloids equally confounding (Butlin, 2002). For instance, the lack of segregation and recombination in the genome of purely asexual lineages should result in its progressive degradation (Kondrashov, 1993; Butlin, 2002) through the effects of transposable elements (Hickey, 1982; Arkhipova and Meselson, 2005), the degeneration of homologous chromosomes (Schurko et al., 2009), the independent evolution of the alleles of a given gene locus [the Meselson effect (Butlin, 2002; Schurko et al., 2009)], and especially the accumulation of deleterious mutations over time (Kondrashov, 1993).

Evidence for the latter from sequence-based analyses of bdelloids is inconclusive, however. Whereas older studies indicated that bdelloids have not accumulated slightly deleterious mutations faster than facultative sexual monogonont rotifers (Welch and Meselson, 2001; Birky et al., 2005), two newer studies using more elaborate and extensive sampling strategies indicated the possible existence of this effect (Barraclough et al., 2007; Swanstrom et al., 2011). However, the results of these latter studies, which examine the rate of historical accumulation of deleterious mutations, are complicated by several confounding factors. For instance, the latest study showed no increased accumulation in bdelloid rotifers when comparisons were made between monogononts and bdelloids from the same habitat (Swanstrom et al., 2011). Additional potential confounding factors include methodological aspects [e.g. sample-size effects (Swanstrom et al., 2011)], the long divergence time separating bdelloids and monogononts (Barraclough et al., 2007), natural factors [e.g. severely deleterious mutations that will not go to fixation (Barraclough et al., 2007)], and the frequency and impact of any repair/recombination events (i.e. desiccation in bdelloids, sex in monogononts). These difficulties notwithstanding, the long-term existence of bdelloids as a group [at least 40 million years (Ricci and Fontaneto, 2009)] would indicate that some mechanism(s) to prevent genome degradation is present in these animals.

In investigating the latter, a more direct, profitable route is to check the susceptibility and/or ability of bdelloids to repair point mutations by inducing them directly instead of looking for their historical footprints. This can be done via UVB irradiation, which causes lesions in the DNA, with cyclobutane-pyrimidine dimers (CPDs) being the most frequent form (Mitchell and Nairn, 1989). Here, adjacent pyrimidine nucleotides become linked via a four-carbon (cyclobutane) ring, thereby disrupting the base-pairing with...
the other strand and altering the DNA configuration. In so doing, CPDs inhibit gene transcription and DNA replication by obstructing both RNA and DNA polymerases (Sauerbier and Hercules, 1978; Protic-Sabljić and Kraemer, 1985). In addition, the otherwise stable cytosine bases are unstable in CPDs and readily deaminate to uracil, which, in turn, gives rise to C→T and CC→TT mutations (reviewed in Ikehata and Ono, 2011).

In this paper, we investigate how the bdelloid Philodina roseola Ehrenberg 1832 handles the threat of possible mutations by inducing CPD lesions via UVB exposure. We quantified both the number of CPDs produced and the rate of their repair under both constantly hydrated conditions and conditions where desiccation, and therefore DNA repair, could occur. For comparison, we performed analogous experiments on the facultative sexual monogonont Brachionus rubens Ehrenberg 1838. Furthermore, we ascertained any fitness effects associated with this DNA damage by recording the reproductive outputs of control and irradiated individuals of both species with and without the ability to repair the DNA (via desiccation or sex, respectively). Our data represent the first of their kind for bdelloid rotifers, providing another important clue towards how they have survived as ancient asexuals despite the many disadvantageous aspects of this mode of reproduction.

MATERIALS AND METHODS

Rotifer and algal cultures

Multiple populations of P. roseola (lifespan of ~45 days, reproductive lifespan to 20–24 days, first eggs at 5–6 days, clutch sizes of ~24 eggs; C.F., personal observation) and B. rubens (lifespan of ~10 days, first eggs at 2–3 days, clutch sizes of ~12 eggs; C.F., personal observation) were cultured under a 9h:15h light:dark regime in 90×15 mm plastic Petri dishes at 20±1°C, with weekly transfers of a small number of individuals from each population to new Petri dishes. The algae used as food source for each species (P. roseola: Cryptomonas sp. SAG 26.80; B. rubens: Monoraphidium minutum SAG 243-1) were each grown in 500 ml Erlenmeyer flasks with COMBO medium (Kilham et al., 1998) under continuous illumination (13 W/840 Daylight neon tubes, Osram, Munich, Germany).

Each species was used for two series of experiments, the first focusing solely on CPD accumulation and its repair and the second on the impact of irradiation on the reproductive capability of the animals.

Experiment I: Quantifying CPD accumulation and repair

Rotifers were filtered using a 60 μm mesh and transferred to algae-free COMBO medium for roughly 1 h before batches of 50 animals in 2 ml of medium were transferred into one of 16 even-sized pie-shaped chambers (maximum capacity of 5.5 ml) arranged uniformly on a round plate. Each species was assigned randomly to half the secondary DNA repair), (3) irradiated animals and (4) irradiated species (including controls) exposed to UVB irradiation for 4 h. An additional 20 batches of 50 animals were likewise irradiated for 4 h, with the chambers subsequently covered by UV-opaque Plexiglas for 1 or 2 h to enable light-dependent repair of any UV damage. Immediately following irradiation and any repair time, the vessels were put on ice and 2.5 ml of 99% ethanol was added to each chamber to fix the rotifers. After 15 min, the liquid in each chamber was replaced with fresh 99% ethanol and each batch of rotifers was transferred to a separate 1.5 ml tube (Eppendorf, Hamburg, Germany).

Finally, we tested the ability of P. roseola to repair CPDs over longer time periods with or without desiccation. To this end, 40 batches of 50 individuals were irradiated for 4 h at the high UVB intensity (Table 1) and subsequently transferred to 24-well flat-bottom plates (Becton Dickinson, Franklin Lakes, NJ, USA), with each well containing either 2.0 or 0.5 ml of algae-free COMBO medium. The 20 batches in the latter wells were desiccated for 3.5 days (=Protocol D (Ricci et al., 2003)) in custom-built humidor-thermostatic chambers (University of Oldenburg workshops), rehydrated with 1 ml algae-free COMBO medium and maintained for an additional 6 h before ethanol was added as described before. The 20 batches in the higher-volume wells were kept continuously hydrated for 4 days before ethanol was added for fixation.

Experiment II: Quantifying the impact of CPD accumulation and repair on reproductive output

To investigate the potential fitness effects of UVB-induced DNA mutations and their interaction with secondary DNA repair phases (i.e. desiccation or sexual reproduction), we quantified the reproductive output of P. roseola and B. rubens under four treatment conditions: (1) untreated control animals, (2) untreated animals that could undergo desiccation or sex as appropriate for the species (for secondary DNA repair), (3) irradiated animals and (4) irradiated animals that could undergo desiccation or sexual reproduction.

Each treatment group was seeded with a starter population of 50 individuals that was subjected to the following experimental cycle:

| Table 1. Irradiance (W m⁻²) of the different spectral ranges used in the first experimental series |
|-------------------------------|-------------------|-------------------|-------------------|
| Trial | Treatment phase | UVB (280–320 nm) | UVA (320–400 nm) | PAR (400–700 nm) |
| High | Damage | 2.2 | 30 | 195 |
| | Repair | <0.1 | 7.5 | 160 |
| | Control | <0.1 | 10 | 188 |
| Low | Damage | 0.5 | 7 | 134 |
| | Repair | <0.01 | 2 | 120 |
| | Control | <0.01 | 3 | 127 |

PAR, photosynthetically active radiation.
irradiation phase (10 days), DNA-repair phase (7 days), a second irradiation phase (14 days), a second DNA-repair phase (7 days), and no treatment (4 days). For all but the DNA-repair phases, each population was continuously hydrated at 20±1°C in separate Petri dishes (5.5 cm diameter and 1.3 cm deep), each with 15 ml COMBO medium with algae under the standard 9 h:15 h light:dark regime; half of the medium with algae and rotifers was aspirated and renewed every second day. Treatment populations receiving UVB irradiation were exposed in addition to this normal illumination in four 15 min blocks per day, each interspersed by 1 h. UVB irradiation derived from a Bio Block Scientific VL-6.LM UV lamp (Fisher, Illkirch, France) with an intensity of 3.5 W m⁻² UVB emitted at 312 nm as measured with the USB 2000⁺ U-Vis fiber-optic spectrometer.

For the DNA-repair phases, treatment populations of P. roseola were desiccated for 6 days in groups in their Petri dishes following Protocol D (Ricci et al., 2003) in the custom-built humidity-thermostatic chambers after half the medium was aspirated. Reanimation was induced through the addition of COMBO medium with algae. The production of sexual resting eggs by B. rubens occurred throughout the experiment, but was accelerated on the first day of the repair phases by increasing the culture temperature to 26°C. Thereafter, half the medium was aspirated from the Petri dish and the remaining fluid was evaporated within 1 day using the humidity-thermostatic chambers. The dry Petri dishes were then held in the dark at 5–8°C for 4 days before hatching of the resting eggs was induced by adding 15 ml COMBO medium with algae at 20°C. UVB irradiation, where applicable, was first applied the day following the addition of the medium.

After the experimental cycle, 48 newly hatched individuals were randomly chosen from each treatment group and transferred individually into separate wells of either 48-well plates (Becton Dickinson) with 0.5 ml COMBO medium with algae (B. rubens) or 24-well plates (Becton Dickinson) with 1 ml COMBO medium with algae (P. roseola). Reproductive output was counted for 5 days at 12 h control intervals beginning with the first offspring (B. rubens) or eggs (P. roseola). Both offspring and eggs were removed from the wells after counting.

DNA extraction and quantification of CPDs
DNA was extracted at most 1 week after the experiments. Ethanol in the samples was drawn off as much as possible, with the remainder evaporated at 50°C. DNA was extracted for each batch of 50 rotifers using a 70 µl digestion solution of 10% Chelex (Bio-Rad, Munich, Germany) and 0.07 µg µl⁻¹ proteinase K (Carl Roth, Karlsruhe, Germany). Samples were incubated for 30 min at 55°C and heat-inactivated for 10 min at 95°C before being cooled for at least 30 min at 5°C. Following sedimentation of the Chelex at 11,100 g for 15 s, the clear supernatant containing the DNA was transferred into Eppendorf tubes and stored at −18°C until further processing.

CPDs were quantified using an immunofluorescent thymine dimer detection method (Boelen et al., 1999). For each sample, 100 ng DNA, as determined using a NanoQuant plate (Tecan, Männedorf, Switzerland) on an Infinite 200 Pro (Tecan), was denaturated for 10 min at 95°C, cooled for 30 min on ice, and vacuum-blotted in parallel on nitrocellulose membrane (Portran BA79, 0.1 µm pore size, Whatman, Sanford, ME, USA) using a Minifold 1 dot-blot apparatus (96-well, Whatman) and a vacuum pump (KNF Neuberger, Freiburg, Germany). Two calibration series of UV-irradiated calf thymus DNA (Sigma-Aldrich, St Louis, MO, USA) with known CPD concentrations (100 ng–10–320 CPDs per million base pairs) were included on each blot. After vacuum-blotting of the DNA, the membrane was washed with 200 µl phosphate-buffered saline (PBS; Carl Roth) in the dot-blot apparatus. DNA was then fixed to the membrane through heating at 60°C for 2 h. Nonspecific binding sites were blocked with 5% skimmed-milk powder (Sucofin, Zeven, Germany) in PBST (PBS and 0.1% v/v Tween 20; Sigma-Aldrich) for 30 min at room temperature. After washing with PBST (3× for 10 min each), the samples were incubated overnight at 5°C with a monoclonal anti-thymine dimer antibody specific for CPDs (clone H3 produced in mouse, Sigma-Aldrich; 1:3000 diluted in PBST with 0.5% w/v skimmed milk powder). Thereafter, the samples were again washed with PBST (3× for 10 min each) before being incubated with horseradish peroxidase conjugated rabbit anti-mouse (Dako, Glostrup, Denmark) at room temperature for 2 h (1:15,000 diluted in PBST with 0.5% w/v skimmed milk powder). Antibody-labeled CPD complexes were detected by exposing the sealed membrane to photosensitive films (Amersham Hyperfilm ECL, GE Healthcare, Chalfont St Giles, UK) after incubation with ECL western blotting detection reagent (GE Healthcare). Grayscale values of the scanned films were determined in ImageJ1.46r (Rasband, 2012) using a dot-blot analyzer plugin (Carpentier, 2008), with the final CPD concentrations being determined via the two calibration series.

Statistical procedure
All statistical analyses were carried out in SPSS Statistics 20 (IBM, Armonk, NY, USA). The influence on reproductive output (number of offspring) of UV irradiation with or without secondary DNA repair was analysed separately for each species with a full factorial generalized linear model (GLM) using a Poisson error structure with a log as the link function. A post hoc test to disentangle the individual contributions of the factors to a significant, combined effect was carried out in a pairwise fashion using a Holm–Bonferroni (Holm, 1979) correction for multiple comparisons.

RESULTS
The two species showed striking differences in their susceptibility to UVB-induced CPD formation and their ability to repair this damage (Table 2). UVB exposure generated comparable amounts of CPDs in both species in the low-irradiation treatment (two-sample t-test, t=1.825, d.f.=38, P=0.076), with the high-irradiation treatment increasing this amount significantly for B. rubens (two-sample t-
test, \( t=2.963, \) d.f.\( =19.529, P=0.008 \), but not for \( P. \) roseola (two-sample \( t \)-test, \( t=0.962, \) d.f.\( =38, P=0.342 \)).

CPD repair by \( P. \) roseola after 2h was negligible regardless of the irradiation level (low: \( F_{1,58}=0.001, P=0.971, \) adjusted \( r^2=0.017 \); high: \( F_{1,58}=0.136, P=0.713, \) adjusted \( r^2=0.015 \)). Even after 4 days, significant amounts of CPDs still remained in continuously hydrated individuals of \( P. \) roseola exposed to a high-UVB treatment (one-sample \( t \)-test against zero, \( t=4.445, \) d.f.\( =19, P<0.001 \)), although these values were significantly lower than initial values (two-sample \( t \)-test, \( t=5.269, \) d.f.\( =24.162, P<0.001 \)). By contrast, nearly no CPDs were found in individuals that had undergone desiccation (one-sample \( t \)-test against zero, \( t=1.227, \) d.f.\( =19, P=0.217 \)), resulting in a significant difference between these two long-term treatments (two-sample \( t \)-test, \( t=3.347, \) d.f.\( =28.6, P=0.001 \)).

By contrast, \( B. \) rubens repaired almost all induced CPDs over at most 2h, with the repair rate at either irradiation intensity following a linear regression (low: \( r^2=0.453, \) mean \( \pm \) s.e.m. rate of repair=12.1±1.7 CPDs per hour from an initial value of 23.7±2.2 CPDs; high: \( r^2=0.211, \) mean \( \pm \) s.e.m. rate of repair=51.0±12.5 CPDs per hour from an initial value of 101.9±16.1 CPDs).

Reproductive output in \( P. \) roseola was significantly reduced by UV irradiation (Wald \( \chi^2=211.207, \) d.f.\( =1, P<0.001 \)), desiccation (Wald \( \chi^2=83.945, \) d.f.\( =1, P<0.001 \)) and their interaction (Wald \( \chi^2=78.495, \) d.f.\( =1, P<0.001 \); Fig. 1). UV irradiation had no significant influence on its own (mean \( \pm \) s.e.m. difference to the control=–0.21±0.765, \( P=0.785 \)), but increased the negative effect of desiccation significantly when combined with it (UV irradiation alone: mean \( \pm \) s.e.m. difference to the control=–3.23±0.717, \( P<0.001 \); in combination with desiccation: mean difference \( \pm \) s.e.m. to the control=–9.52±0.619, \( P<0.001 \); Fig. 1A). The reproductive output of \( B. \) rubens was, by contrast, only slightly, but significantly, reduced by sexual reproduction (Wald \( \chi^2=0.517, \) d.f.\( =1, P=0.002 \)); otherwise, UV irradiation alone (Wald \( \chi^2=0.055, \) d.f.\( =1, P=0.814 \)) or in combination with sex (Wald \( \chi^2=0.018, \) d.f.\( =1, P=0.893 \)) had no effect (Fig. 1B).

**DISCUSSION**

In dealing with potential DNA damage and mutations from UVB exposure, two main strategies are possible: prevention and/or subsequent repair. Prevention is possible both by behaviorally minimizing exposure to UV irradiation (excluded in our experiments) and through chemical mechanisms such as UV-absorbing compounds [e.g. carotenoids or mycosporine-like amino acids, as in phytoplankton (Hansson and Hylander, 2009)]. Similarly, several potential repair mechanisms exist, including highly efficient and rapid photoreactivation reactions mediated by specific light-dependent photolyases (Sinha and Häder, 2002) or more versatile mechanisms such as nucleotide excision and recombinational repair (Sinha and Häder, 2002). An extension of the latter could include the genome repair breaklods must necessarily undergo following a desiccation event using homologous recombination of sufficiently similar DNA strands of their degenerated tetraploid genomes (Gladyshev and Meselson, 2008). However, all of these latter mechanisms are slower and more energetically expensive than photoreactivation (Quaite et al., 1994) and can also introduce their own mutations, especially if the lesions are frequent and in the relative vicinity of one another (Sinha and Häder, 2002).

Although these two strategies are not mutually exclusive, our results highlight that \( P. \) roseola and \( B. \) rubens employ either one or the other to deal with DNA damage induced by high UVB levels. \( Philodina \) roseola appears to be highly resistant to DNA damage, regardless of irradiation intensity, with carotenoids potentially acting as the UV-absorbing compound, given that this species is often reddish. However, \( P. \) roseola appears largely unable to actively repair any damage that does occur, except through desiccation. The slight reduction observed in the number of CPDs after 4 days likely derives more from the natural deamination of cytosine to uracil (resulting in a \( C \rightarrow T \) mutation) in CPDs (Ikehata and Ono, 2011). Using an intermediate value of 50h for the half-life of a cytosine within a CPD [from published estimates ranging from 2 to 100h (Tu et al., 1998; Burger et al., 2003)], only five CPDs are expected to remain after 4 days based on our initial measured values, which is in good agreement with our observations. The significant reduction in reproductive output observed for irradiated (but not desiccated) individuals also highlights the inefficient or missing DNA repair mechanisms in \( P. \) roseola.

By contrast, \( B. \) rubens showed irradiation-intensity-dependent DNA damage that was largely repaired within a few hours. Interestingly, the rate of repair scaled with the level of damage such that all CPDs were removed in approximately 2h regardless of the initial level of damage. Although our methodology could only reveal the repair of CPDs per se, the lack of any significant reduction in
the reproductive output of irradiated individuals of *B. rubens* indicates that the possible introduction of point mutations during the repair process is small at best. This fact, together with the high repair rate, hints that photolyases are likely used by this species.

Although both species received unnaturally high levels of UVB radiation in our experiments [nearly a maximum of 0.2 W m⁻² in temperate regions (Hansson and Hylander, 2009)], our results are valuable in revealing species-specific strategies. However, the reason behind the use of single, differing strategies is unclear given that a combination of strategies would provide additional protection. The apparent lack of short-term DNA repair in *P. rosea* is especially puzzling, both because asexual species are generally more susceptible to accumulating deleterious mutations and because CPDs are exceptionally detrimental in that they obstruct gene transcription (Sauerbier and Hercules, 1978; Protic-Sabljic and Kraemer, 1985). However, this species might not normally be confronted with significant UVB levels. For instance, irradiation intensity attenuates relatively fast in water (Häder et al., 1998) and bdelloids often occur in habitats such as mosses or soil that (partly) shield them against UV irradiation (Ricci and Fontaneto, 2009). Nevertheless, increased exposure to UV irradiation in bdelloids should occur during the lead-up to anhydrobiosis, when the desiccating conditions remove any protecting water layer as well as limit any behavioral avoidance. At this time, the immediate repair of any lesions via photolyases is unlikely given that the metabolism is being progressively silenced, and passive protection by UV-absorbing compounds would represent an effective strategy.

In addition, our findings are consistent with the ‘sleeping beauty’ hypothesis for bdelloid rotifers (Ricci et al., 2007), which states that individuals of these species regularly need to undergo desiccation to repair any DNA damage that they accumulate, but cannot repair while hydrated. Indeed, the apparent lack of DNA repair mechanisms might underlie the fitness decay observed in continuously hydrated bdelloids (Ricci et al., 2007). However, desiccation does not appear to be a cure-all for DNA damage given that our irradiated animals still showed reduced fitness following desiccation, presumably due to some CPDs naturally deaminating to form point mutations beforehand or the genome repair via homologous recombination possibly introducing and/or spreading new mutations via gene conversion (Johnson and Jasin, 2000; Chen et al., 2007). Thus, the frequency of desiccation in combination with the level of accumulated damage might determine how successful any DNA repair at this time is. Nevertheless, selection after desiccation should act to purge those individuals with reduced fitness and high mutation loads (Kondrashov, 1993), with new populations being founded by the surviving, fitter individuals. Altogether, this scenario could explain why robust indications for the expected faster accumulation of (slightly) deleterious mutations in bdelloids remain missing, as well as the increased tendency to find these animals in desiccation-prone habitats (Ricci, 2001).

By contrast, fast and effective DNA repair might represent the more profitable strategy for the planktonic *B. rubens*, which might be exposed to higher UV irradiation at the water surface. Importantly, in the absence of any immediate DNA repair and insufficient protection from irradiation, any residual damage will be exposed in the haploid males at the initiation of sex (Wallace et al., 2006), which, if severe enough, would be disadvantageous for long-term survival.

It is important to stress that our results hold only for the two species examined and the trends should not be extended to all monogonont and bdelloid rotifiers. Indeed, UV-absorbing compounds have been documented in the monogonont rotifers *Polyarthra dolichoptera*, *Synchaeta grandis*, *Synchaeta pectinata* and *Keratella cochlearis* (Obertegger et al., 2008). In addition, as shown in a related context by Swanstrom et al. (Swanstrom et al., 2011) and implicit in our arguments, habitat is a potentially important confounding factor. As such, investigations should be expanded to include other rotifer species, with a view toward comprehensive sampling in terms of both habitat and evolutionary relatedness. In addition, given that our experiments focused on DNA lesions through UVB irradiation, it would be instructive to explore how bdelloids repair DNA damage to their genomes in general and deal with point mutations derived from other sources, what kinds of damage can be repaired and when such repair is possible (i.e. is it necessarily restricted to when the genome is reconstructed following a desiccation event?).

### LIST OF ABBREVIATIONS

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
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<tbody>
<tr>
<td>CPD</td>
<td>Cyclobutane-pyrimidine dimer</td>
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<tr>
<td>GLM</td>
<td>Gene conversion: mechanisms, evolution and human disease.</td>
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<td>PAR</td>
<td>Photolyase</td>
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<td>PBS</td>
<td>Photobiochemistry and photobiology.</td>
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<td>PBST</td>
<td>Photoinhibition and photoreactivation.</td>
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<td>UV</td>
<td>Ultraviolet</td>
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<td>UVA</td>
<td>Ultraviolet A</td>
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<td>UVC</td>
<td>Ultraviolet C</td>
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### AUTHOR CONTRIBUTIONS

C.F. designed the project with input from the other authors, with W.H.v.P. and A.G.J.B. teaching C.F. the methods for the detection of CPDs. All research was performed by C.F., as were all analyses, with input from W.H.v.P. All authors discussed results. The manuscript was drafted by C.F. with input from O.R.P.B.-E. All authors contributed to the finalization of the manuscript.

### COMPETING INTERESTS

No competing interests declared.

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### REFERENCES


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