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# Interspecific differences in carotenoid content and sensitivity to UVB radiation in three acanthocephalan parasites exploiting a common intermediate host

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ABSTRACT

Few endoparasite species are pigmented. Acanthocephalans are an exception however, with several species being characterised by yellow to orange colouration both at the immature (cystacanth) and adult stages. However, the functional and adaptive significance of carotenoid-based colourations in acanthocephalans remains unclear. One possibility is that the carotenoid content of acanthocephalan cystacanths acts as a protective device against ultra-violet radiation (UVR) passing through the translucent cuticle of their crustacean hosts. Indeed, acanthocephalans often bring about behavioural changes in their aquatic intermediate hosts that can increase their exposure to light. Carotenoid composition and damage due to ultra-violet – B (UVB) radiation were investigated in three acanthocephalan parasite species that induce contrasting behavioural alterations in their common intermediate host, the crustacean amphipod *Gammarus pulex*. The fish acanthocephalans *Pomphorhynchus laevis* and *Pomphorhynchus tereticollis* both induce a positive phototaxis in gammarids, such that infected hosts spend more time out of shelters, while remaining benthic. The bird acanthocephalan *Polymorphus minutus*, on the other hand, induces a negative geotaxis, such that infected hosts typically swim close to the water surface, becoming more exposed to UV radiation. We show that differences in cystacanth colouration between acanthocephalan species directly reflect important differences in carotenoid content. The two fish parasites exhibit a contrasting pattern, with *P. tereticollis* harbouring a large diversity of carotenoid pigments, whereas *P. laevis* is characterised by a lower carotenoid content consisting mainly of lutein and astaxanthin. The highest carotenoid content is found in the bright orange *P. minutus*, with a predominance of esterified forms of astaxanthin. Exposure to UVB radiation revealed a higher susceptibility in *P. laevis* larvae compared with *P. tereticollis* and *P. minutus*, in terms of sublethality (decreased evagination rate) and of damage to DNA (increased cyclobutane pyrimidine dimers production). Although we found important and correlated interspecific differences in carotenoid composition and tolerance to high UVB radiation, our results do not fully support the hypothesis of adaptive carotenoid-based colourations in relation to UV protection. An alternative scenario for the evolution of carotenoid accumulation in acanthocephalan parasites is discussed.



## 1. Introduction

Ever since the work of Alfred Russell Wallace (1905), the adaptive value of animal colours has stimulated much empirical and theoretical work (Cott, 1940; Endler, 1990; Lozano, 1994; Bradbury and Verhencamp, 1998). Little attention, however, has been paid to the colouration of parasites. This may stem from the fact that a large number of parasites live inside their hosts, have reduced sense organs, and therefore do not seem to benefit from possessing photoreflecting pigmentation. One noticeable exception, however, is found among acanthocephalan parasites, where several species show bright yellow to orange colouration, particularly at the cystacanth larval stage (Crompton and Nickol, 1985). Orange colouration in animals is generally due to the presence of carotenoids. Animals are unable to synthesise carotenoids de novo and, therefore, must obtain them from their food (Lozano, 1994; Lesser, 2006). Previous studies (Van Cleave and Rausch, 1950; Barrett and Butterworth, 1968, 1973; Ravindranathan and Nadakal, 1971; Gaillard et al., 2004; Duclos, 1996. Functional significance of pigment in larval *Corynosoma constrictum* Van Cleave, 1918 (Acanthocephala: Polymorphidae). Ph.D. Dissertation, University of Nebraska, USA) have indeed shown that the yellow to bright orange colouration of a number of acanthocephalan parasites depends upon carotenoid pigments that they obtain from their hosts. Although carotenoids may not be essential for the adult acanthocephalan parasite in the intestine of their vertebrate final host (Barrett and Butterworth, 1968), their role and function in cystacanths infecting crustacean intermediate hosts is of evolutionary interest (Moore, 2002).

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Acanthocephalan parasites have complex life cycles, and typically exploit arthropods as intermediate hosts. A large proportion of acanthocephalan parasites with coloured cystacanths (the stage at which the parasite becomes infective to its definitive host) exploit amphipods as intermediate hosts, and in most cases the coloured cystacanths are visible through the host's translucid cuticle. Two adaptive functions have been proposed for the presence of carotenoid-based colourations in acanthocephalan cystacanths (Bakker et al., 1997; Moore, 2002). It was initially proposed that the colouration of cystacanths may act to enhance trophic transmission of the parasite to its final host, through increased conspicuousness of infected intermediate hosts (Bakker et al., 1997). However, a recent study (Kaldonski et al., 2009) indicates that the orange colouration of fish acanthocephalans actually plays no role in the increased vulnerability of infected gammarids to predation by fish. In addition, Bethel and Holmes (1977) pointed out that cystacanths of *Polymorphus minutus*, an acanthocephalan species using amphipods as intermediate hosts and wildfowl as definitive hosts, are bright orange, although ducks do not rely on vision to capture their crustacean prey. Another adaptive explanation relies on the well known function of carotenoids in photoprotection, either as reactive-oxygen species (ROS) scavengers or as ultra-violet (UV)-screening compounds (Cockell and Knowland, 1999; Stahl and Sies, 2003), particularly in aquatic species (Lamare and Hoffman, 2004; Hansson and Hylander, 2009). Among variable selection pressures, carotenoid accumulation may therefore be interpreted as an adaptation to UV threat, in particular in zooplankton (see Hansson and Hylander, 2009 for a review). Although a photoprotective function of carotenoids in pigmented acanthocephalans has been hypothesised (Barrett and Butterworth, 1973; Bakker et al., 1997), direct evidence is lacking. However, this hypothesis might be particularly relevant for acanthocephalan species that, through manipulating their host behaviour to make them more vulnerable to predation by appropriate definitive hosts (Cézilly et al., 2000; Moore, 2002), become exposed to more UV radiation. Indeed, acanthocephalan species with yellow-orange cystacanths tend to exploit intermediate hosts with translucid cuticles such as crustacean amphipods whereas, noticeably, white species of acanthocephalan generally occur in intermediate hosts with dark opaque cuticle (such as *Moniliformis moniliformis* in cockroaches, *Plagiorhynchus cylindraceus* in woodlice or *Acanthocephalus anguillae* in asellids). UVB radiation may penetrate several metres in clear water (Smith et al., 1992) and, therefore, acanthocephalan cystacanths infecting amphipods in rivers and streams might be exposed to UV radiation.

Little is known about the variation in pigmentation among acanthocephalan species, and even less about the potential role of carotenoid pigments in the protection of acanthocephalan cystacanths against UV radiation. According to the photoprotection hypothesis, the carotenoid content of cystacanths should be related to the risk of exposure to UV radiation (UVR) at the interspecific level. Thus, an important step to validate the adaptive function of cystacanth colouration in relation to UV protection is to experimentally investigate the effects of UVR on the survival of acanthocephalan cystacanths with different carotenoid content (see Cywinska et al., 2000; Armstrong et al., 2002; Lamare and Hoffman, 2004) and differential risk of exposure to UVR (Hansson and Hylander, 2009).

Here, we present new data on the carotenoid content and resistance to UVB in cystacanths belonging to three pigmented acanthocephalan species that all infest the amphipod *Gammarus pulex* (Fig. 1), but bring about contrasting behavioural alterations in their common intermediate host (Cézilly et al., 2000; Kaldonski et al., 2007; Perrot-Minnot et al., 2007). The two pomphorhynchid parasites *Pomphorhynchus laevis* and *Pomphorhynchus tereticollis* reverse the phototaxis of their intermediate host *G. pulex*, making infected amphipods increasingly attracted to light (Cézilly et al., 2000; Tain et al., 2006), while remaining benthic when uninfected (Perrot-Minnot, unpublished data) or, at least, slightly less benthic (*P. laevis*; Cézilly et al., 2000). In constrast, the bird polymorphid parasite *P. minutus* reverses geotaxis in its intermedidate host, resulting in infected *G. pulex* spending more time at the water surface (Cézilly et al., 2000; Perrot-Minnot, unpublished data). The negative phototaxis of *G. pulex* is not reversed by *P. minutus* (Tain et al., 2006; Perrot-Minnot, unpublished data) or only moderately so (Cézilly et al., 2000). Because exposure to UV is maximal at the water surface (Gáspár et al., 1996), *P. minutus* are presumably exposed to more UVR than the two fish acanthocephalans. We assessed the carotenoid content of cystacanths using HPLC analysis, and investigated the effects of UVB radiation in vitro on both the survival and genomic integrity of cystacanths. We discuss our results in relation to the role of carotenoids as photoprotective compounds, and to the evolutionary significance of their accumulation in larvae of acanthocephalan parasites.

## 2. Materials and methods

### 2.1. Collection of samples

Parasite samples were collected from three different rivers (Bèze, Ouche, Tille) in Burgundy (eastern France). Parasites were sampled by collecting infected *G. pulex* which were easily recognised in the field by the presence of a yellow-orange dot that corresponds to the cystacanth, visible through the translucid cuticle of the host. Parasite species were identified based on morphological characteristics and prior genetic analyses of the sampled populations (see Perrot-Minnot, 2004). For HPLC analysis of carotenoid content, cystacanths were rinsed in deionized water after dissection in saline, and quickly dried on absorbant paper. Between 16 and 25 conspecific individuals were then pooled together in a tube and stored at −80 °C. The exposure of live cystacanths to UVB radiation in vitro was performed directly after dissection in saline.

### 2.2. Extraction of carotenoids and HPLC analysis

Extraction of carotenoids was achieved following the protocol of Gaillard et al. (2004), using Matrix Solid Phase Dispersion (MSPD). Briefly, frozen parasites were crushed on dry ice and mixed with 200 mg of Isolute MSPD grade C18 sorbent material (International Sorbent Technology Ltd., UK). One-hundred microlitres of internal standard (2 ng/μL of lycopene in 50% methyl-*tert*-butyl ether (MTBE)/50% methanol) were applied on the column, before the pigments were eluted with 500 μL of methanol first and then 500 μL of MTBE. By using a combination of polar and non-polar solvents in the MSPD procedure, the complete extraction of carotenoids including xanthophylls and carotenes was achieved (Gaillard et al., 2004). After evaporation under nitrogen in the dark, parasite extracts were re-dissolved in 50 μL of 50% MTBE/50% methanol and injected into the HPLC column.

HPLC separations were performed according to Gaillard et al. (2004) on a 250 × 4.6 mm stainless steel ProntoSIL C30 reversed-phase column (3-μm particle size and 200-Å average pore diameter; Bischoff, Leonberg, Germany). Chromatographic analyses were conducted on a Waters system (Milford, MA, USA) controlled by Millenium$^{32}$ software. Separation of 50 μL carotenoid extracts was carried out using a mixture of MTBE, methanol and deionized water as the mobile phase, following these steps: 10 min with methanol/MTBE/water (81:15:4 v/v/v) followed by a 40 min linear mobile phase gradient from 81:15:4 to 10:90:0 methanol/MTBE/water v/v/v, and a 20 min gradient back to initial conditions. These conditions allowed a complete elution of pigments from xanthophylls to non-polar carotenoids in one step (Gaillard et al., 2004). Absorption spectra were collected from 250 to 600 nm with a