Local adaptation to UV radiation in zooplankton: a behavioral and physiological approach

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Abstract. Ultraviolet radiation (UVR) is recognized as a driving force for phenotypic divergence. Here, we aim at assessing the ability of zooplankton to induce UVR tolerance and disentangle the relative importance of local adaptations behind the expression of such tolerance. Two populations of Daphnia pulex, derived from environments strongly differing in UVR conditions, were exposed to UVR for 70 d to induce production of photo-protective compounds and changes in behavioral responses. We expected greater toler- ance to UVR in individuals from the high-UVR (H-U) environment as well as a refuge demand inversely related to the level of pigmentation. However, the complementarity between physiological and behavioral strategies was only observed on animals from the Low-UVR environment (L-U). L-U animals developed photo-protective compounds and decreased their refuge demand when re-exposed to UVR, that is, tolerated more UVR, compared to their control siblings. Conversely, UVR-exposed individuals from the H-U environment even having developed higher levels of photo-protective compounds increased their refuge demand staying deeper in the water column compared to the control animals, likely expressing an evolutionary memory to seek refuge in deeper waters irrespective of the UVR level. Stronger changes were observed in the H-U population compared to the L-U population; thus, our results suggest that although changes in tolerance after UVR exposure were evident for both populations, the strength of the inductions was more related to local adaptation independently of the rearing environment, showing that UVR tolerance is dependent on the evolutionary history of each population.

Key words: Daphnia; evolutionary memory; local adaptation; photo-protection; ultraviolet radiation; vertical migration.

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INTRODUCTION

The evolutionary history of a species is determined by the set of phenotypic and genetic changes of their characters and abilities over generations. Any successful population has evolved in a complex setting with a multitude of environmental factors shaping their habitat, such as temperature, water chemistry, food quality and quantity, seasonality, and predation pressure. This may result in a diverse set of adaptations to key environmental drivers and prevailing environmental conditions.

As most species cover different geographical areas, a given genotype often has the capacity to express a range of phenotypes in response to the
aquatic organisms are very sensitive to UVR (Williamson et al. 2001, Williamson and Zaragóz 2003, Häder et al. 2011, 2015). UVR tolerance among zooplankters is here defined as the ability to withstand UVR, including physiological (pigmentation) and behavioral responses (avoidance) as complementary traits to avoid or reduce UVR damage. This tolerance varies across zooplankton taxa, being species/taxon specific (Leech and Williamson 2000, Hansson and Hylander 2009), and may be dependent on local adaptations and ontogenetic trajectories. Organisms from high-UVR environments are particularly useful for studies of local adaptations to endure UVR. The tropical Andean lakes of South America are naturally exposed to high-UVR levels (Campero et al. 2011) due to their high altitude (Blumthaler et al. 1992, Pfeifer et al. 2006) and low ozone levels (Zaratti and Forno 2003, Liley and McKenzie 2006), making UVR a potential natural selective force in these environments (Fernández et al. 2018).

In order to assess the relative importance of local adaptations on the expression of UVR tolerance, we performed experiments with two Daphnia pulex populations, each of them from contrasting UVR environments. Environmental factors were controlled in a common garden setup, thereby eliminating other environmental sources of phenotypic variation between populations, which therefore had identical ontogenetic trajectories. We induced changes in pigmentation and behavior (avoidance; assessed by 3D tracking using nano-fluorescent labeling) of both populations, exposing them to non-lethal UVR doses for five generations. We expected to have populations with different sensitivity to the same stimuli (UVR), therefore expressing differences in UVR tolerance (physiological or behavioral) from which it is possible to infer the importance of local adaptations. On the contrary, if the phenotypic divergence in the expression of UVR tolerance was null or low between populations, ontogenetic drift may have compensated for origin differences within a few generations. Based on this hypothesis, we predicted that organisms from the High UVR environment would express more tolerance (more pigmentation and less avoidance behavior) than the organisms from the Low-UVR environment independently of the rearing environment.
**METHODS**

**Daphnia populations**

Two different populations of *Daphnia pulex* were chosen from high- and low-UVR environments, respectively, and raised under laboratory conditions. Both species were morphologically determined to *D. pulex*, although the species complex is still under taxonomic revision (Mergeay et al. 2008). The population from a high-UVR environment (H-U) was hatched from resting eggs sampled at several sites in Lake T’uturu Qucha, located in the Central Andes of Bolivia (17.46° S – 65.63° W) at 3730 m a. s. l., while the population from a low-UVR environment (L-U) was a population that was kept in the laboratory for more than four years (more than 100 generations) originating from Lake Dalby quarry, located in southern Sweden (55.66° N – 13.40° E) at approximately 95 m a. s. l. The tree cover around both lakes was negligible; that is, trees are not providing any refuge from UVR for the animals. Both populations were cultured separately in multiple flasks in dechlorinated tap water at 20°C and 12:12-h light:dark regime. *Daphnia* were fed *ad libitum* three times a week with an algal culture mainly composed by *Scenedesmus* sp.

**UVR exposure treatment**

To induce the expression of tolerance to UVR on both populations, newborn animals from each population were divided into two groups in multiple flasks: exposed and unexposed to UVR (hereafter referred to as UVR-exposed and Control treatments, respectively). Ultraviolet irradiation (135 μW/cm²; daily dose of 64.8 KJ/m²) and illumination (30.3 μmol·m⁻²·s⁻¹) were provided by three UVA fluorescent lamps (UVA-340; Q-panel) and four cool white fluorescent lamps (PAR; Aura Ultimate Life 36 W), respectively. Both treatments were in the same cabinet, UVR exposure was allowed or restricted covering the experimental flasks with a UVR-permeable Plexiglass (Röhm GS 2458; Röhm, Darmstadt, Germany) allowing UVR to penetrate, or with an UVR-screening Plexiglas (Röhm GS 233; Röhm) that cuts off UVR radiation. For a full spectral transmittance of the Plexiglasses, see Hansson et al. (2007). The daily UVR dose provided was selected based on previous studies that successfully exhibit non-lethal effects of UVR (Ekvall et al. 2015, Fernández et al. 2018).

Animals were kept under treatments for approximately 70 d (induction time). The first clutch of each flask was always discarded; offspring of the second and third clutches were transferred to new flasks to start a new generation. At the end of the induction time, adult females from the fifth generation with similar size were collected from each treatment for photo-protective compound analyses and behavioral experiments, avoiding males (due to their small size) and egg-bearing females.

**Photo-protective compounds analysis**

Prior to the analysis of photo-protective compounds, animals were transferred to filtered tap water and kept there for 2 h, allowing gut evacuation in order to avoid interference from phytoplankton photo-protective molecules. Effective gut evacuation was tested by scanning all samples within the chlorophyll peak absorbance (Gitelson 1992).

Three photo-protective compounds were analyzed: melanin, carotenoids, and mycosporine-like amino acids (MAAs). Three samples per photo-protective compound, each composed of 40 animals, were taken from each treatment and then stored at -80°C until the analysis. Carotenoid content was extracted adding 1.5 mL of ethanol (96%) to each sample and sonicating on ice for 30 s (VWR Ultrasonic Cleaner USC300D, VWR International bvba/sprl, Leuven). All samples were incubated in the dark at room temperature for 5 h and then centrifuged for 5 min at 1008 g. Supernatants were transferred to UV silica cuvettes, and carotenoids were measured at maximum absorbance of astaxanthin (3,3’-dihydroxy-β,β-carotene-4,4’-dione; 470 nm), quantitatively the most prevalent carotenoid in crustaceans (Coral-Hinostroza and Bjerkeng 2002). Carotenoid content was then calculated according to Tolasa et al. (2005) and related to the estimated dry mass (DM) of the sample (Dumont et al. 1975).

MAAs were extracted in a two-step extraction according to Tartarotti and Sommaruga (2002). 600 μL of 25% methanol was added to each sample; then, all samples were sonicated on ice for 30–40 s (VWR Ultrasonic Cleaner USC300D, VWR International bvba/sprl, Leuven), and
MAAs were extracted for 2 h at 45°C. Subsequently, samples were centrifuged at 13,000 rpm for 15 min at 4°C. 500 µL of the supernatant was collected and stored in a fridge. Then, another 400 µL of 25% methanol was added to each sample and the content was extracted at 45°C for two more hours. Both extracts were then mixed and centrifuged at 18,928 g and 4°C for 15 min. Samples were then scanned by a Cary 100 BIO UV–VIS spectrophotometer (Varian, California, USA) within the range of maximum absorption of MAAs (310–360 nm waveband; Sinha et al. 2007). The concentration of MAAs in each sample (µg/mg DM) was obtained from the equation

\[ C = \frac{D \cdot V \cdot 10^4}{E \cdot W} \] (1)

where \( C \) is the MAA concentration in the sample, \( D \) is the peak absorbance (\( \sim 334 \) nm), \( V \) is the extract volume in mL, \( E \) is the extinction coefficient for a 1% (weight:volume) solution, set as 42300 at 334 nm (Karentz 2001), and \( W \) is the estimated dry mass of the sample in mg.

Melanin content of each sample was extracted for 16 h at 65°C in 1 mL of 1 mol/L NaOH and 10 µL of H₂O₂ (3% aqueous solution), based on Herbert and Emery (1990) and Hobæk and Wolf (1991). All samples were centrifuged at 11,200 g for 1 min, and absorbance of the supernatant was measured spectrophotometrically (Cary 100 BIO UV–VIS spectrophotometer, Varian, California, USA) at 350 nm. Concentrations were calculated by using a reference curve from synthetic melanin (Sigma, St. Louis, Missouri, USA; M-8631). The concentrations were standardized to animal dry mass (DM) according to Dumont et al. (1975).

**Behavioral assay**

Diel vertical migration of zooplankters as a behavioral response to UVR has been observed in natural systems (Leech and Williamson 2001, Aguilera et al. 2006), as well as under laboratory conditions (Hylander and Hansson 2010, Ekvall et al. 2015). A behavioral assay was set up to record *Daphnia* migration responses to UVR by three-dimensional (3D) tracking using fluorescent nanoparticles (quantum dots; Qdots) according to Ekvall et al. (2013). For this, we used four synchronized digital cameras (Pike F-210C, Allied Vision Technologies GmbH) coupled to a 0.2 × 0.2 × 0.85 m aquarium and arranged as two stereo pairs. The setup allowed us to record instantaneous and individual movements in three dimensions at six frames per second (see Palmér et al. 2016).

Adult organisms of each treatment were isolated and kept in clean water for about 1 h before the assay for food evacuation. Labeling was performed according to Ekvall et al. (2013). Briefly, we transferred *Daphnia* individuals to a 2 mL poly-L-lysine-conjugated fluorescent Qdots solution for 30 min. Excess conjugated nanoparticles were then removed by rinsing the individuals three times with filtered water. After random labeling, two *Daphnia* (one UVR-exposed and its respective control) were introduced into the aquarium arena filled with 30 L of water (0.75 m water column). After an acclimation time of 15 min under blue light to excite the fluorescence in Qdots, we started recording a three-phase routing for 9 min. The first 3 min was recorded under blue light only; then, UVR at the top of the aquarium was turned on for 3 min (360 µW/cm² intensity; UVA LED array; VANQ Technology), and the last 3 min UVR was again turned off. The procedure was repeated twenty times per treatment with different *Daphnia* individuals in each trial. In total, 8 of the recordings were removed from the statistical analysis due to that air bubbles were trapped under the carapace of the animals, making them unable to dive.

**Data treatment and statistical analysis**

Differences in concentration of pigments between H-U and L-U populations were tested using two-way ANOVA and Tukey post hoc analysis in R v3.4.1 (R Core Team 2016). The image processing was performed according to Ekvall et al. (2013) and Palmér et al. (2016). All recorded videos were transformed to three-dimensional (3D) positions at six frames per second. Reflexes and noise (coming from, e.g., air bubbles in the water) were removed from the image, and each individual's position was then tracked by triangulating the data of all cameras using MatLab R2017b (MathWorks 2017).

To assess behavioral differences in UVR avoidance between treatments, depth position of each individual was analyzed as refuge demand. This is defined as the integrated vertical position of each organism over time in a non-UVR:UVR: non-UVR routine (Hansson et al. 2016). Larger
values of refuge demand represent longer time at deeper positions in the water column. Differences in refuge demand were assessed by two-way ANOVA. Only tracks with a clear pattern over the time were used in this analysis, and the number of tracks is specified in Fig. 3.

Due to missing data points in some tracks in some timeslots (no image recorded in some pixels for unknown reasons), Montecarlo bootstrap (resampling with reposition) was applied on the median vertical position and swimming 3D speed per minute to get a balanced database (50 replicates per treatment). To analyze position and speed over the time, mixed models were applied, where population (H-U/L-U), treatment (UV exposure/Control), and UVR phase (before/during/after) were used as fixed factors. The individual identification (ID) nested within the recording number (since we tracked two individuals at the same time), and the minute within the UVR phase were used as random factors. All statistical analyses were made in R v3.4.1 (R Core Team 2016).

**RESULTS**

**Photo-protective compounds**

Carotenoid pigmentation varied significantly in both *Daphnia* populations at the end of the induction time (Fig. 1). There was a UVR exposure effect (two-way ANOVA, $F_{1,8} = 15.31$, $P < 0.010$), as well as a difference in response between populations (two-way ANOVA, $F_{1,8} = 66.61$, $P < 0.001$). The concentrations of carotenoids were undetectable in the control samples of the low-UVR site (L-U) population. Tukey post hoc analyses showed that there were no differences in carotenoid concentrations between exposed animals of the L-U population and control animals in the high-UVR site (H-U) population ($P = 0.160$). Concentrations in the UVR-exposed animals were three times higher in H-U than in the L-U population ($0.18$ and $0.06$ μg·mg$^{-1}$·dry mass [DM], respectively).

Mycosporine-like amino acid (MAA) values from control individuals were $0.28$ and $0.30$ μg·mg$^{-1}$·DM in L-U and H-U populations, respectively. UVR exposure affected both populations, increasing the MAA content but at different levels (two-way ANOVA; Population Treatment, $F_{1,8} = 28.80$, $P < 0.001$), being higher in the H-U than in the L-U population ($0.70$ and $0.52$ μg·mg$^{-1}$·DM, respectively, Fig. 1).

Regarding melanin, L-U showed different patterns compared with the H-U population (two-way ANOVA; Population Treatment, $F_{1,8} = 29.98$, $P < 0.001$). The mean amount of melanin in the H-U population was nearly 10 times higher than for L-U *Daphnia* ($0.73$ and $0.06$ μg·mg$^{-1}$·DM, respectively; Fig. 1) in control treatments. Melanin concentration was not significantly affected by the UVR exposure in the L-U *Daphnia*. In contrast, the H-U population showed strong responses to the UVR exposure increasing the melanin concentration from $0.73$ (control) to $2.19$ (UVR-exposed) μg·mg$^{-1}$·DM, that is, with 200%.

**Behavior**

Mixed-models ANOVA revealed significant differences between patterns of vertical position (Treatment Population UVR phase, $F_{2, 1733} = 12.24$, $P < 0.001$) and swimming speed (Treatment Population UVR phase, $F_{2, 1733} = 8.89$, $P < 0.001$) of both populations within the 9-min routine (Table 1, Figs. 2, 3a). Depth preferences of *Daphnia* exhibited high variability in the absence of UVR in our experiment. On average, animals from both populations preferred shallow to medium depths during the first three minutes (no UVR), except for individuals from H-U; UVR-exposed treatment, which preferred to remain in deeper waters (Fig. 3a). During the UVR exposure (minute 4–6), animals from all treatments responded strongly by descending to deeper waters. During the recovery time (last 3 min), the average behavior was to return to shallow waters.

The median swimming velocity (speed) initially increased in the UVR-exposed *Daphnia* of both populations at UVR exposure (see Table 1, Fig. 2). The behavior of control animals differed between populations; H-U *Daphnia* exhibited a rapid reaction to UVR exposure increasing their speed during the first minute of exposure. The L-U *Daphnia*, on the other hand, maintained the same speed when moving to deeper waters.

In terms of refuge demand, the UVR-exposed treatment had different effects on the L-U and H-U populations (two-way ANOVA; Population Treatment, $F_{1,41} = 4.25$, $P < 0.040$).

The UVR treatment had no significant effect on refuge demand in the L-U population (Tukey
post hoc test, $P = 0.740$). On the other hand, UVR-exposed *Daphnia* from the H-U population increased the total refuge demand compared to the animals in the control treatment (Fig. 3b). These differences were more evident during the UVR phase (Fig. 3c). *Daphnia* from the control treatment of both populations increased their refuge demand during the UVR phase, but only H-U *Daphnia* maintained this behavior among the UVR-exposed animals (Fig. 3c).

**DISCUSSION**

Our study provides a test of the hypothesis that local adaptation determines the expression of tolerance to ultraviolet radiation (UVR) in terms of pigmentation and behavior in *Daphnia*. We tested this hypothesis by using *D. pulex* populations from environments with contrasting UVR levels. None of the lakes had tree cover along the rim, and no major land-use changes have, to our knowledge, affected the lakes, suggesting that the refuge from UVR has been negligible, ensuring considerably different UVR threat between lakes over the past decades, that is, during evolutionary time scale for these animals. Previous studies have provided evidence for phylogenetically controlled susceptibility (survival test) of *D. melanica* to UVR in function of the transparency of their native ponds (Miner and Kerr 2010). Our results show that not only tolerance, but also the responses to minor changes in UVR are different among populations.

Our study shows that it is possible to induce both the production/accumulation of photo-protective compounds and changes in migration behavior of *Daphnia pulex* within relatively short time scales (70 d) and relatively low-UVR doses (UVA: 64.8 KJ/m² day), exposing the strong effect of the environment in activating the plastic capacity of populations. We also observed a shift in the response at the population and individual levels independently of the rearing environment, enhancing the role of local adaptations in the expression of tolerance to UVR.

Regarding photo-protective pigmentation, the carotenoid concentrations were low in both our populations and even below detection limits in the L-U control (Fig. 1), which is in line with previous studies showing that those pigments are completely absent, or at best, present in very low concentrations.
concentrations in cladocerans (Rautio et al. 2009). Nevertheless, even at low concentrations, the induction of carotenoids by UVR was evident, being three times as higher in H-U compared to L-U Daphnia. It is worth to note that H-U Daphnia presented low concentrations of carotenoids even without UVR exposure. Similar studies have reported carotenoids in ovaries and eggs of cladocerans exposed and non-exposed to UVR (Green 1957, Siebeck 1978). Life-history traits in both populations have been documented in a previous work, where early maturation and high fecundity of the H-U population were proposed as an adaptive strategy against UVR (Fernández et al. 2018). Given that growth capacity (Niu et al. 2012, 2014, Zhang et al. 2013), immune responses (Supamattaya et al. 2005), and reproductive performance (Bjerkeng 2008, Schneider et al. 2016) are parameters influenced by carotenoid concentrations in crustaceans, the difference in carotenoid concentrations we found here may be related with reproductive trade-offs in this population. In our experiments, we used UVA radiation that, besides being less harmful than UVB, could be beneficial in the repairing processes (Gonçalves et al. 2002, Banaszak et al. 2003). In this sense, the possibility of trade-offs in allocation of energy in protective pigmentation or repair mechanisms is worth considering, but it was beyond the scope of this study.

Similar to carotenoids, the accumulation of mycosporine-like amino acids (MAAs) was also promoted by exposure to UVR, but in very low concentrations (Fig. 1). However, in contrast to carotenoids, MAA concentrations did not differ between populations and were negligible in control treatments. Few studies have shown the presence of MAAs in cladocerans (Hansson et al. 2016), and they are generally undetectable (Tartarotti et al. 2001, 2004, Gonçalves et al. 2002) or only present in trace amounts (Persaud et al. 2007). This indicates that the accumulation of MAAs is not a major strategy used by Daphnia to cope with damaging UVR. Apparently, the enzymatic uptake and retention system to accumulate MAAs are not as efficient in cladocerans as in copepods (Tartarotti et al. 2001), leading to far lower MAA levels and an unclear function in cladocerans (Hessen and Sørensen 1990).

Cuticular melanin, on the other hand, is the only pigment that cladocerans can produce themselves (Luecke and O’Brien 1983, Herbert and Emery 1990), and it has been shown to improve survival when animals are exposed to UVR (Zellmer 1995, Rautio and Korhola 2002). Fishless environments tend to favor melanin production over behavioral responses to diminish the effects of UVR on Daphnia (Tollrian and Heibl 2004), but the concentration of melanin is generally declining if UVR is removed (Hessen 1996, Hansson et al. 2007), suggesting that as melanin has to be synthetized in every molt (Herbert and Emery 1990), its production has a metabolic cost. Hessen (1996) suggested a trade-off between growth rate and UVR protection in a melanic morph of Daphnia pulex, agreeing with

| Variable | Factor                          | df     | F       | P     |
|----------|---------------------------------|--------|---------|-------|
| Depth    | Treatment                       | 1, 1733| 5.59    | 0.0200|
|          | Population                      | 1, 1733| 70.15   | <0.0001|
|          | UVR phase                       | 2, 6   | 10.18   | 0.0100|
|          | Treatment:Population             | 1, 1733| 13.74   | <0.0010|
|          | Treatment:UVR phase             | 2, 1733| 15.15   | <0.0001|
|          | Population:UVR phase            | 2, 1733| 6.69    | 0.0010|
|          | Treatment:Population:UVR phase  | 2, 1733| 12.24   | <0.0001|
| Speed    | Treatment                       | 1, 1733| 12.71   | 0.0004|
|          | Population                      | 1, 1733| 0.22    | 0.6400|
|          | UVR phase                       | 2, 6   | 1.39    | 0.3200|
|          | Treatment:Population             | 1, 1733| 121.47  | <0.0001|
|          | Treatment:UVR phase             | 2, 1733| 5.59    | 0.0040|
|          | Population:UVR phase            | 2, 1733| 3.93    | 0.0200|
|          | Treatment:Population:UVR phase  | 2, 1733| 8.89    | 0.0001|

**Note:** Factors include Treatment (UVR exposed, Control), Population (H-U, L-U), UVR phase of the routine (off–on–off).
observations by Weider (1987), who observed delayed age at first reproduction and smaller clutch size in *Daphnia*. However, life-history experiments on both of our populations (same experimental conditions) revealed that even if fecundity and the number of offspring were negatively affected by UVR, the H-U population had a higher reproductive rate than the L-U population both in the absence and in the presence of UVR (Fernández et al. 2018).

The extent to which daphnids respond behaviorally to UVR is generally inversely related to their pigmentation (Rhode et al. 2001), possibly because synthesis or accumulation of photo-protective substances has an energetic cost (Vincent and Neale 2000). In general, the H-U population displayed greater amounts of pigments in comparison with the L-U animals. Thus, given the complementarity between physiological and behavioral strategies (Vincent and Neale 2000), lower migration activity would be expected in H-U than in L-U populations. This counterbalance between behavior and photo-protection has previously been observed when comparing cladoceran species (Hansson et al. 2016). However, in spite of the melanic response in the H-U population to UVR exposure (from 0.73 to 2.19 µg mg⁻¹ DM), individuals did not migrate less than their control siblings, as would have been expected. On the other hand, the L-U population behavior was in line with previous observations on *D. magna*, which expressed less migration when re-exposed to UVR after an acclimatizing period under UVR (Hylander et al. 2014), demonstrating that ontogenetic processes could change the expression of tolerance in terms of less migratory behavior within a few generations.

Fig. 2. Speed of *Daphnia* from high- and low-UVR environment cultured under UVR (UVR-exposed) or regular PAR-light (Control). Purple bands represent UVR exposure for 3 min (min 4–6). Top numbers = number of tracks used in each minute analysis; black lines within the boxes = median of the minute; blue line = mean; dots = outliers.
It is important to note that following the initial reflex to swim down when UVR was turned on, most individuals ascended after approximately one minute, while still being under UVR exposure, whereas the animals from the Control treatment of the H-U population behaved more cautious and generally remained deeper down in the water column until the end of the UVR exposure (Fig. 3a). Theoretically, poorly pigmented populations from high-UVR environments (as the H-U population) may have an evolutionary memory of photoreception that forces them to make stronger migrations even at low-UVR levels. Daphnia possess the ability to perceive UVR through photoreceptors that should result in appropriate depth selection when exposed to UVR (Smith and Macagno 1990), but whether or not they have fine-scaled power discrimination is still an open question.

The H-U control Daphnia reacted and swam faster than animals in any other treatment when UVR was turned on (Fig. 2). This suggests that populations from high-UVR environments may compensate the absence of physiological photoprotection with a fast-behavioral reaction, even without previous UVR experience. Again, this suggests evolutionary imprinted memory, as this fast reaction did not occur in L-U population.

We are aware that different studies (Mergeay et al. 2008, Held et al. 2016) have identified genetic differences between American and European D. pulex and that our results may reflect genetic distance instead of local adaptation. In order to disentangle this issue, we performed a complementary experiment with two different species (D. pulex and D. peruviana) from the same UVR regime (Tutuра Qucha Lake in Bolivia and its vicinity), strongly differing in

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**Fig. 3.** Vertical positions and refuge demands (complete tracks only) of Daphnia from high- and low-UVR environment cultured under UVR (UVR-exposed) or regular PAR-light (Control) in a non-UVR:UVR:non-UVR recording routine. (a) Average (red lines) and individual (overlapping gray areas) vertical positions. Purple areas represent the UVR phase of the routine. (b) Total refuge demand (mean ± SE) in a 9-min routine. (c) Specific refuge demand (mean ± SE) before, during, and after the UVR exposure. Closed and open symbols represent H-U and L-U populations, respectively. H-U, Control n = 10; H-U, UVR-exposed n = 10; L-U, Control n = 12; L-U, UVR-exposed n = 13 individuals.
melanization (D. peruviana being the most mela-
nic). We measured their downward migration
with and without UVR stimulus (see Appendix S1: Fig. S1) and found that, while both
species were at similar depths in the absence of
UVR, both strongly migrated downwards at
UVR exposure, with D. peruviana migrating dee-
per than D. pulex, despite it is heavily melanized
and hence more protected against UVR. This
shows that even different species with identical
evolutionary history expose similar behavioral
responses despite different pigmentation and
thus reinforce our observations that local adapta-
tions can strongly drive behavior, despite genetic
distance.

It should be noted that responses to the presence
or absence of UVR were immediate, indicating
that UVR-avoiding migration does not necessarily
follow a daily pattern, but rather responds rapidly
to changes in UVR (Hansson et al. 2016). In this
sense, migration behavior could be more complex
as it interacts with other factors such as tempera-
ture (Rhode et al. 2001) and predation (Hylander
et al. 2009). Finally, local adaptations and ontoge-
netic drift can lead to different responses to UVR
within a species or a species cluster. Therefore, we
may conclude that tolerance can be induced
depending on the environmental context, whereas
the evolutionary history and local adaptations
likely control its expression.

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