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Adaptation to local ultraviolet radiation conditions among neighbouring *Daphnia* populations

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Understanding the historical processes that generated current patterns of phenotypic diversity in nature is particularly challenging in subdivided populations. Populations often exhibit heritable genetic differences that correlate with environmental variables, but the non-independence among neighbouring populations complicates statistical inference of adaptation. To understand the relative influence of adaptive and non-adaptive processes in generating phenotypes requires joint evaluation of genetic and phenotypic divergence in an integrated and statistically appropriate analysis. We investigated phenotypic divergence, population-genetic structure and potential fitness trade-offs in populations of *Daphnia melanica* inhabiting neighbouring subalpine ponds of widely differing transparency to ultraviolet radiation (UVR). Using a combination of experimental, population-genetic and statistical techniques, we separated the effects of shared population ancestry and environmental variables in predicting phenotypic divergence among populations. We found that native water transparency significantly predicted divergence in phenotypes among populations even after accounting for significant population structure. This result demonstrates that environmental factors such as UVR can at least partially account for phenotypic divergence. However, a lack of evidence for a hypothesized trade-off between UVR tolerance and growth rates in the absence of UVR prevents us from ruling out the possibility that non-adaptive processes are partially responsible for phenotypic differentiation in this system.

Keywords: evolution; population structure; genetic drift; natural selection; local adaptation

1. INTRODUCTION

A central challenge in evolutionary biology concerns understanding the historical processes that generated current patterns of phenotypic differentiation within spatially subdivided populations of a single species [1,2]. Mechanisms underlying phenotypic divergence among populations fit into two broad categories: phenotypic plasticity without underlying genetic divergence between populations [3,4], or heritable genetic divergence in traits [5]. In the latter case, population divergence can be the result of: (i) an *adaptive* process in which natural selection is the primary force generating divergence, (ii) a *non-adaptive* process in which genetic drift generates divergence via such mechanisms as founder effects and/or the random fixation of alleles, or (iii) a combination, in which adaptive and non-adaptive processes are jointly responsible. In this third and arguably most common case, the relative importance of both kinds of processes must be evaluated, and considerable effort has been dedicated to this challenge [6,7].

Felsenstein [8] argued that historical relationships among species could confound studies using the comparative method; a similar concern exists for comparisons of closely related populations of a single species [9]. Particularly for populations that have diverged recently, inferences regarding the role of environmental factors in driving evolution that assume statistical independence among populations may be misplaced. When assessing adaptive phenotypic differentiation, the structure of evolutionary relationships among populations must be considered and included in a null model of non-adaptive divergence. For example, the Q_{ST}/F_{ST} approach compares quantitative genetic variation with neutral genetic variation to detect the influence of natural selection on quantitative genetic traits, and is useful when its assumptions are satisfied [10]. However, the Q_{ST} metric does not explicitly consider the structure of relationships among populations, and is inappropriate in many situations [11], such as when comparing among populations with recent and unknown colonization history [12]. Therefore, alternative approaches should be considered to measure the relative importance of adaptive and non-adaptive processes in the evolution of phenotypic divergence among populations [10,11].

Understanding evolutionary processes in subdivided populations has been particularly challenging in the case of freshwater invertebrates. Many aquatic taxa have high dispersal capacity during resting stages [13,14], yet typically exhibit pronounced genetic structure even among neighbouring populations, a phenomenon labelled the 'dispersal–gene flow paradox' [15]. The conflict between apparent high-dispersal capacities and significant population subdivision has been resolved in two ways. First, estimating rates of gene flow from neutral allele frequency divergence can be misleading when populations are founded by a small number of individuals and then grow rapidly [16]. This is a characteristic of populations

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of zooplankton and other aquatic taxa that have high growth rates and often have small founding populations [15]. In such cases, founder effects can lead to significant genetic structure among populations that is not the result of an adaptive process. Second, many aquatic species are capable of adapting to local environmental conditions within a small number of generations, a phenomenon that may limit subsequent gene flow between populations that experience differing selection pressures [15]. However, whether this second *adaptive* component is necessary to explain natural patterns of neutral allele frequencies has been questioned [17,18]. To fully address these issues requires a study system and a statistical methodology in which phenotypic divergence, population structure and evidence for adaptation can be jointly investigated.

Here we used natural variation in ultraviolet radiation (UVR) transparency among shallow subalpine ponds to explore the scope and limits of adaptation in the freshwater zooplankter *Daphnia melanica*. We asked whether the UVR tolerance of organisms from different populations was positively related to the UVR threat in their native pond even after accounting for the structure of population relationships using a combination of population-genetic and statistical techniques. Additionally, we investigated a possible fitness trade-off between UVR tolerance and growth rate, as the existence of that trade-off could provide an adaptive explanation for the limited spread of the most tolerant phenotypes. We found that an environmental stressor such as UVR predicts phenotypic divergence in a manner consistent with adaptation, and that this result holds even when historical relationships between populations are taken into account. However, we did not find evidence of a fitness trade-off of UVR tolerance, suggesting that both adaptive and non-adaptive processes may have influenced phenotypic evolution in our study populations.

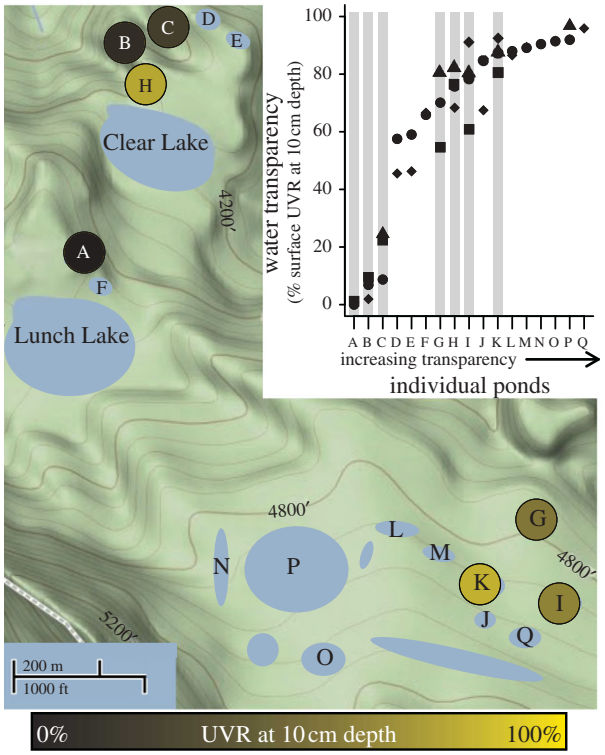

Figure 1. Map of the field site, with study ponds labelled in ascending alphabetical order of water transparency. The maximum distance between any two ponds (D–Q) is approximately 1500 m, and the maximum elevation difference is 245 m (800 inch). Ponds overlaid with black/yellow circles are those from which we collected live *Daphnia* for laboratory experiments, and the degree of shading from black to yellow represents water transparency in 2008. Inset: water clarities of all 17 ponds, estimated from water samples taken in late August of multiple years (filled diamonds, 2006; filled triangles, 2007; filled squares, 2008; filled circles, 2009). Transparencies are the estimated percentage of surface UVR at 10 cm depth. Ponds highlighted in grey match those with overlaid circles on the map. The average coefficient of variation (CV) among all ponds within years is 48%, while the average CV for individual ponds across years is 24%.

2. MATERIAL AND METHODS

(a) *Field sampling and laboratory culture*

We conducted field sampling of water transparency in July–September of 2006–2009 in the Seven Lakes Basin region of Olympic National Park in northwest Washington State. We sampled small (<0.25 ha), shallow (depth < 2 m) ponds in an ecological transition zone from montane to subalpine habitat (figure 1). The amount of terrestrial vegetation in the landscape surrounding each pond was variable, a factor known to affect the concentration of dissolved organic matter and therefore the UVR transparency of a water body [19]. To estimate UVR transparency, we measured absorbance of 440 nm light passed through a 10 cm path length quartz cuvette containing approximately 30 ml of filtered (0.02 µm pore size) pond water in a UV-2100 spectrophotometer (Shimadzu America, Columbia, MD, USA). Using an established relationship for Pacific Northwest ponds [19], we estimated UV-B attenuation coefficients (K_d) to calculate the corresponding UV-B intensities at a reference depth of 10 cm. We collected water samples in each of 4 years to compare interannual variation in water transparency.

We collected zooplankton in horizontal tows at approximately 10 cm depth with a 125 µm conical tow net, isolated live *Daphnia*, and transported them to the laboratory within three days. We preserved additional *Daphnia* in 95 per cent ethanol for DNA analyses, and others in 10 per cent formalin for melanin extractions. We identified all study populations as *D. melanica* by comparing sequences of the mitochondrial *ND5* gene to those from an existing phylogeny for the *Daphnia pulex* group [20]. Our populations nested within the previously sampled populations of *D. melanica*.

We established laboratory cultures with animals collected in a single field visit in August 2008. We raised clonal lineages starting from individual field-collected *Daphnia* females. We kept cultures in laboratory incubators (Percival Scientific, Perry, IA, USA) on a 16 L : 8 D light cycle at 12°C to mimic summer field conditions, thus maintaining parthenogenesis. We maintained *Daphnia* in FLAMES, an artificial freshwater medium developed for Cladocera from soft-water localities [21]. We fed cultures two to three times per week with vitamin-supplemented *Cryptomonas ozolinii* (UTEX LB 2782).

(b) *Ultraviolet radiation tolerance experiments*

We conducted laboratory UV-B trials in a temperature-controlled incubator outfitted with a UV-lamp phototron, modified from the design of Williamson *et al.* ([22],

see electronic supplementary material). Each experimental trial contained cohorts of egg-bearing adult *Daphnia* that were each 28–35 days old. A single experimental replicate consisted of 15 animals in 80 ml of FLAMES medium [21] containing *C. ozolinii* (*ca* 4.5–5.5 mg l^{-1} dry mass) in an uncovered beaker placed on the outer rim of the rotating wheel. Experimental trials consisted of 12 h of UV-B exposure during a 16 h day of visible and UV-A radiation at 12°C. The total UV-B dosage measured under such experimental trials ranged from *ca* 27–33 kJ m^{-2}. Control treatments were maintained on a second-tier rotating wheel just below the first and blocked from UV-B radiation. Following UV-B exposure, we moved each replicate into 500 ml of the same algae/medium mix and maintained these individuals under visible and UV-A radiation (16 L : 8 D) at 12°C for an additional seven days before scoring survivorship. We added fresh food every 2–3 days during the period between UVR exposure and survival measurement.

For a given *Daphnia* clone, we tested one to three UVR-exposed and one to three control (unexposed) replicates in each experimental trial. All trials of a particular clone contained at least one control treatment. We tested multiple clones in each trial and ran multiple trials over a period of several months (March–November 2009). We conducted UVR exposure trials with *Daphnia* clones from seven different ponds selected to represent the full range of water transparencies present in this system (figure 1). We tested one to five clones from each of these ponds, for a total of 21 clones. The number of replicates per clone ranged from 1 to 14, with a median of six replicates. Mortality in control treatments was rarely more than 10 per cent, and we excluded from our analyses the few replicates (both UVR and control) from runs where the control animals for that clone had more than 10 per cent mortality. In our statistical modelling of the influence of source pond on survival under UVR, we included data only from UVR-exposed *Daphnia*, excluding the control survival data, which did not differ among clones.

(c) *Melanin assay*

We measured melanin, a known photoprotective mechanism in zooplankton exposed to UVR, in both field-collected and laboratory cultured animals, using protocols described by Scoville & Pfrender ([23], see electronic supplementary material).

(d) *Population genetics*

We performed population-genetic analyses by surveying five polymorphic nuclear microsatellite loci. We extracted DNA from individual ethanol-preserved animals using the CTAB extraction protocol [24]. We collected data from five microsatellite markers (see electronic supplementary material, tables S3 and S4 for primer sequences and polymerase chain reaction (PCR) conditions). We diluted PCR products to appropriate concentrations and mixed with formamide before denaturing at 95°C for 2 min and cooling on ice. We then measured the fluorescence of fragments in the ABI 3730 in the Comparative Genomics Center at the University of Washington. We included negative controls and a known sample as a size standard in each fragment-analysis run. We manually measured sizes of fluorescent peaks by viewing fragment analysis trace files in GeneMapper (Applied Biosystems, Foster City, CA, USA).

We tested for Hardy–Weinberg equilibrium and linkage disequilibrium and conducted an Analysis of Molecular Variance using Arlequin v. 3.5 [25]. To estimate the number of ancestral populations (k) and individual ancestry coefficients (Q values), we used the Bayesian Markov Chain-Monte Carlo method implemented in the program Structure v. 2.3 [26,27]. We ran Structure under the admixture model with correlated allele frequencies [27], and we used sampling locations as prior information to assist the clustering algorithm (LOCPRIOR model; [28]). Because the use of a location prior may increase the algorithm's ability to find population clusters [28], we view this as a conservative approach given that our goal was to test for the role of habitat characteristics in predicting phenotypes *after* accounting for population structure. We estimated the total probability of the data over a range of k values [2–8], averaged the total probabilities from three independent runs of each k value, and chose the value of k with the highest mean probability. We created graphical displays of individual inferred ancestry coefficients (Q values) using the program Distruct [29].

(e) *Life-table assay*

We assessed life-history characteristics in the absence of UVR using a standard experimental design [1,30] and used these data to calculate r, the intrinsic rate of increase. The number of replicates for each clone ranged from 1 to 4. For further details, see electronic supplementary material.

(f) *Statistical analyses*

To assess the statistical significance of our experimental results, we used generalized linear mixed-effects models (GLMMs) created with *R* [31]. For all models, we included *Daphnia* clone ID as a random effect nested within the locality (pond) of origin. We tested the significance of melanin relationships with pond transparency and growth environment (field versus laboratory) as fixed effects. Because many of our samples contained no melanin, we used a Poisson error structure with a log-link function. For experimental UVR trials, our response variables were the counts of dead and surviving animals; therefore, we used a binomial error distribution with a logit link. We used four models that differed in fixed effects. In one pair of models, we did not incorporate population-genetic structure. Thus, the null model contained no fixed effects, and contained only the random effects of locality and clone within locality. In contrast, the UVR transparency model contained the measured UVR transparency (in August 2008, see figure 2*a*) for each pond as a continuous predictor. In our second pair of models, we used the same random effects but included as fixed effects q values based upon individual ancestry coefficients (Q values) from the Structure output, where $0 \leq q \leq 1$, $q_1 = Q_1$ and

$$q_i = \frac{Q_i}{1 - \sum_{j=1}^{i-1} Q_j} \quad \text{for } i \in \{2, 3, \ldots k-1\}.$$

The structured null model contained only random effects plus the fixed effects of population structure. The structured UVR transparency model included fixed effects of population structure and the measured UVR transparency of each pond. The formula for the most general model was $\hat{S}_{cp} = \text{logit}(\alpha + \beta_0 u + \sum_{i=1}^{k-1} \beta_i q_{i,cp} + \varepsilon_{1,p} + \varepsilon_{2,cp})$, in which the subscript p represents the source pond, the subscript c represents clonal lineage within pond, *number surviving* $\sim$ *binom*($\hat{S}_{cp}$, N), $\hat{S}_{cp}$ is the fitted survival, the q_i values are as

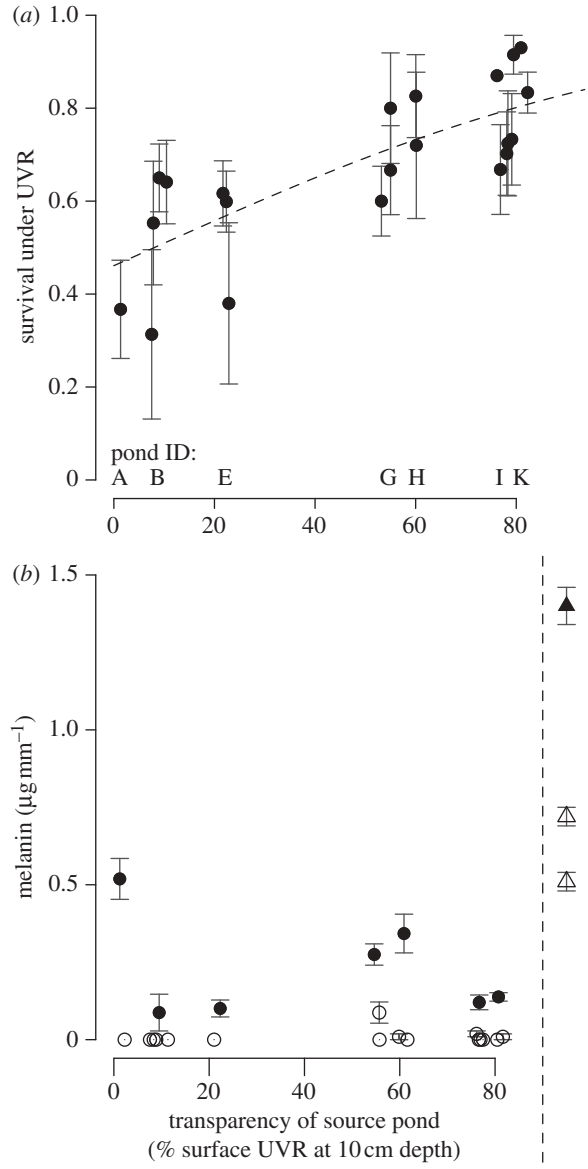

Figure 2. (*a*) Survival (mean ± s.e.) following ultraviolet radiation (UVR) exposure for *D. melanica* clones from seven source ponds reared in a common garden. Each point represents a unique *Daphnia* clone; there are multiple clones per pond. The dashed line represents the UVR transparency model of table 1. (*b*) Estimated melanin content (mean ± s.e.) of both field-collected and laboratory-raised *D. melanica*. Olympic laboratory clones are those used in subsequent laboratory experiments. Melanin measures for Sierra Nevada *D. melanica* [23] are included for reference and have no *x*-values. Filled circles, Olympic field samples; open circles, Olympic laboratory cultures; filled triangles, Sierra Nevada field samples; open triangles, Sierra Nevada laboratory cultures.

described above, u is the estimated percentage of surface UVR at 10 cm depth for each pond, $\varepsilon_{1,p}$ represents the random effect of source pond and $\varepsilon_{2,cp}$ represents the random effect of clone within the pond, where $\varepsilon_{1,p} \sim N(0, \mathrm{var}_{pond})$ and $\varepsilon_{2,cp} \sim N(0, \mathrm{var}_{clone})$ in which var_{pond} and var_{clone} are the variances associated with the random effects of locality and clone, respectively. All models had matching formulae and differed in which β values were set to zero. We evaluated the relative fit of our data to each of these four models using Akaike's information criterion (AIC). We also performed likelihood ratio tests (LRTs) between competing models. The single exception to our GLMM framework was our analysis of the life-table experiment, where we tested for a relationship between UVR tolerance and *r* estimates using a linear regression.

Table 1. Generalized linear mixed-effects models of survival under laboratory ultraviolet radiation (UVR). UVR transparency models include the UVR transparency of each pond as a continuous fixed effect; Structured models include individual ancestry coefficients for the inferred population clusters as fixed effects; all models include random effects of *Daphnia* clone ID nested within the locality (pond) of origin. The UVR transparency model, in italic, represents the best-fit model with the fewest parameters. k is the number of parameters in each model; all models had an n of 108. Parameter values for the best-fit model were $\alpha = -0.135$, $\beta_0 = -0.013$, $\beta_1 = \beta_2 = 0$, $\mathrm{var}_{pond} = 3.57 \times 10^{-14}$ and $\mathrm{var}_{clone} = 8.21 \times 10^{-2}$.

model	k	log-likelihood	AIC	ΔAIC
structured UVR transparency	6	−276.5	565.1	0[a,b]
UVR transparency	*4*	*−278.7*	*565.4*	*0.03*[b,c]
structured null	5	−281.6	573.1	8.0[a]
null	3	−283.8	573.5	8.4[c]

[a]Likelihood ratio test between these two models $p = 0.002$.
[b]Likelihood ratio test between these two models $p = 0.11$.
[c]Likelihood ratio test between these two models $p = 0.002$.

3. RESULTS

Olympic ponds varied considerably in transparency to UVR (figure 1), consistent with previous observations in this region [19]. Interannual variation in water transparency within ponds was much lower than variation in transparency among ponds (figure 1, inset). For the seven focal ponds from which we collected zooplankton at approximately 10 cm depth in 2008 for laboratory culture and experiments, estimated UV-B at 10 cm among ponds ranged from 1 to 81 per cent of surface radiation (figure 1, inset square points shaded in grey). When exposed to UVR in the laboratory, *Daphnia* from ponds with greater water transparency (thus higher UVR) had significantly greater survival than did those from less transparent ponds (figure 2*a* and table 1). The best-fit statistical model included water transparency as a continuous and positive predictor of UVR tolerance (UVR transparency model, table 1 and dashed line in figure 2*a*: LRT against null: $p = 0.002$).

Because photoprotective melanin pigmentation is a known mechanism that often underlies variation in UVR tolerance among *Daphnia* populations [23,32], we measured melanin content in both wild-caught and laboratory-raised animals. Wild-caught animals had slightly more melanin than those raised in the laboratory (LRT against null model: $p = 0.0009$; figure 2*b*) but melanin levels bore no relationship to the transparency of the source pond (LRT against previous model: $p = 0.53$; figure 2*b*). Melanin content of our *D. melanica* was much lower than those of *D. melanica* populations from the Sierra Nevadas (figure 2*b*; [23]).

To assess population-genetic structure, we genotyped between 24 and 28 individual *Daphnia* from each focal pond at five nuclear microsatellite loci. All loci were

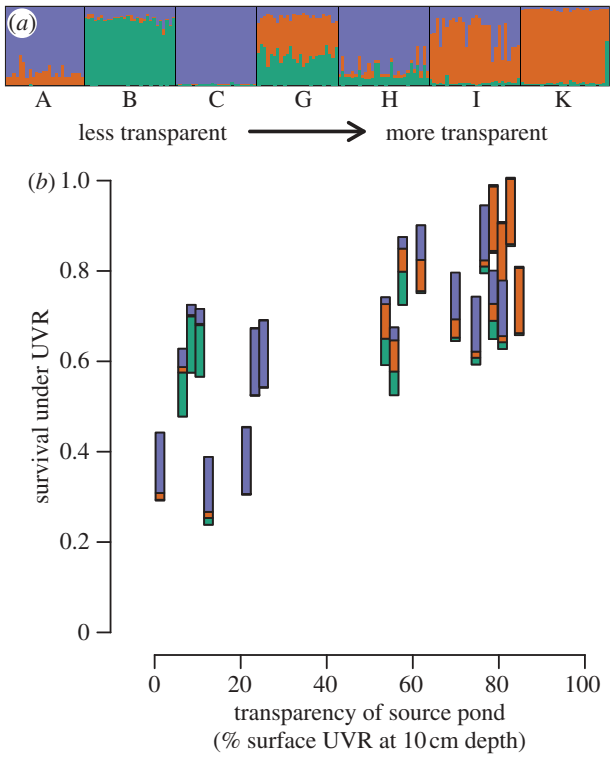

Figure 3. (*a*) Inferred ancestry of individual field-collected *Daphnia* from seven ponds, based on allelic variation at five nuclear microsatellite loci. Vertical bars represent individual animals; the purple proportion represents the ancestry coefficient for cluster 1, orange for cluster 2 and green for cluster 3. Ponds are arranged from left to right in ascending order of ultraviolet radiation transparency; labels correspond to those in figures 1 and 2. (*b*) Integrated presentation of habitat, phenotype and genetic data. Each vertical bar represents a unique *Daphnia* clone, with the *x*- and *y*-location of the bars as in (*a*). The shading of each bar represents each clone's ancestry coefficients for the three inferred population clusters, as in figure 2*a*.

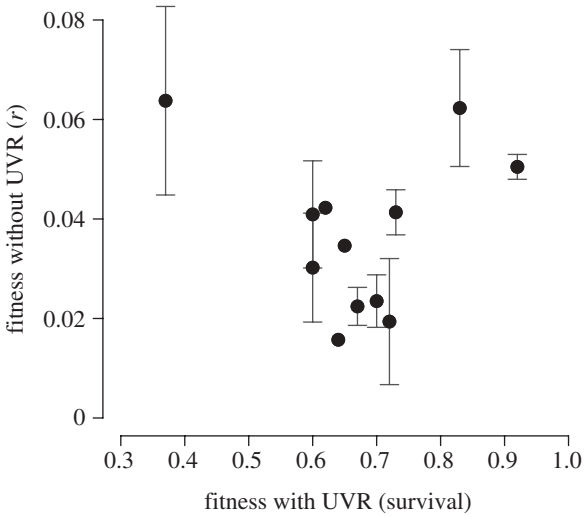

Figure 4. Estimates of *r*, the intrinsic rate of population increase, in the absence of ultraviolet radiation (UVR) for a subset of the clones from the UVR tolerance experiments. We use *r* as a proxy for fitness and find that under standard laboratory conditions there is no relationship between *r* and a clone's UVR tolerance (linear regression: $\beta = -0.005$, $p = 0.89$, $r^2 = 0.002$).

polymorphic in at least one population, and mean allelic richness did not vary among populations (see electronic supplementary material, table S1). Most loci were in Hardy–Weinberg and linkage equilibrium within most populations, but significant deviations did occasionally exist (see electronic supplementary material, table S1), though these were unrelated to water transparency. We found significant genetic differentiation among populations, with an AMOVA ϕ_{ST} value of 0.23 ($p < 0.00001$ in a permutation test) and pairwise F_{ST} values that ranged from 0.02 to 0.51 (see electronic supplementary material, table S2). A Bayesian clustering analysis of genetic structure resulted in a best-fit model with three ancestral population clusters ($k = 3$; see electronic supplementary material, figure S1). Individual ancestry coefficients for each of the three clusters were largely similar among individuals within populations, but varied considerably among populations (figure 3*a*).

The relationship between UVR exposure and population ancestry coefficients was non-random (figure 3*b*): one cluster in particular (orange in figure 3) was associated with greater UVR exposure and greater UVR tolerance. To account for this clustering, we incorporated individual ancestry coefficients from the genetic clustering analysis into our statistical modelling of phenotypic divergence. However, models that included population structure as a fixed effect predicting UVR tolerance had similar AIC values to corresponding models without population structure (table 1), demonstrating that the addition of population clustering parameters does not improve model predictions of phenotype (structured UVR transparency versus UVR transparency models, table 1; LRT $p = 0.11$).

If present, a fitness trade-off between UVR tolerance and growth rate would provide an adaptive explanation for why the most tolerant *Daphnia* genotypes are not present in all populations. However, *Daphnia* lineages with greater UVR tolerance did not have lower population growth rates than less-tolerant lineages in the absence of UVR. The relationship between UVR tolerance and the intrinsic rate of population increase (*r*), estimated from life-table assays in the absence of UVR, was extremely weak and not significant (linear regression: $\beta = -0.005$, $p = 0.89$, $R^2 = 0.002$; figure 4).

4. DISCUSSION

Our *D. melanica* populations displayed clear evidence of adaptation to local UVR conditions. *Daphnia* from ponds with greater UVR transparency were more tolerant of UVR in laboratory trials than were populations from low-transparency ponds (figure 2*a*). From one perspective, this selective pattern is expected because UVR is a potent stressor that damages DNA in living tissue [33]. Yet, as these populations are close together (<1 km apart) and may experience frequent dispersal among ponds, population differentiation in UVR tolerance was not a foregone expectation, even in the face of UVR selection. Our population-genetic analysis found that the populations display significant structure at neutral loci, suggesting that they do not in fact exist as a single panmictic population (figure 3*a*). The association between population structure and water transparency was non-random (figure 3*b*), yet individual ancestry coefficients

explained little of the variation in UVR tolerance among *Daphnia* lineages (structured versus unstructured models in table 1). We were therefore motivated to consider the possibility that phenotypic divergence was merely a consequence of population structure and was not driven by UVR. To do so, we incorporated the outcomes of our population-genetic analysis as predictors in our statistical models of UVR tolerance. However, the incorporation of population structure in this manner did not improve predictions of UVR tolerance phenotypes beyond those based upon water transparency alone (table 1). The best-fit model shows that only environmental factors significantly predict phenotypic divergence.

The degree of neutral genetic differentiation among *Daphnia* populations was considerable, with pairwise F_{ST} values as high as 0.51 (electronic supplementary material, table S2). Such levels of differentiation are typical of *Daphnia*, even among neighbouring populations [1,18,34,35]. Among the seven ponds in our study, the inferred number of ancestral population clusters was 3 (figure 3*a*). Each pond displayed a distinct signature of inferred ancestry coefficients and within-population variability was minimal (figure 3*a*). Although the only ponds with notable coefficients for cluster 2 (orange) tended to have higher UVR exposure and higher UVR tolerance (figure 3*b*), the reverse did not hold: not all high-UVR, high-tolerance populations belonged primarily to cluster 2. An important counterexample is Pond H: located in the lower basin, it had a transparency comparable with the high-UVR ponds in the upper basin (figure 2*a*), likely owing to a combination of large size and small drainage area. In this pond, *Daphnia* had relatively high UVR exposure (figure 1) and were quite tolerant of UVR (figure 2*a*), but genetically clustered with individuals from neighbouring low-UVR ponds (figure 3*a*). Here the environmental influence of UVR exposure predicts phenotypes more accurately than neutral expectations based upon population structure.

Surprisingly, our study populations were minimally pigmented with melanin (figure 2*b*), which contrasts with *Daphnia* in most high-UVR habitats [32,36–40] and all previously studied *D. melanica* [23,41,42]. *Daphnia* that coexist with visual predators typically have diminished melanin pigmentation, even in high-UVR habitats [23], but our small study ponds do not contain such predators. Melanin pigmentation is protective of UVR in both laboratory and field conditions [36,37], but carries a growth-rate cost [36] and requires induction by UVR [23,38,43]. Although our populations can synthesize melanin (like all *Daphnia*, they deposit melanin in the eye and in the ephippium encasing diapausing eggs), the genetic pathways required to develop concentrated pigmentation in the carapace could be missing. Natural variation in melanin pigmentation in many insects results from changes at a small number of genes [44–46], some of which may also regulate pigmentation in *Daphnia* [23]. Also, photoprotective pigmentation may not be adaptive in the habitats we studied because of its significant growth rate costs [36]. Although behavioural avoidance of UVR has been demonstrated in non-melanic *Daphnia* in deep lakes [47], this would be of limited benefit in our shallow (<1.5 m depth) study ponds, so an alternative tolerance mechanism must be present. Here the role of phenotypic plasticity may be critical [48,49], as pigmentation intensity is plastic on the time scale of carapace moulting (several days) [23,32]. In principle, the expression and activity of an enzymatic response to UVR could react on a shorter time scale (minutes to hours), minimizing the lag-time of the induced phenotype. Because an enzymatic response might have lower physiological costs (no melanin synthesis) and reversibility on shorter time scales, such a mechanism could be favoured in moderate-UVR habitats with variable exposure intensities [49]. We characterize the UVR environment of our Olympic study sites as moderate because *D. melanica* populations of the Sierra Nevadas are found at elevations that are approximately 1500–2000 m higher than our Olympic sites [41] and UV-B radiation at the Earth's surface increases approximately 20 per cent per 1000 m of elevation [50]. In the habitats of highest UVR intensity (at high elevation or arctic latitudes), pigmentation may be the only viable phenotype regardless of costs [23].

Because our study populations demonstrated phenotypic differentiation for UVR tolerance (figure 3), we expected to find evidence for a fitness cost to UVR tolerance in the absence of UVR, as is the case for *Daphnia* adapted to UVR via the pigmentation mechanism [36]. Such a cost would explain why the genotypes with the highest UVR tolerance have not spread to all ponds, and would fit a pattern of local adaptation [51]. However, using a standard life-table design [1,34], we were unable to detect a significant growth-rate cost of the most UVR-tolerant phenotypes in the absence of UVR (figure 4). Of course, our experimental design might have failed to detect an existing cost; we measured growth rates in a well-fed common garden in the absence of competition or external stressors, and fitness trade-offs can sometimes be obscured in the presence of abundant resources [52]. Also, the source ponds probably differ in multiple variables, such as food quality and quantity (unfortunately, a field reciprocal transplant is not possible in this system because of National Park Service restrictions and access limitations). However, the possibility that no fitness trade-off exists (even in natural conditions) is worth considering. If this is the case, an alternative mechanism is needed to explain why the most UVR-tolerant genotypes have not spread to low-transparency ponds, where they may be selectively neutral. A critical point is that the frequency distributions of neutral alleles are often not in equilibrium in zooplankton populations; this is because of strong initial founder effects followed by rapid growth to large population sizes that buffer gene frequencies against the homogenizing effects of immigrant alleles [16]. Such founder effects can result from the purely random sampling of genotypes that are dispersed into novel habitats, or can be owing to a non-random selective process that favours certain genotypes dispersing to, or establishing in, novel habitats (termed the 'favoured founder' by Quinn *et al.* [53]). The phenomenon of non-equilibrium gene frequencies in zooplankton populations is particularly pronounced in previously glaciated areas of North America that contain relatively young populations, where realized dispersal rates could simply be low enough that insufficient time has passed for gene frequencies to reach equilibrium [17]. Given that our study populations exist in a

mountain range that was entirely glaciated less than 20 000 years ago [54], and at a regional elevation that had year-round snow cover until much more recently, this is a reasonable possibility.

To effectively evaluate the relative importance of selection pressures, historical population relationships, and putative fitness trade-offs in generating current patterns of phenotypic divergence among neighbouring populations requires a study design that considers each of these factors in a single system, such as that presented here. Our results demonstrate that in naturally subdivided populations, environmental variables can have significant influences on phenotype even when the specific structure of population relationships is integrated into statistical models of phenotypic divergence. Furthermore, that we did not find clear evidence for a fitness trade-off suggests that the limited geographic spread of certain phenotypes may not be the result of an adaptive process [16]. A combination of a short post-glaciation time span, low dispersal rates and directional selection for UVR tolerance is, in principle, sufficient to explain the present-day patterns in this system. Our results demonstrate that adaptive processes are important in phenotypic evolution, and raise the possibility that non-adaptive processes also play a role. However, this latter speculation merits further study before definitive conclusions can be made. Regardless, this work highlights the importance of explicitly incorporating population structure when testing adaptive hypotheses in nature.

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