

Supporting Information

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SI Text

Adaptive Value of Plasticity in Melanin. Consistent with the hypothesis that melanin provides protection from UV, populations without fish experience a smaller increase in mortality when exposed to UV than populations with fish. This pattern was assessed through ANOVA, where adjusted clutch failure (clutch failure under UV – clutch failure under non-UV) was modeled as a function of population nested within fish history and fish history. Before analysis, adjusted clutch failure was transformed by taking the square root of $1 +$ adjusted clutch failure to eliminate a significant right skew and platykurtosis in the residuals. Fishless populations experienced a significantly smaller adjusted clutch failure than the less melanic fish populations ($F_{1,44} = 5.03$; $P = 0.03$). We analyzed adjusted clutch failure because clutch failure in non-UV conditions is not normally distributed.

Consistent with the hypothesis that melanin is costly to produce in the absence of UV, increased production of melanin is associated with a longer time to reach maturity. We assessed this pattern first through ANOVA, predicting the natural log of time to maturity as a function of clone nested within population, population nested within fish history, fish history, UV, and fish history by UV interaction, with clone treated as a random effect. A log transformation was used to eliminate significantly unequal variances between UV and non-UV treatments before analysis. Of these factors, only population ($F_{2,46} = 15.42$; $P < 0.0005$) and fish history by UV interaction ($F_{1,46} = 4.28$; $P = 0.04$) were significant. The significant fish history by UV interaction indicates that fishless populations, which exhibit a greater increase in melanin under UV, also exhibit a greater increase in time to maturity under UV (Table S1). In addition, a GLM predicting the natural log of time to maturity as a function of population and melanin revealed a significant positive effect of melanin ($F_{1,92} = 7.35$; $P = 0.008$). Together, our data on clutch failure and time to maturity support an adaptive nature of the ancestral reaction norm of melanin to UV.

Neither clutch size nor body size at maturity appear to be related to melanin production at a significance level of $P < 0.05$. Clutch size was measured as the number of eggs in the brood pouch at maturity. We analyzed these two traits in the same manner as time to maturity. ANOVA revealed no significant fish history by UV interaction (clutch size: $F_{1,157} = 2.16$; $P = 0.14$; body size: $F_{1,157} = 0.41$; $P = 0.52$). A GLM including all significant factors, as assessed by ANOVA, plus melanin revealed a positive but nonsignificant association between melanin and clutch size ($F_{1,110} = 3.61$; $P = 0.06$) and melanin and body size ($F_{1,111} = 2.91$; $P = 0.09$). Neither clutch size nor body size at maturity satisfied equality of variances between each combination of population and UV treatment, as assessed by Levene's test. However, differences among variances were slight and would act only to inflate type II error in detecting an effect of population or fish treatment. In concordance with earlier findings (1), both population and fish history had highly

significant effects on clutch size (population: $F_{2,157} = 14.71$; $P < 0.0005$; fish history: $F_{1,157} = 35.24$; $P < 0.0005$) and body size (population: $F_{2,157} = 200.54$; $P < 0.0005$; fish history: $F_{1,157} = 742.59$; $P < 0.0005$) and were included in the GLMs used to assess the association with melanin. Body size and clutch size were smaller in populations coexisting with fish. Residuals from ANOVAS and GLMs performed on these two traits did not deviate from normality.

Genetic Independence of Populations. To assess population structure and test for independence of evolutionary changes in the populations exposed to fish predation, we examined allelic variation in six polymorphic nuclear loci. We applied a Bayesian model-based clustering method using STRUCTURE v. 2.2 (2) to place our populations coexisting with fish (Puppet and Evelyn lakes) in the context of neighboring populations (Alcatraz, Golden, Lower Goethe, Middle Skelton, Source, and Snowpole; Frog data were unavailable due to human error). STRUCTURE allowed us to infer the number of distinct populations, or clusters (K). For any particular number of clusters, it also allowed us to infer, for each individual, the probability of assignment to each cluster. For each population we used 20 multilocus genotypes based on six microsatellite loci (3) (Table S2). To estimate the number of clusters, we used an admixture model that assumed correlated allele frequencies among populations (2). For each level of clustering ($K = 1-8$) we used a burnin period of 30,000 steps, 100,000 MCMC reps, and five separate runs. The STRUCTURE analysis suggested $K = 8$ (average $\ln P(D) = -1347.6$). This result indicates that the genetic architecture of each population is largely independent of the genetic architecture of neighboring populations (Fig. S1). At $K = 8$ the two populations co-occurring with fish have nonoverlapping cluster membership. As we force a reduction in the number of clusters these nonoverlapping cluster memberships are retained until K is constrained to 4, and they remain largely separated even at $K = 3$ (Table S3).

To examine the relationships among populations, we used microsatellite genotypes to construct a pairwise matrix of corrected average difference ($(\pi_{xy} - (\pi_x + \pi_y)/2)$) values using Arlequin ver. 3.11 (4). We used this matrix to construct a UPGMA phenogram using MEGA ver. 4.0 (5) (Fig. S2); this analysis confirms that our two fishless populations are not closely related in comparison with surrounding populations. Data from Snowpole, a fishless population that is sister to the fish population Evelyn (Fig. S2), reveal that *Daphnia* from this lake resemble other fishless populations, including Source and Middle Skelton lakes, in both phenotypic and life-history traits (1). Taken together, our STRUCTURE analysis and the relationships between Puppet and Evelyn lakes in our population phenogram support the conclusion that these two lakes are on independent evolutionary trajectories (i.e., their resemblance is not due simply to gene flow).

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2. Pritchard JK, Stephens M, Donnelly M (2000) Inference of population structure from multilocus genotype data. *Genetics* 155:945–959.
3. Colbourne JK, Robison B, Bogart K, Lynch M (2004) Five hundred and twenty-eight microsatellite markers for ecological genomic investigations using *Daphnia*. *Mol Ecol Notes* 4:485–490.

4. Excoffier L, Laval G, Schneider S (2005) Arlequin ver.3.0: An integrated software package for population genetics data analysis. *Evol Bioinform Online* 1:47–50.
5. Tamura K, Dudley J, Nei M, Kumar S (2007) MEGA4: Molecular Evolutionary Genetics Analysis (MEGA) software version 4.0. *Mol Biol Evol* 24:1596–1599.

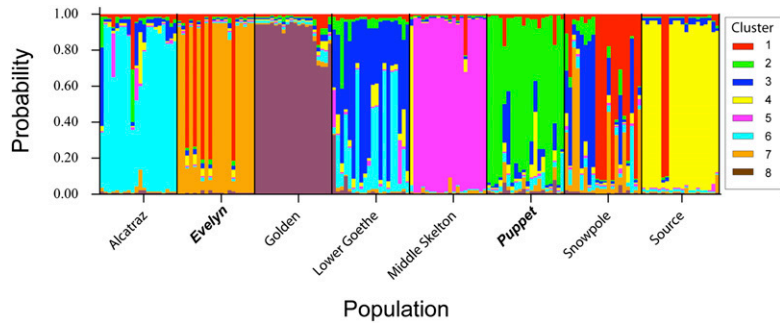


Fig. S1. Probability of cluster membership for individual genotypes in eight populations based on an analysis of six microsatellite loci using STRUCTURE, v. 2.2. Each individual is represented by a thin column that is partitioned into colored segments representing the individual's estimated probability of assignment to each of the eight clusters ($K = 8$). Black lines separate different populations, which are labeled below the figure. *Daphnia* populations coexisting with fish are shown in bold italics.

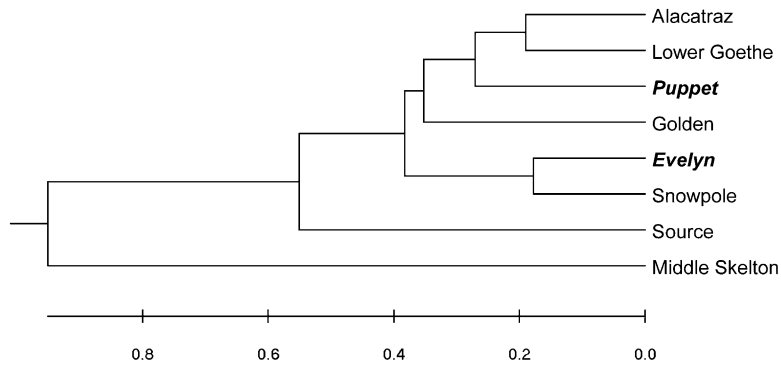


Fig. S2. Phenogram of eight populations based on six microsatellite loci. The scale bar below the phenogram represents the corrected average difference. *Daphnia* populations coexisting with fish are shown in bold italics.

Table S1. Means and SEs

Trait	Population	Exposure to fish (years)	Treatment			
			No UV		UV	
			<i>N</i>	Mean (SE)	<i>N</i>	Mean (SE)
Melanin ($\mu\text{g}/\text{mm}$)	Source	0	13	0.51 (0.03)	10	0.96 (0.07)
	Frog	0	15	0.72 (0.03)	14	1.11 (0.05)
	Puppet	53	13	0.42 (0.03)	12	0.50 (0.04)
	Evelyn	91	10	0.20 (0.02)	11	0.38 (0.03)
Clutch failure	Source	0	11	0.01 (0.01)	11	0.17 (0.06)
	Frog	0	15	0.08 (0.03)	15	0.29 (0.05)
	Puppet	53	13	0.04 (0.03)	12	0.38 (0.07)
	Evelyn	91	12	0.14 (0.04)	12	0.45 (0.07)
Time to maturity (days)	Source	0	13	8.28 (0.11)	11	8.99 (0.39)
	Frog	0	16	8.95 (0.15)	15	10.40 (0.40)
	Puppet	53	13	9.56 (0.26)	12	10.07 (0.70)
	Evelyn	91	13	8.16 (0.13)	11	8.30 (0.48)
Number of eggs at maturity	Source	0	13	4.75 (0.35)	11	3.10 (0.36)
	Frog	0	17	5.24 (0.38)	15	4.39 (0.30)
	Puppet	53	13	4.37 (0.25)	12	3.36 (0.27)
	Evelyn	91	13	2.64 (0.18)	11	2.35 (0.18)
Body size at maturity (mm)	Source	0	13	1.95 (0.04)	11	1.98 (0.02)
	Frog	0	17	1.97 (0.02)	15	1.99 (0.02)
	Puppet	53	13	1.76 (0.03)	12	1.77 (0.02)
	Evelyn	91	13	1.29 (0.02)	11	1.32 (0.02)
<i>Ddc</i> expression	Source	0	6	0.41 (0.12)	6	0.19 (0.06)
	Frog	0	6	0.55 (0.09)	6	0.34 (0.03)
	Puppet	53	6	0.32 (0.02)	6	0.46 (0.09)
	Evelyn	91	6	0.38 (0.05)	6	0.40 (0.06)
<i>Ebony</i> expression	Source	0	6	0.46 (0.06)	6	0.39 (0.08)
	Frog	0	6	0.33 (0.07)	6	0.34 (0.02)
	Puppet	53	6	0.61 (0.08)	6	0.50 (0.06)
	Evelyn	91	6	0.45 (0.05)	6	0.57 (0.05)

Clutch failure is given as a proportion of total clutches. Gene expression is a relative measure, given as a proportion of the highest expression sample in the data set. *N* refers to the number of clones. In the case of melanin, number of eggs at maturity, and body size at maturity, each clone is represented by measurements on 1–3 replicates in each UV treatment. For all other traits, a single clone was measured within each UV treatment.

Table S2. Microsatellite loci used in this study, including primer names, oligonucleotide sequence, and annealing temperature

Locus	Primer sequence	Annealing temp
CAA2	5'-CAC CTT GGA ACA CTC TGA CT-3'	52
	5'-AGG GTC GCA TTT GAC CAT CT-3'	
CAA8	5'-CAT CTG CGA AGA GTA TCG TCA-3'	56
	5'-CCA CAT GGC AGA ACA GTC AA-3'	
CAA14	5'-GAA TTT CTC GAG GCG ATT GA-3'	48
	5'-CCT TTC TGT ATC GCC AGC TC-3'	
CAA27	5'-CCA AGT TCT ACG TCA CTA TCA TC-3'	60.5
	5'-TCG CGG GTC TGT CCG TTC-3'	
GTT3	5'-TTA TCA CCT CCT CAA CTT C-3'	48
	5'-CTT CTT CCT TCA CTT CTC C-3'	
1-41	5'-GGT GGT CGT GAA GGT CTC AT-3'	52
	5'-CCA ACC GAG TTA CGG ACT GT-3'	

All primers are from ref. 3.

Table S3. Cluster membership from STRUCTURE analysis

Population	Cluster no.								K
	1	2	3	4	5	6	7	8	
Evelyn	5						15		K = 8
Puppet		19		1					
Evelyn			5		15				K = 7
Puppet		20							
Evelyn				20					K = 6
Puppet	20								
Evelyn					20				K = 5
Puppet		19		1					
Evelyn		20							K = 4
Puppet	16	2	2						
Evelyn	20								K = 3
Puppet		2		18					
Evelyn	20								K = 2
Puppet	20								

For the two populations co-occurring with fish (Evelyn and Puppet lakes), we note how many genotypes are assigned to each cluster, based on their highest inferred assignment probability. This distribution is assessed over a range of clusters as we force the number of clusters (K) to be smaller (from $K = 8$ to $K = 2$, shown to the right). Alternate shading groups results for the two lakes for each particular number of clusters. At $K = 5$ and greater the two lakes have nonoverlapping cluster assignments. At $K = 3$ and $K = 4$ the two populations have minimal overlap, with only 2 individuals from Puppet Lake assigned to clusters containing all of the Evelyn Lake individuals. Only at the lowest number of clusters ($K = 2$) are all individuals in these two populations assigned to the same cluster.

Table S4. Primers used for quantitation of gene expression using real-time quantitative PCR

Locus	Primer sequence	Annealing temp.
<i>α-tubulin</i>	5'-CAC CTT GGA ACA CTC TGA CT-3'	59
	5'-AGG GTC GCA TTT GAC CAT CT-3'	
<i>Ddc</i>	5'-CAT CTG CGA AGA GTA TCG TCA-3'	64
	5'-CCA CAT GGC AGA ACA GTC AA -3'	
<i>ebony</i>	5'-GAA TTT CTC GAG GCG ATT GA-3'	64
	5'-CCT TTC TGT ATC GCC AGC TC-3'	
<i>EF-1α</i>	5'-CCA AGT TCT ACG TCA CTA TCA TC-3'	59
	5'-TCG CGG GTC TGT CCG TTC-3'	
<i>G3PD</i>	5'-TTA TCA CCT CCT CAA CTT C-3'	59
	5'-CTT CTT CCT TCA CTT CTC C-3'	
<i>RNAP II</i>	5'-GGT GGT CGT GAA GGT CTC AT-3'	64
	5'-CCA ACC GAG TTA CGG ACT GT-3'	

All primers yielded amplification efficiency greater than or equal to 97%, as assessed through amplification of a standard curve in triplicate.

Table S5. MANOVA on all traits

Effect	df	<i>F</i>	<i>P</i>
Clone	100, 82	1.75	0.005
Population	10, 32	4.46	0.001
Fish	5, 16	30.51	0.000
UV	5, 16	29.00	0.000
UV x fish	5, 16	7.32	0.001

MANOVA was conducted on melanin, clutch failure, natural log of time to maturity, and expression of *Ddc* and *ebony*. Clone is nested within population, and population is nested within fish history. Statistics are based on Wilks' criterion. The *F* value for clone is approximate. Degrees of freedom are shown as numerator, denominator. Significant results ($P < 0.05$) are indicated in bold.