

The heritable basis and cost of colour plasticity in coastrange sculpins

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Abstract

Both genetic and plastic traits contribute to adaptation in novel environments. Phenotypic plasticity can facilitate adaptation by allowing for existence in a wider range of conditions and a faster response to environmental change than gene-based selection. Coastrange sculpins (*Cottus aleoticus*) colonize new and variable streams arising in the wake of receding glaciers in south-east Alaska, and substrate-matching plasticity may enhance colonization success by reducing detection by visual predators. As part of a long-term study of the fitness consequences of colour plasticity and its capacity to respond to both positive and negative selection, we investigated whether it is heritable and costly. We raised full-sib broods of sculpins in the laboratory: one half of each brood was raised in white containers, the other half in black. After 4 months, we digitally analysed their colour and found significant but weak heritability in both baseline colour and colour plasticity. To investigate the cost of colour plasticity, we compared the growth and mortality rates of juvenile sculpins reared under constant substrate colours to those reared on substrates that changed colour frequently, and compared growth rates among sculpin that differed in their colour change ability. We found evidence of small costs of plasticity, consistent with other studies of natural populations. Evidence of heritable genetic variation for plasticity and small costs to its maintenance and expression contributes to explanations of how plasticity is variable and persistent among wild populations and underscores its ability to respond both positively and negatively to selection in variable habitats.

Introduction

The role phenotypic plasticity plays as an adaptive strategy in the face of environmental unpredictability has received a surge of interest in recent years (Agrawal, 2001; Ghalambor *et al.*, 2007; Chevin *et al.*, 2010; Beldade *et al.*, 2011). As a result, we have gained a better understanding of its role in evolutionary change, including genetic accommodation (West-Eberhard, 2003; Pigliucci *et al.*, 2006; Crispo, 2007), how it affects speciation and diversification (Pfennig *et al.*, 2010; Thibert-Plante & Hendry, 2011) and proximate mecha-

nisms, such as its heritability (Scheiner & Lyman, 1989; Scheiner, 1993; Flatt, 2005), and limits to and costs of its maintenance and expression (Auld *et al.*, 2010).

It is now evident that phenotypic plasticity may be an important adaptive strategy in response to rapid climate change (Crozier *et al.*, 2008; Hendry *et al.*, 2008; Sih *et al.*, 2011). Although many populations emigrate or go locally extinct in the face of rapid environmental change, others may persist in their original range through phenotypic plasticity or genetic adaptation (Holt, 1990). The challenge to the latter is that the rate of mutational genetic change may be unable to keep up with the rapid environmental change taking place. In this case, phenotypic plasticity, when adaptive, can allow the population to locally persist by 'buying time' for the adaptive phenotype to assume an

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underlying genetic basis; a process known as a type of genetic accommodation (Baldwin, 1896; West-Eberhard, 2003; Pigliucci *et al.*, 2006; Crispo, 2007; Ghalambor *et al.*, 2007). Populations that use phenotypic plasticity as an adaptive response to rapid climate change are especially likely to be found in habitats in high elevations and latitudes, where the rate and ecological impacts of climate change are largest (Parmesan, 2006).

One high latitude region currently experiencing rapid, large-scale environmental change is south-east Alaska. Although this region contains one of the largest collections of mountain glaciers in the world, it is also experiencing rapid and accelerating rates of glacial recession (Arendt *et al.*, 2002). One consequence of this is the generation of new stream habitats in the wake of receding glaciers. Coastal species that colonize these new glacier-fed streams are provided with an increasing supply of new habitat but are also faced with a tremendous degree of habitat unpredictability. Despite long-term studies of the dynamics of ecological succession in streams from the recently deglaciated ecosystem of Glacier Bay National Park in south-eastern Alaska (Chapin *et al.*, 1994; Milner *et al.*, 2000, 2007, 2008), we still do not understand the evolutionary strategies that allow for their successful colonization.

Populations of coastrange sculpin (*Cottus aleuticus*), a small-bodied (adult size is 50–100 mm TL), amphidromous species, have been colonizing coastal glacial streams throughout south-east Alaska since deglaciation began at the end of the last ice age, and more recently, as glacial retreat has accelerated over the last ~225 years (Milner *et al.*, 2000). Although dispersal occurs during a brief marine phase after larvae leave their natal streams, post-settled juveniles and adults reproduce and spend the rest of their lives in freshwater streams (Mason & Machidori, 1976), many of which are glacial. These glacial streams exhibit variation in their substrate colours as a function of stream age: younger streams are dominated by black, grey, and white rocks and pebbles with very little organic matter, whereas older streams have rocks and pebbles covered with red and green algae and wooden debris (Robertson & Milner, 2006). Coastrange sculpin integument colour matches the colour of their stream substrate (Whiteley *et al.*, 2009), although there is variation in baseline colour, colour change ability and integument patterning within and among stream populations (Whiteley *et al.*, 2009, 2011). Substrate matching is largely due to colour plasticity, common in fish, amphibians and reptiles, which is stimulated by visual input, triggering hormonal and nervous responses that affect the dispersion and density of pigment molecules and melanophores in the integument (Nery & Castrucci, 1997; Sugimoto, 2002). Visual piscivores frequent streams in south-east Alaska (Milner *et al.*, 2000), and it is likely that colour plasticity in coastrange sculpin

reduces the likelihood of detection by enhancing crypsis (Whiteley *et al.*, 2009, 2011).

Given the potential benefits of colour plasticity in this species, it might be expected that plasticity would be ubiquitous. However, there is considerable variation in the expression of plasticity among and within stream populations in the wild (Whiteley *et al.*, 2009, 2011). On a common laboratory substrate, adult sculpin from two streams with different substrate colours converged in coloration towards the colour of the laboratory substrate, thus demonstrating colour change plasticity (Whiteley *et al.*, 2009). However, colour convergence was variable and incomplete, suggesting that there might be either genetic variation for colour plasticity and/or inherent costs that limit its expression and maintenance, which are common across taxa (Relyea, 2002). The likely adaptive benefit plasticity provides in the way of enhanced crypsis in heterogeneous environments means that positive selection for plasticity could exist if it has a genetic basis. Subsequently, there may be differences in strength of positive selection for plasticity among habitats as a function of environmental heterogeneity. Furthermore, if plasticity comes at a physiological cost, it may result in negative selection against heritable plasticity (Snell-Rood & Papaj, 2009) in relatively homogeneous streams. Assessing whether this cost exists in coastrange sculpin, and if so to what degree, is crucial to our interpretations of the evolutionary significance of colour plasticity, and the potential role genetic accommodation plays in adapting to a changing climate. To assess the potential for colour plasticity to respond both positively and negatively to selection, here we investigated (i) whether there is evidence of a heritable basis to baseline colour and colour plasticity in this species and (ii) whether there are detectable costs to its production and maintenance.

Materials and methods

Heritability of colour and colour plasticity

To investigate the heritable basis of colour and colour plasticity, we conducted an experiment with full-sibling split-brood families reared on two colour treatments. We sampled one discrete egg cluster from each of 25 different males' nests near the mouth of Salmon Creek (Juneau, Alaska) in May 2008. Individual egg clusters, each containing approximately 100 fertilized full-sibling eggs, were carefully removed from the underside of rocks by hand and transported to the laboratory. Each cluster was placed separately into a tray of a standard salmon incubation rack. Each incubation tray was refit with 500- μ m mesh to accommodate the small size of sculpin eggs relative to salmon eggs. Fresh 5 °C water constantly flowed through the incubation rack.

Within days prior to hatching, eggs were transferred to Petri dishes maintained at 5 °C to prevent mixing

between families. Petri dishes were kept in the same refrigerator on the same rack, and we changed the water in each dish twice daily with water from the same source until the eggs hatched (for approximately 2 days). Upon hatching, larval sculpin were transferred to five-gallon black containers. All of these containers were housed together in the same large tank (4 m²; 0.5 m deep) with a 50 : 50 mix of fresh and salt water. Each container had four equidistant holes cut into its sides, each with a diameter of 12 cm and covered with 250- μ m mesh. Each family was housed in a separate container, and containers had their position randomly rotated within the large tank each day to avoid local environmental effects. Mesh screening allowed exchange with the water in the larger tank, but to ensure that toxins did not build up in the containers, we cleaned the bottom of the containers each day with a syphon and performed a 20% water change. Larval fish were fed three times daily with 100–300- μ m live rotifers (*Brachionus plicatilis*). After 10 days post-hatch, larval fish were fed rotifers and newly hatched *Artemia* shrimp. Larval fish were planktonic until day 55, at which point they settled to the bottom of the container. At day 55, approximately half of the full-sibs from each family were kept in the original black container (black environment) and the other half transferred to white containers of the same size and construction (white environment), to create two rearing treatment colours for each family. All black and white buckets were kept in the same large tank.

Fish were photographed at four separate ages (days post-hatch): day 78 ($N = 307$ fish in 25 families), day 97 ($N = 228$ fish in 18 families), day 116 ($N = 213$ fish in 15 families) and day 249 ($N = 137$ fish in five families). Not all families survived for the duration of photograph sessions, and some families only had survivors in black containers. Photography was performed by placing each fish in a glass box on a photographic light board with constant lighting and a colour standard in each image. Photography and colour analysis were conducted exactly as in previous studies in this system (see Whiteley *et al.*, 2009, 2011 for details). Briefly, digital images were taken with a Canon Digital Rebel XT (Canon USA, Inc., Lake Success, NY, USA). Images were quantified in Photoshop CS4 (Adobe Systems Inc., San Jose, CA, USA), using the 'curves' correction to standardize black and white colour values relative to standards on an X-rite colorchecker card (X-rite, Inc., Grand Rapids, MI, USA). We used the CIE 1976 $L^*a^*b^*$ colour space model to quantify fish colour, which consists of three orthogonal axes: lightness (L^*), which varies between white (high L^*) and black (low L^*), and a^* and b^* axes, which vary between red and green, and yellow and blue, respectively (Stegen *et al.*, 2004). We manipulated substrate colour only between white and black, and the primary response of fish colour occurred along this colour axis (A.R. Whiteley & C.A. Bergstrom,

unpublished); therefore, we only present results for L^* . Furthermore, L^* is one of the primary sources of colour variation among natural stream substrates encountered by coastrange sculpin (Whiteley *et al.*, 2009).

At all photograph sessions, means for L^* were calculated for each family in both colour treatments. Because most fish were between 5 and 7 mm in length, they were too small to tag so we could not track individuals within families among photograph sessions. To investigate the ontogenetic trajectory of L^* axis colour expression in response to black and white rearing environment colours, we used a repeated measures ANOVA with family means repeated through time (age of the family among photograph sessions) as well as between rearing environment colours (each family is represented in two rearing environment colours).

To investigate the degree to which variation in colour along the L^* axis among families was due to genetic components or plasticity, we used fish from the subset of families that had survivors in both rearing environments at each age (day 78: eight families, day 97: six families, day 116: six families, day 249: four families). Full-sib families were assumed to represent distinct genotypes. With individuals from this subset of families, we performed a two-way mixed-model ANOVA with environment (E: rearing environment colour) as a fixed factor and genotype (G: family) as a random factor. We performed separate ANOVAs on the fish at each of the four different ages at which they were photographed. A significant rearing environment colour factor would indicate colour plasticity and a significant family factor would indicate genetic variation in mean baseline colour. A significant interaction term ($G \times E$) would indicate heritable variation in plasticity among families. We tested the significance of the random $G \times E$ interaction terms both with the mixed-model ANOVA's and with likelihood-ratio tests that test the contribution of the random interaction term by comparing the fit for models with and without it (Pinheiro & Bates, 2000; Lee & Peterson, 2002; Bolker *et al.*, 2009). The likelihood-ratio tests provided a more conservative approach to testing the significance of random interaction terms. For these, we used the *lmer* function in the R program package *lme4* (available at <http://www.R-project.org>) to perform a linear mixed-effects model with colour as a fixed factor (E), family as a random factor (G), and colour \times family ($G \times E$) as a random factor. We allowed correlation between the random slopes and intercepts. We did not use likelihood-ratio tests to compute P -values for the fixed colour factor or random family factor, following Pinheiro & Bates (2000).

A significant genotype factor in the ANOVA's mentioned earlier may indicate a heritable basis to colour, but it could also indicate random noise associated with container effects, as due to space constraints each family/container colour combination was only represented once. To investigate this, we estimated pairwise

correlations of L^* means between black and white environments among families, using the same subset of families represented in both container colours at each age. We calculated both parametric Pearson's correlations (r) as well as nonparametric Spearman rank correlations (ρ) due to the likelihood that our small number of families ($n = 4$ at 249 days old through $n = 8$ at 78 days old) would depart from a normal distribution. If there is a heritable basis to colour, we predicted that families that were relatively dark among white environment treatments would also be relatively dark among black environment treatments, which would result in positive correlations among containers.

Costs of colour plasticity

After the heritability experiment was concluded (249 days), we kept the juveniles for additional experiments that would assess some components of colour plasticity cost. This included five remaining families, four of which still had survivors in both rearing colours and one that had survivors only in the black containers.

Each fish ($N = 127$) was placed alone in an individually labelled, 500-mL, clear glass jar with a 1-mm mesh lid in one of 10 replicate water baths at ambient temperature (5 °C). The mesh lid allowed water exchange with the water bath. Water going into the water baths was flow-through filtered water from the same local creek used during the previous rearing period in the laboratory. Water baths were large, plastic cylindrical bins 0.5 m in diameter. The bottom of each water bath was covered in a round piece of plastic sheeting that was half black and half white, and the colour was clearly visible through the glass bottom of the individual fish containers that were set on the plastic sheets. We placed about 12 individual fish jars in each water bath, with families split up evenly among water baths to control for tank effects. Immediately prior to placing each fish in its labelled jar, it was measured for total body length and mass (average total length: 24.4 mm \pm 2.4 SD; average mass: 105.4 mg \pm 30.9 SD). Mass was

measured by gently dabbing off excess water with a paper towel and measuring to the nearest 0.1 mg on a fine balance. A random subset of 20 individuals were measured twice to calculate the average measurement error (% of trait size) of length and mass (0.76% for length and 1.67% for mass). Repeatabilities were also calculated (see Lessells & Boag, 1987) for both measures and were 0.96 for length and 0.97 for mass.

Fish were kept on either (i) a constant colour background, (ii) a background colour that was changed every 2 days (fast-change treatment), or (iii) a background colour that was changed every 2 months (slow-change treatment) (Fig. 1). Separation of the fast-change and slow-change treatments was done to see whether there were different costs associated with morphological colour change versus physiological colour change (Whiteley *et al.*, 2011). Morphological (slow) colour change occurs over weeks to months and involves the synthesis or apoptosis of melanophore cells, whereas physiological (fast) colour change occurs in the order of minutes and involves the dispersion of pigment molecules within the melanophores (Nery & Castrucci, 1997; Sugimoto, 2002). Fish were randomly assigned to one of these three treatments, and those to be kept on a constant colour were kept on the same colour on which they had been reared.

Fish chosen for the fast-change treatment were initially assigned randomly to one of the two substrate colours, and then changed to the alternate substrate colour every 2 days by sliding their jar to the other colour on the same piece of plastic sheeting. Fish chosen for the slow-change treatment were placed on the opposite of their earlier rearing colour to induce an initial colour change, and then switched every 2 months (initial colour substrate switch, once after 2 months, once after 4 months, then stopped at 6 months). All fish, regardless of treatment, had their position on the plastic sheeting reshuffled every 2 days to equalize handling. Fish were fed freshly hatched *Artemia* every other day and had the bottoms of their clear glass containers cleared of detritus so that it did not interfere with the visibility of the

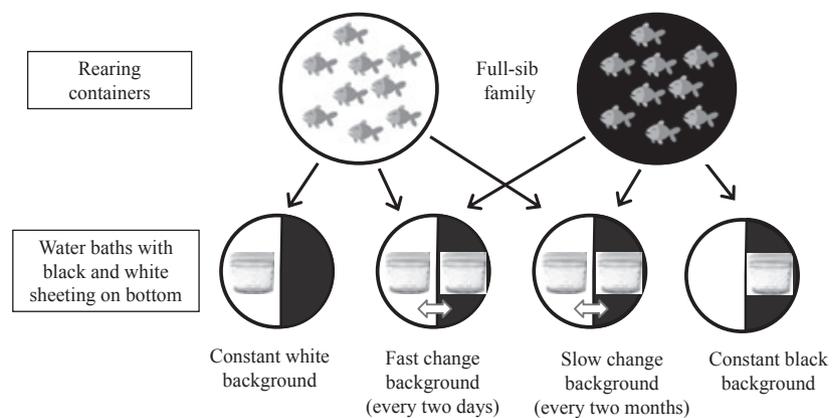


Fig. 1 Experimental design to test for cost of colour plasticity. Individual full-sibs reared in either white or black containers were placed in glass jars with mesh lids and placed in one of three treatments: constant background (same as colour reared on), fast change background, or slow change background. Each family was split evenly among treatments and water baths.

white or black plastic sheeting. Lights were kept on for 24 h a day.

At the end of the 6-month experiment, the total body length and body mass of each surviving fish was remeasured. Using Fulton's condition factor ($K = \text{body mass} \times \text{length}^{-3}$), we calculated an initial body condition index, a final body condition index and the difference between the two. We compared changes in body condition among plasticity treatments using a two-way ANOVA with body condition change as the dependent, plasticity treatment as a fixed factor and family as a random factor. Significant treatment effects would show that expressing plasticity in different environments (substrate colour) affects body condition. If costs to colour plasticity expression are present in coastrange sculpin, those fish on changing colour backgrounds will have reduced growth rates compared with those on constant backgrounds.

As an additional fitness proxy, we compared the total rates and frequencies of mortality among treatment levels throughout the 6-month experiment. Kaplan–Meier survival curves (Pollock *et al.*, 1989) were generated and differences in curves among treatment levels tested for statistical significance with log-rank X^2 tests. To compare total mortality frequencies at the end of the experiment, we ran a three-way log-linear analysis, using backwards elimination for model selection and included family (five families), mortality (lived or died) and treatment level (constant, fast or slow change) in the model.

All surviving individuals used to measure costs for the fast-change treatment also had their colour change plasticity quantified at the end the 6-month period to allow us to indirectly measure the cost of plasticity capacity in this subgroup (substrate colour changed every 2 days). All individuals were placed in a one-gallon tank in complete darkness for 10 min (see Whiteley *et al.*, 2011 for detailed protocol). They were then immediately placed on a white background surrounded by a white box and photographed every minute for 30 min. Colour was analysed digitally (Whiteley *et al.*, 2011) and used to determine the maximum range of colour expression each individual showed over the 30-min period. All fish lightened to some degree over this period. Magnitude of colour plasticity was calculated by subtracting the lowest lightness value from the highest value expressed for each fish during the 30-min period. We quantified colour change successfully in 23 fish from four families.

We analysed these data using family means with the multiple genotype approach to assess plasticity costs modified for use among individuals that are not genetically identical, such as full-sibs (DeWitt, 1998; DeWitt *et al.*, 1998; Relyea, 2002). We used a multiple regression with fitness (mean family change in body condition) as the dependent and trait plasticity (difference between starting and ending colour) and trait values (starting or ending colour) as independent

variables. Trait value was included due to the possibility that it could both affect fitness and be correlated with plasticity. This correlation can cause collinearity problems in the multiple genotype approach, biasing estimates of selection coefficients and making accurate estimates of plasticity costs difficult (Auld *et al.*, 2010, 2011). Therefore, we ran collinearity diagnostics in each of our multiple regressions and considered collinearity to be a problem when tolerance values were <0.1 .

In the case of collinearity problems between the magnitude of colour change and trait values, we calculated an additional metric of fast-change plasticity: T_{\max} . This was the amount of time each fish took to reach its maximum colour change. This metric was used as an independent measure of plasticity in a separate set of multiple regressions as described earlier. T_{\max} was log-transformed to achieve normality. If costs to the capacity for fast colour change exist, greater magnitude of colour change and faster T_{\max} will have a significant negative effect on fitness in the multiple regressions.

We also reran this analysis with individual fish instead of family means, using an ANCOVA model with family as a random factor, and colour plasticity and both trait values (starting or ending colour) as covariates. Because we only had four families with low sample sizes of survivors, this provided more statistical power while still accounting for among-family variance.

Except for the likelihood-ratio tests, which were run in R, all statistical analyses were run in PASW Statistics 17.0.2 (SPSS Inc., 2009, Chicago, IL, USA, www.spss.com).

Results

Heritability of colour and colour plasticity

Overall developmental changes in colour within families

We observed differences between black and white rearing environments as the families aged from 78 to 249 days old. Families reared in white containers showed little change in L^* values as they aged, but families reared in black containers showed a sharp reduction in L^* from 78 to 116 days old, after which L^* values levelled off (Fig. 2). In the repeated measures ANOVA, there was no overall significant main effect of age on L^* values ($F = 1.31$, $P = 0.35$), but both environment colour and the interaction between age and environment colour were significant ($F = 90.79$, $P = 0.01$; $F = 5.87$, $P = 0.03$, respectively).

Genotype \times Environment interaction

Our data revealed strong colour plasticity in captive-reared coastrange sculpin during post-settlement development. At all four ages and for almost every full-sib family, sculpin reared in the white containers exhibited lighter

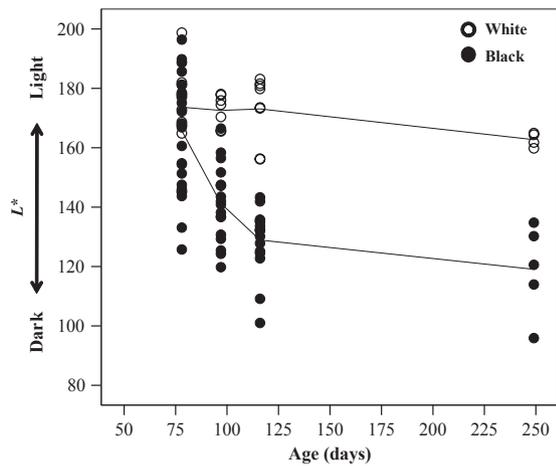


Fig. 2 Developmental changes in colour in captive-reared coastrange sculpin. Data points are family averages of fish colouration measured as L^* (y -axis) across time from 78 to 249 days old (x -axis), and between white and black rearing containers. Interpolation lines show means for each age/container colour combination.

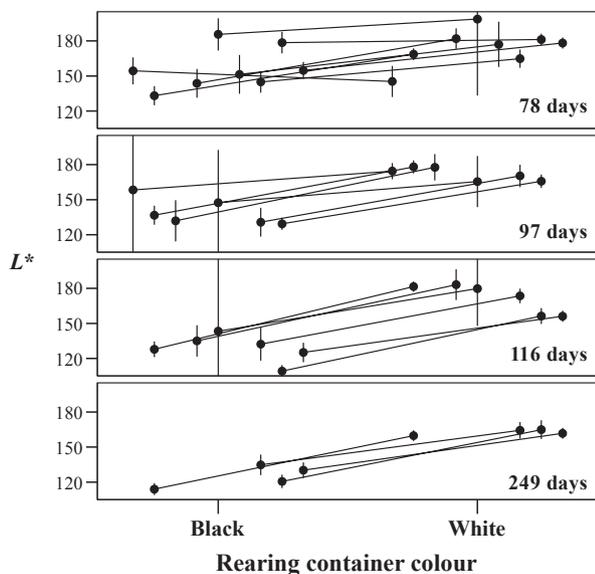


Fig. 3 Genotype (family) by environment (rearing container colour) patterns of colour development (L^*) in captive reared coastrange sculpin from 78 to 249 days old. Only those families that had split clutches present in both white and black rearing containers at a given age were included. Data points and error bars are family means \pm 1 SE.

colour than their full-siblings reared in black containers (Fig. 3), and the overall effect of substrate colour was statistically significant at all four ages (Table 1). There was significant heritability of plasticity ($G \times E$ interaction terms) at some ages. Heritability of plasticity was statistically significant at day 78 and day 249 and approached sig-

Table 1 Mixed-model two-way ANOVA's with L^* means as the dependent variable, family (G = genotype) as a random effect, rearing environment colour (E = environment) as a fixed effect and interaction terms ($G \times E$) as random effects. Significant terms are in bold print. Likelihood-ratio test results (χ^2 and P) also given for interaction terms.

Age	Source: L^* values	d.f.	F	P	χ^2	P
249	Family (G)	3	1.57	0.36		
	Colour (E)	1	82.40	<0.01		
	Family \times Colour ($G \times E$)	3	3.74	0.01	7.07	0.029
116	Family (G)	5	5.52	0.04		
	Colour (E)	1	93.18	<0.001		
	Family \times Colour ($G \times E$)	5	3.03	0.01	5.34	0.069
97	Family (G)	5	1.85	0.26		
	Colour (E)	1	62.53	<0.001		
	Family \times Colour ($G \times E$)	5	1.64	0.15	0.01	0.995
79	Family (G)	5	2.05	0.29		
	Colour (E)	1	13.16	0.01		
	Family \times Colour ($G \times E$)	7	5.21	<0.001	16.39	<0.001

nificance at day 116 (Table 1). These significant $G \times E$ interactions were evident graphically in crossing reaction norms of full-sib family groups (Fig. 3). Furthermore, differences between environments among families became more congruent with age (lines became more parallel; Fig. 3), despite the interactions remaining significant.

Correlations in sculpin colour between black and white rearing environments

Although none of the correlations in coastrange sculpin colour between split clutches reared in the black and white containers were statistically significant (Table 2), all of them were positive (four of four) regardless of age (78, 97, 116 and 249 days old; Fig. 4). Slopes became more positive as the fish aged. Positive correlations between rearing environment colours indicates that families that are relatively dark among white treatments are also relatively dark among black treatments. The lack of significance in individual correlations may be due to the small sample sizes (number of families) that survived in both rearing environments.

Table 2 Correlation between colour of captive-reared coastrange sculpin in white and black environments for the L^* colour axis across time from 78 to 249 days old. Both parametric Pearson's r and nonparametric Spearman's ρ given for each.

Age	n	Pearson's r	P	Spearman's ρ	P
249	4	0.50	0.50	0.40	0.60
116	6	0.76	0.08	0.66	0.16
97	6	0.15	0.77	0.09	0.87
78	8	0.23	0.59	0.21	0.46

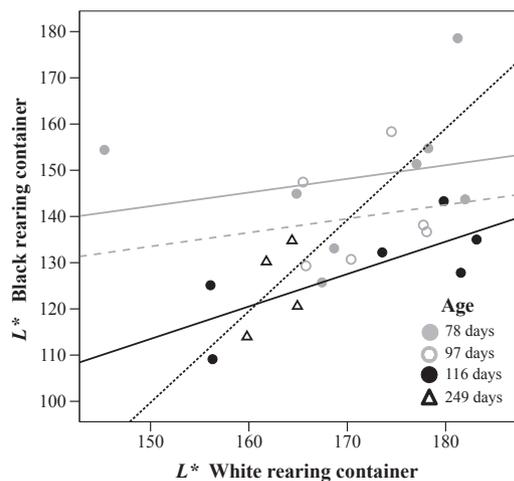


Fig. 4 Correlation of full-sibling fish colouration measured as L^* between those reared in white (x -axis) and black (y -axis) containers across time from 78 to 249 days old. Each data point represents mean colour of a single clutch split and reared in either black or white containers. Lines are best fit linear regressions for each age: 78 days old (solid grey line), 97 days old (broken grey), 116 days old (solid black), 249 days old (broken black).

Cost of colour plasticity

Comparisons among treatment levels

Of the 127 fish used in the experiment, 79 of them (62%) survived for the entire 6-month experiment. Of those 79, 14 increased body condition and the remaining 65 lost body condition. However, we found no significant changes in body condition among the three treatments ($F = 1.76$; $P = 0.208$) and slight but nonsignificant differences among families ($F = 2.92$; $P = 0.091$).

However, there were differences in the rate of mortality among treatment groups. After 20 days of the cost experiment, there was a sharp reduction in survival in the fast and slow-change treatments, but a relatively minor reduction in survival in the constant control group (Fig. 5). After 40 days, the slow-change treatment continued to decline in survival, whereas the fast-change and constant control group levelled off. Mortality rates were not recorded again until the end of the 6-month period. Kaplan–Meier survival curve analyses indicated overall differences were not statistically significant (Log-Rank $\chi^2 = 3.88$; d.f. = 2; $P = 0.144$). However, pairwise curve comparisons between the constant control and slow-change group closely approached significance (Log-Rank $\chi^2 = 3.70$; d.f. = 1; $P = 0.054$), whereas that between the control and fast-change group was nonsignificant (Log-Rank $\chi^2 = 0.30$; d.f. = 1; $P = 0.585$).

Consistent with this, total endpoint mortality frequencies differed among treatment levels at the end of the 6-month period (Fig. 5). The constant colour treatment had the highest percentage of survivors (75.0%), fol-

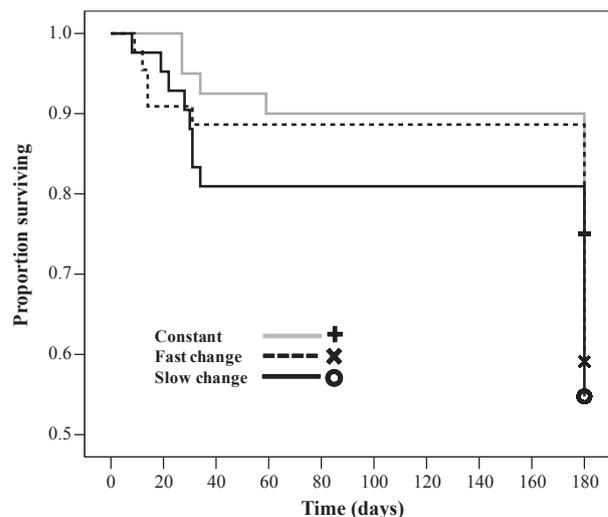


Fig. 5 Kaplan–Meier survival curves over 6 months for fish in three different plasticity treatments. Symbols at 180 days mark total mortality frequencies at the end of the experiment.

Table 3 Three-way log-likelihood analysis of frequency of mortality among plasticity cost experimental treatments and families.

	Likelihood ratio (G)	d.f.	P
Treatment \times Mortality	3.35	2	0.187
Mortality \times Family	19.69	4	0.001
Treatment \times Family	3.34	8	0.911
Treatment \times Mortality \times Family	7.41	8	0.493

lowed by the fast-change treatment (59.1%) and the slow-change treatment (54.8%). Total mortality rates did not differ significantly among all three treatment levels (Treatment \times Mortality term; Table 3). However, pairwise comparisons between the constant control and slow-change group approached significance ($G = 3.08$; d.f. = 1; $P = 0.080$), whereas between the control and fast-change treatment did not ($G = 1.89$; d.f. = 1; $P = 0.170$). Total mortality rates differed significantly among families, but the mortality rate among treatment levels was consistent among families, as evident by the nonsignificant three-way interaction term (Table 3).

Fast-change results

The multiple regression models testing for costs of fast-change ability (measured as the magnitude of colour change) were nonsignificant when using either starting colour or ending colour for trait value (both models: $F < 27.00$, $P > 0.14$). Tolerance was low (both < 0.05), suggesting that collinearity between starting trait colour and colour plasticity was present. Resulting partial r -values for plasticity, starting colour and ending colour were all nonsignificant (all $P > 0.346$).

The multiple regression models testing for costs of fast-change ability (measured as T_{\max}) were also non-significant when using either starting colour or ending colour for trait value (both models: $F < 32.00$, $P > 0.12$). However, tolerance was high (both > 0.69), suggesting that collinearity between starting trait colour and colour plasticity was absent. Regardless, resulting partial r -values for plasticity, starting colour and ending colour were still all nonsignificant (all $P > 0.10$).

We analysed this with an ANCOVA model, using family as a random factor and magnitude of colour plasticity as well as both trait values (starting and ending colour) as covariates. We first ran the model to investigate significant interaction terms involving family. When this was run for starting colour as the trait value, none of the interaction terms involving family were significant (all $P > 0.75$) so we reran the model excluding these interaction terms. None of the remaining main effects had a significant effect on body condition changes (plasticity $F = 0.183$; $P = 0.675$; starting colour or ending colour $F < 0.001$; $P > 0.987$; family $F = 1.863$; $P = 0.179$, plasticity \times starting colour $F = 0.734$; $P = 0.405$).

Discussion

Colour plasticity in coastrange sculpin appears to have a genetic component and to come at a slight cost. This, combined with the highly variable cryptic coloration in recently colonized populations in south-east Alaska, their ability to match markedly different substrate colours of streams (Whiteley *et al.*, 2009), and the prevention of visual detection by predators that crypsis provides (Whiteley *et al.*, 2011) lend support to the hypothesis that colour plasticity in these wild populations can respond to selection.

The pattern and timing of the ontogeny of colour expression in the laboratory is consistent with the ecology of coastrange sculpin. Full-sibs in white and black environments did not differ in coloration shortly after metamorphosing into their benthic juvenile form, but colour divergence increased as the fish aged (Fig. 2). The lack of divergence in colour for the youngest full-sibs in different colour treatments is consistent with the fact that this species is planktonic in the ocean for the larval life stage. In the wild, the planktonic period lasts for approximately 30 days, after which larvae settle to the bottom and seek freshwater streams (Mason & Machidori, 1976). There would be no spectral stimulus from any benthic substrate before this point, so plastic capacity may not be initiated until after settlement. In our experiment, fish did not settle to the bottom of rearing containers until approximately 55 days of age. This delay could have been due to a large number of factors that differ between the wild and our controlled settings, including temperature and amount and quality of food. The first time point in our analysis of juvenile colour corresponded to approximately 20 days follow-

ing settlement. Larvae were transparent upon hatching and at the first photographic time point had a tan/yellowish coloration, with few dark spots indicative of melanophores. Fish in the black substrate treatment produced increasing numbers of melanophores, corresponding to the observed darkening between 78 and 249 days post-hatch (Fig. 2). Fish in the white substrate environment generally produced few melanophores and maintained light coloration (tan/yellowish) throughout the experiment (Fig. 2).

Heritability of colour and colour plasticity

The full-sib analysis suggests weak heritability of both mean baseline coloration of coastrange sculpin as well as colour plasticity at the post-settled juvenile life stage (Figs 3 and 4). The likely proximate mechanism for this plastic response is differential synthesis or destruction of melanophores combined with synthesis or destruction of melanosomes within melanophores (Sugimoto, 2002). Comparisons among families within black or white colour treatments provided evidence for a genetic component to colour variation, and variance among families within colour treatments at least at one time point (116 days old) despite the relatively small number of families examined. Although we had a decreasing number of families as fish got older, the results from the black treatment provide a compelling argument for a genetic basis to colour variation. In fact, if we examine the larger set of families from the black rearing environment alone we had 24 families surviving at 78 days post-hatch, 18 families at 97 days, 16 families at 116 days and five families at 249 days. There were significant differences in mean L^* among families in black containers at each of these four ages (all $P < 0.001$). Increased colour variance both among and within families in the black treatment (Fig. 3) is consistent with the hypothesis that an increase in melanophore number (and a likely increase in melanosomal content within those melanophores) causes greater inter-individual variation. These results are consistent with those from another study that showed increased variance in colour plasticity following 2 month's conditioning on black substrates relative to the fish that were preconditioned on white substrates (Whiteley *et al.*, 2011). Therefore, reduced melanophore production and melanosomal content is likely responsible for the lack of variation among individuals and families that received the white rearing treatment in the current study.

The consistent pattern of positive correlations between clutches split between environments suggests a genetic basis to colour. Although correlations were not statistically significant, the consistent pattern is conservative because heterogeneity due to tank effects would only introduce noise into this correlation. However, we were unable to calculate accurate estimates of heritability due to our use of full-sib clutches (Falconer

& Mackay, 1996). Furthermore, we cannot determine the extent to which maternal effects may have influenced our results, and there is a very slight possibility that families in our analysis shared parents, which could have reduced variance among clutches and therefore reduced the signal of a heritable basis to colour. However, females deposit discrete clusters of all of their eggs in the nests of freshwater sculpin males, and each cluster differs in colour according to developmental stage (Fiumera *et al.*, 2002). We sampled one clearly distinct egg cluster from each male's nest, which makes it highly likely that each family had unique parents.

We detected significant evidence of heritable plasticity at all time periods except at 97 and 116 days (which approached significance). Reaction norm lines intersected more for younger fish and became more parallel as fish aged. Thus, our estimates of genetic variation for plasticity changed depending on the ontogenetic stage of this population, and this is likely because of decreased variance for colour among fish as they age. We hypothesize that expression of L^* is more variable early in ontogeny due to asynchronous timing in the development of melanophores. As the development of these pigment molecules proceeds, variation among individuals and among families may become reduced, especially in families reared on white backgrounds where the extent of melanophore development is minimized.

Heritable genetic variation for plasticity indicates that this trait could evolve by natural selection (Via & Lande, 1985). The study population for this experiment resides in a stream that is approximately 1000 years old and has a well established community of algae covering the rocks and pebbles. Similar studies of fish collected from streams that differ in their substrate spectral qualities will be needed to test hypotheses related to the adaptive significance of colour plasticity and whether it covaries with stream environmental heterogeneity. Greater colour plasticity in fish from streams with lots of spectral variation than from streams that are relatively homogeneous would support the hypothesis that natural selection via visual predators could impose positive directional selection for colour plasticity following colonization. However, despite the presence of heritable variation for plasticity, it would be unlikely to be selected *against* even in homogeneous streams unless a cost was present. Variability in plasticity among and within populations of coastrange sculpin (Whiteley *et al.*, 2009, 2011) is consistent with our data showing heritability and costs of plasticity, helping to demonstrate that colour plasticity in this species may have the capacity to respond to both positive and negative selection.

Costs of colour plasticity

We tested the cost of colour plasticity in two ways: (i) by comparing mortality rates, frequencies and changes in body condition between individuals on a constant col-

oured background to those on slow- and fast-changing coloured backgrounds, and (ii) by comparing changes in body condition among individuals that differ in the magnitude of their fast-change plasticity but that were all on the fast-changing coloured background. The first test combined production costs (cost of *expressing* plasticity) with maintenance costs (energetic maintenance of *ability* to generate environmentally induced phenotypes in response to a stimulus (Relyea, 2002)). The second test, however, assessed primarily maintenance costs as all fish were induced to express plastic responses to a common stimulus, but their ability to do so varied (the magnitude and time taken to reach their maximum fast colour change ability). Maintenance costs are environment independent and can be incurred in the absence of environmental stimuli, whereas production costs are environment dependent and will only be incurred when plasticity is expressed in response to a stimulus (Auld *et al.*, 2010). There are conflicting reports as to which type of cost will affect the evolution of plasticity to the greatest degree, and few studies have empirically addressed both (but see Scheiner & Berrigan, 1998).

We found a slight increase in mortality rate and endpoint mortality frequency over 6 months in fish in the slow-change treatment, but there was no signal of a cost in our fast-change plasticity test (primarily the cost of plastic ability, or maintenance costs). This indicates a production cost to the development or apoptosis of melanophore cells that cause slow colour change over a period of weeks to months, as opposed to the dispersion of pigment within those melanophores that cause fast colour change.

Our finding of a weak signal of plasticity cost is consistent with Van Buskirk & Steiner's (2009) review that demonstrated that plasticity costs are likely to be common but small in natural populations. Assuming that costs of colour change plasticity occur but are small in this species, this may allow for plasticity to persist in populations for some time even in relatively homogeneous habitats. Positive selection for plasticity by visual predators on heterogeneous substrates would lead to the persistence of this trait, which is conceivable given the indication of heritable variation for its expression. The variation in plasticity among populations could then be the result of variation in strength of positive and weak negative selection among glacial stream habitats, consistent with our demonstration of costs and genetic components to colour plasticity early in the juvenile life stage.

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