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BACKGROUND MATCHING AND COLOR-CHANGE PLASTICITY IN COLONIZING FRESHWATER SCULPIN POPULATIONS FOLLOWING RAPID DEGLACIATION

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Anthropogenic-induced change is forcing organisms to shift their distributions and colonize novel habitats at an increasing rate, which leads to complex interactions among evolutionary processes. Coastrange sculpin (*Cottus aleuticus*) have colonized recently deglaciated streams of Glacier Bay in Alaska within the last 220 years. We examined divergence among populations in background matching coloration and tested the hypothesis that observed variation is due to morphological color plasticity. To examine how color-change plasticity has interacted with other evolutionary processes, we also determined the influence of colonization on neutral genetic diversity. We observed clinal variation in substrate-matching fish color along the chronological continuum of streams. Microsatellites provided little evidence of genetic subdivision among sculpin populations. Fish color was significantly correlated to substrate color, but was not correlated to neutral population genetic structure. Furthermore, a laboratory experiment revealed that morphological color plasticity could explain much, but not all, of the observed fish color divergence. Our study demonstrates that sculpin in Glacier Bay have colonized and adapted to recently deglaciated habitat and suggests that color change plasticity has aided in this process. This research, therefore, highlights the important role phenotypic plasticity may play in the adaptation of species to rapid climate change.

KEY WORDS: **Adaptation, climate change, colonization, gene flow, glacial recession, phenotypic plasticity.**

Anthropogenic change is currently causing a planet-wide selection experiment (Gienapp et al. 2008). Under current predictions of climate change (IPCC 2007), organisms will be forced to colonize new habitats at an increasing rate, either through population establishment in newly created habitats within existing ranges or expansion to areas formerly outside of ranges (Parmesan 2006).

To predict the consequences of colonization, it is necessary to consider the interaction of drift, selection, gene flow, and mutation (Rosenblum et al. 2007). Genetic drift following population bottlenecks might lead to a large role played by random processes during colonization (Lee 2002). Colonization may also promote rapid adaptive evolution (Reznick and Ghalambor 2001; Ghalambor et al. 2007). Directional selection caused by new ecological conditions, such as new biophysical environmental factors or new competitive/predation regimes, combined with an

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opportunity for population growth, often leads to adaptive responses following colonization (Reznick and Ghalambor 2001). However, gene flow, if strong enough, may prevent rapid adaptive evolution (Lenormand 2002; Nosil and Crespi 2004). Over short-time scales, mutation is likely to play a less important role.

Phenotypic plasticity may be a particularly important mechanism by which organisms quickly adjust to the new environmental conditions encountered following colonization (Agrawal 2001; West-Eberhard 2003; Ghalambor et al. 2007). The idea that phenotypic plasticity can lead to evolutionary change has received increased attention recently (West-Eberhard 2003; Caruso et al. 2006; Brookes and Rochette 2007; Ghalambor et al. 2007; Hendry et al. 2008). Situations where "phenotype acts as the leader," rather than genes leading to phenotypic change, may be more common in nature than previously appreciated (West-Eberhard 2003). Phenotypic plasticity that occurs in the same direction of increased fitness may be a key step in the process that promotes adaptation by organisms to new conditions during colonization (Ghalambor et al. 2007). What begins as an environmentally induced response can become genetically accommodated, that is, gain a genetic basis (West-Eberhard 2003; Crispo 2007; Ghalambor et al. 2007), and then be subject to interactions between selection, drift, and gene flow.

Regions where active glacial recession is taking place provide natural experimental conditions to study the interaction of evolutionary processes during colonization. As ice sheets recede, it is possible to compare how evolutionary processes are occurring in replicate populations that have known chronologies. One of the most important locales for the study of successional dynamics in a recently deglaciated ecosystem is Glacier Bay National Park (hereafter Glacier Bay) in southeastern Alaska (Chapin et al. 1994; Fastie 1995; Milner et al. 2000; Engstrom and Fritz 2006, Milner et al. 2007, 2008). Glacier Bay is an approximately 100-km fjord that was completely covered with ice 220 years ago. Glacial recession has created a chronological continuum of streams with known ages (Fig. 1; Milner et al. 2000). Streams closest to the edge of receding ice sheets are a few decades old whereas those close to the mouth of Glacier Bay are about 200 years old (Table 1). Biophysical aspects and successional stages of newly formed streams are well described (Flory and Milner 1999; Engstrom et al. 2000; Flory and Milner 2000; Milner et al. 2001; Robertson and Milner 2006; Milner et al. 2007, 2008). Younger streams are more turbid, colder, more variable in flow, and offer less habitat for macroinvertebrates and fishes due to less woody debris (Milner et al. 2000; Robertson and Milner 2006). Older streams are less turbid, warmer, more constant in flow, and have more habitat for aquatic organisms due to a more heavily forested riparian zone and contributions of large woody debris (Milner et al. 2001). For the purposes of this study, another important environmental difference among streams involves their substrate

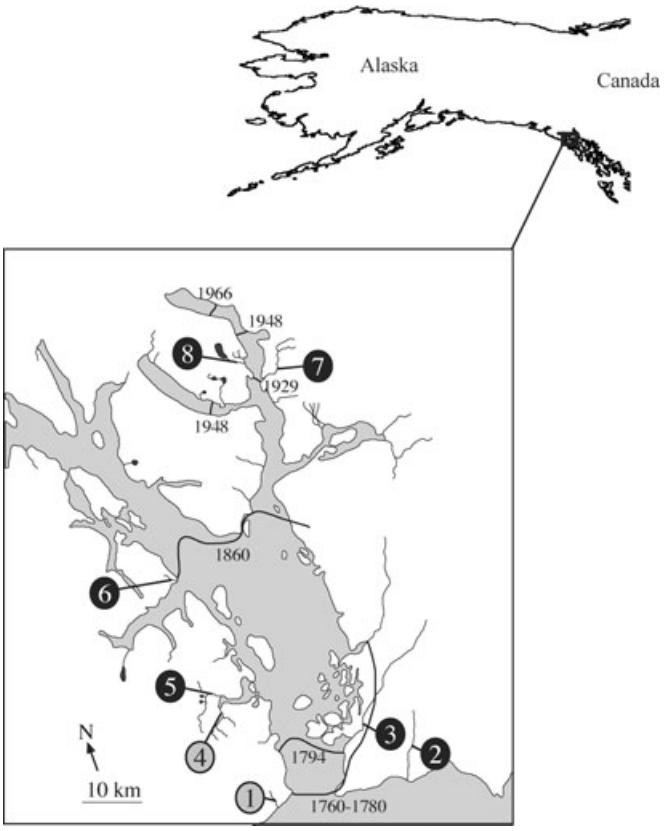

Figure 1. Map of sample sites in Glacier Bay, Alaska. Site numbers correspond to Table 1 and increase towards upper portions of the bay. The six sites used for color analyses are shown with black circles. Locations of maximum ice advance are shown for various dates by black lines. Adapted from Milner et al. (2000).

color. Younger, less productive streams have very low algae cover (Robertson and Milner 2006) and are dominated by white, gray, and black colored rocks in continuous riffle/run habitat. Older streams are more productive and have greater algae and macrophyte cover (Robertson and Milner 2006), which produce red or green coloration in pool/riffle sequences.

Within the rapidly changing landscape of Glacier Bay, the coastrange sculpin (*Cottus aleuticus*) is well suited for examining the evolutionary consequences of colonization. This small (adult size is 50–100 mm), bottom-dwelling fish quickly colonizes streams following deglaciation, most likely through oceanic dispersal of larvae (Mason and Machidori 1976). Coastrange sculpin exhibit highly cryptic pigmentation patterning that matches stream substrate color. Thus, colonizing sculpin may be rapidly adapting to substrate color differences in new streams. Furthermore, preliminary observations indicate that coloration in this species is phenotypically plastic, where individuals can change color both rapidly (on the order of minutes) and gradually (on the order of months). This color changeability appears to be related to increased crypsis rather than for communication

Table 1. Sample information for coastrange sculpin collected from eight streams in Glacier Bay National Park, Alaska. Sample number corresponds to Figure 1. Stream age is from Milner et al. (2000) and adjusted to 2008. *N* corresponds to sample size, H_S to mean expected heterozygosity, *AR* to mean allelic richness (mean number of alleles/locus scaled to the smallest sample size; *N* = 25). Asterisks indicate sites for which color data were not collected.

Sample no.	Stream name	Stream age (years)	*N*	H_S	*AR*
1*	Carrolus R.	1385	25	0.76	9.2
2	Salmon R.	232	40	0.76	9.7
3	Bartlett R.	208	33	0.80	9.9
4*	Berg Bay South Cr.	175	36	0.80	8.8
5	Berg Bay North Cr.	175	39	0.77	8.8
6	Oystercatcher Cr.	145	34	0.78	9.0
7	Nunatak Cr.	70	31	0.77	9.7
8	Wolf Point Cr.	59	36	0.76	9.4

purposes (e.g., Stuart-Fox and Moussalli 2008). As in other examples of color change (Kats and Vandragt 1986; Heinen 1994; Endler 1995; Arigoni et al. 2002; Garcia and Sih 2003), crypsis likely allows this species to evade predation by fish, birds, and mammals.

Coloration and adaptive pigment patterning are classic traits for studies of adaptation and the generation and maintenance of phenotypic diversity (Sheppard 1951; Kettlewell 1955; Camin and Ehrlich 1958). Recent studies of coat color in mice have revealed connections between genes, gene expression, and phenotype in an adaptive evolutionary context (Bennett and Lamoreux 2003; Nachman et al. 2003; Hoekstra 2006; Steiner et al. 2007). However, those studies were conducted with animals that have fixed color patterning. Many fishes, reptiles, and invertebrates can change pigmentation patterns within their adult lifespan (Maia Nery and de Lauro Castrucci 1997; Sugimoto 2002). Rapid color change that occurs on the order of minutes is termed physiological color change and is mediated by signals from the nervous or endocrine systems that determine the extent of dispersion of pigment molecules within chromatophores, or pigmentation cells (Maia Nery and de Lauro Castrucci 1997; Sugimoto 2002). If pigment molecules are dispersed, the organism appears darker than if these molecules are aggregated at the center of the chromatophore. Gradual color change that occurs on the order of weeks or months is termed morphological color change and occurs via persistence of the same signaling mechanism but takes weeks or months for synthesis or apoptosis of chromatophores (Sugimoto 2002).

In this article, we examined interactions among evolutionary processes during colonization of several recently deglaciated rivers in Glacier Bay by the coastrange sculpin. We examined neutral genetic subdivision at eight microsatellite loci. We also examined substrate matching fish coloration across the chronological gradient of streams. In addition, we kept fish from two natural populations in a common laboratory environment to test the hypothesis that observed color variation is due to morphological color plasticity.

Methods

SAMPLE COLLECTION

Approximately 25 to 40 individuals were collected from each of eight sites in Glacier Bay (Table 1, Fig. 1). Kick nets were used to capture fish throughout the lower reaches of the streams. Fin tissue was collected and stored in 95% ethanol until DNA extraction. Individuals from six of the eight sites were photographed in the field for color analysis (Table 1). Forty-five individuals were collected from Oystercatcher Creek (site 6; stream age: 145 years) and 52 from Wolf Point Creek (site 8; stream age: 59 years) and transported to Juneau, Alaska, for laboratory experiments.

SUBSTRATE COLOR AND FISH BACKGROUND MATCHING

Coastrange sculpin exhibit a highly cryptic saddle pattern typical of small benthic fishes that live in uneven rocky substrates (Armbruster and Page 1996). This patterning appears to be an example of disruptive coloration, where dark saddles are the color of shadows and gaps between rocks and lighter colored regions between the saddles are the color of the rocks. This color pattern is likely to be adaptive, given its wide taxonomic occurrence and its phenotypic convergence in ecologically similar yet taxonomically distant fishes (Armbruster and Page 1996).

We analyzed the color of fish and substrates from Salmon, Bartlett, Berg Bay North, Oystercatcher, Nunatak, and Wolf Point Creeks, which span in age from 59 to 1385 years but vary geographically by approximately 100 kilometers or less (Table 1, Fig. 1). We used digital photography to quantify coloration. Digital photography is now a standard method for quantifying animal coloration (Stevens et al. 2007). For substrate analyses, we captured frames from underwater video footage. We used a laminated black and white card placed against the substrate while videotaping for standardization. The black and white card was itself standardized to an X-Rite mini colorchecker card (X-Rite Inc., Grand Rapids, MI) with squares of known color values as a standard. We videotaped both standards together immediately prior to collecting the underwater footage. This allowed us to standardize underwater still frames to the other digital images used for color quantification of fish. For fish color analysis, we photographed each individual as soon as possible upon capture in the field. Fish were held on their natural substrate prior to photographs to reduce physiological color change. The same X-Rite mini colorchecker appeared in all images. We used the same plastic container with

the same small rocks and filled it with water for the background of all digital images, so that this background could be quantified and standardized across photographs. In other experiments, we confirmed that rapid placement on a rock substrate followed by an immediate photograph allowed us to capture natural fish color prior to any physiological color change (data not shown). Digital images were taken with a Canon Digital Rebel XT (Canon, Inc., Lake Success, NY) in RAW format, with manual white balance and the same camera settings for each image. Lighting was controlled in the field by photographing under an umbrella with two opposing external flashes, each at a 45° angle to the surface of the water to eliminate glare. A polarizing filter was also used to eliminate glare. Following transportation to the laboratory, fish were kept in 35-gallon aquaria with natural rock substrate from a nearby stream. All fish were fed the same diet of bloodworms (Simuliidae) while in captivity. Photographs were repeated after five months on the common laboratory substrate. The same natural rock background used in the field was used for this photo session.

Digital images were quantified with Photoshop CS3 (Adobe Systems Inc., San Jose, CA). The "curves" correction was used to standardize all photographs relative to the black and white standards on the X-Rite colorchecker card. For substrate analyses, 10 still frames per substrate were analyzed. For each fish, histograms were produced for the entire dorsal surface. We used the CIE 1976 $L^*a^*b^*$ color-space model, which consists of three orthogonal axes: the lightness axis (L^*) stands vertically and a^* and b^* axes lie in the same plane and provide color coordinates for the measured color in a two-dimensional color circle (Stegen et al. 2004). Positive a^* values indicate red coloration, negative a^* values indicate green. Positive b^* values indicate yellow, negative b^* values indicated blue. An advantage of using the $L^*a^*b^*$ color space is that Euclidean distances (*ED*) can be calculated because the three axes are orthogonal (CIE 1986).

For statistical analyses of stream substrate and fish color along the chronological continuum of streams in Glacier Bay, we first analyzed L^*, a^*, and b^* color values, followed by principal components analysis (PCA). For stream substrate color, we used a multivariate analysis of variance (MANOVA) with L^*, a^*, and b^* values as dependent variables and stream as the independent factor. For fish color, we used multivariate analysis of covariance (MANCOVA) with L^*, a^*, and b^* values as dependent variables, stream (population) as the independent factor, and, to test for an effect of size on fish color, fish length as a covariate. We used univariate correlation analyses to test the relationship between stream substrate color, fish color, and stream age. Correlations were performed separately on mean population values of Darkness (D^*, calculated as 100 – lightness for heuristic purposes), the a^* and b^* color axes, and scores on principal component axis 1 (PC1). Darkness was used instead of lightness in this case for simplicity of interpretation. PC scores were generated from a PCA of dorsal surface D^*, a^*, and b^* values and was based on the correlation matrix. We controlled the false discovery rate (FDR) for multiple tests according to Benjamini and Hochberg (1995). We also performed a univariate analysis of variance (ANOVA) with PCA factor scores as the dependent variable and stream as the independent factor. Following a significant result, we used Tukey's HSD tests to determine which groups occupied different regions of color space.

GENETIC ANALYSES

DNA was extracted from each fin clip with a standard salt precipitation procedure. Target sequences were PCR amplified for eight microsatellite loci: *COTT100*, *COTT255*, *COTT635*, *COTT687*, *LCE29*, *COTT127*, *COTT582*, and *COTTES19* (Nolte et al. 2005) in two multiplexes. We used Qiagen multiplex buffer (Qiagen Inc., Valencia, CA) and the manufacturer recommended thermal-cycler profile for microsatellite amplification. An ABI 3130xl capillary sequencer was used to size PCR fragments. GeneMapper (Applied Biosystems, Inc., Foster City, CA) was used to score individual genotypes based on the LIZ 600 size standard run with each individual. Genotypic data are available from the authors upon request.

We used GENEPOP version 3.4 (Raymond and Rousset 1995) and FSTAT version 2.9.3.2 (Goudet 2001) to estimate population genetic parameters. These parameters included: allele frequencies, deviations from Hardy–Weinberg expectations, composite gametic disequilibrium, observed (H_O) and expected (H_E) heterozygosity per locus and population, mean within-population expected heterozygosity (H_S), mean allelic richness per population (*AR*; mean number of alleles scaled to the smallest sample size), exact tests for genic differentiation, *F*-statistics, and pairwise F_{ST} values. We used Micro-Checker to test for the presence of null alleles (Van Oosterhout et al. 2004). We used θ (Weir and Cockerham 1984) for estimates of F_{ST}. Confidence intervals (95%) for multilocus F_{ST} estimates were generated by bootstrap sampling over loci (Goudet et al. 1996). Significance of pairwise F_{ST} values was determined with 1000 permutations using GENETIX version 4.0 (Belkhir 1999). We used F_{ST} instead of R_{ST} because F_{ST} estimates are more conservative when relatively few microsatellite loci are used (<20) and populations have diverged recently (Gaggiotti et al. 1999). For multiple tests of Hardy–Weinberg expectations, gametic disequilibrium, and pairwise F_{ST}, we again controlled the FDR.

We tested for a correlation between stream age and within-population genetic diversity (H_S and *AR*). Bottlenecks associated with colonization would be expected to leave a signature of reduced within-population genetic variation. We tested the prediction that newer streams (Table 1) would have reduced within-population genetic diversity with linear regression where stream

age was the independent variable and either H_S or AR was the dependent variable. Each population was also tested for heterozygosity excess compared to that expected at mutation-drift equilibrium. We used the program Bottleneck (Cornuet and Luikart 1996; Piry et al. 1999) with the two-phase mutation model of microsatellite evolution (DiRienzo et al. 1994) with 10% of the infinite allele model and 90% of the stepwise mutation model (White and Searle 2007). A one-tailed Wilcoxon test for heterozygosity excess was used as the test for a bottleneck.

Genetic data were further analyzed with Structure version 2.1 (Pritchard et al. 2000; Falush et al. 2003). No prior population information was used for each structure analysis. We used 100,000 replicates and 20,000 burn-in cycles under an admixture model where we applied the "infer α" option (α is the Dirichlet parameter for degree of admixture) with a separate α for each population under the F model. We used the correlated allele frequencies model under the $\lambda = 1$ option, where λ parameterizes the allele frequency prior with a Dirichlet distribution of allele frequencies. We allowed F to assume a different value for each population and allowed for different rates of drift among populations. We ran five replicates runs for each of $K = 1$ to 8.

CORRELATION BETWEEN SUBSTRATE COLOR, FISH COLOR, AND GENETIC DIVERGENCE

To test whether variation in fish color was better explained by substrate color or by genetic divergence, we used matrix correspondence tests (MCTs), which test the association among distance metrics for a variety of data types (Smouse et al. 1986; Thorpe et al. 1996; Rosenblum 2006). We constructed matrices based on pairwise population comparisons of fish color (phenotype matrix), substrate color (habitat matrix), or genetic differentiation (genetic matrix). For fish and substrate color, we calculated pairwise ED values for fish and substrates based on mean L^*, a^*, and b^* scores for each population according to the following formula: $ED = \sqrt{((L_1 - L_2)^2 + (a_1 - a_2)^2 + (b_1 - b_2)^2)}$, where subscripts correspond to one member of each pair of sites. Alternatively, we used the absolute values of linear distances between population means along PC1, following Rosenblum (2006). The genetic distance matrix consisted of pairwise estimates of F_{ST}. We performed both pairwise and partial MCTs. Pairwise MCTs tested the correspondence between pairwise combinations of phenotypic, habitat, and genetic matrices. Partial MCTs were used to test the correspondence between the phenotype matrix and the habitat matrix while controlling for genetic subdivision. This method uses partial regression to test the correlation between two matrices while controlling for the effects of the third (Rosenblum 2006). We used 1,000 permutations implemented in XLSTAT (Addinsoft USA, New York, NY) to test for significance of all MCTs.

COLOR CHANGE EXPERIMENT

For the color change experiment, we used fish transported to the lab from Wolf Point and Oystercatcher Creeks, two creeks that differ in age and substrate color. We examined color determined from photographs at the time of capture (t_0) and after five (t_5) months in the common laboratory environment with MANOVA and discriminant function analysis (DFA). Our goal was to test (1) whether fish color approached the color of the common laboratory substrate and (2) if the color of fish from each of the sites became more similar over time. Tests were performed on group means because, due to logistical constraints, we were unable to maintain individual fish identities following transportation from the field. We tested for differences between group means in occupied color space among time periods and populations with MANOVA of dorsal surface L^*, a^*, and b^* values as dependent variables. Following an overall significant result, we tested four a priori defined contrasts: for goal (1) Wolf Point Cr. t_0–Wolf Point Cr. t_5 and Oystercatcher Cr. t_0–Oystercatcher Cr. t_5, and for goal (2) Wolf Point Cr. t_0–Oystercatcher Cr. t_0 and Wolf Point Cr. t_5–Oystercatcher Cr. t_5. We used a linear DFA to visualize differences among samples in color space relative to the natural and laboratory substrates. To examine color differences between populations at both time points, we calculated mean squared Mahalanobis distances and the proportion of misclassified individuals. To test for an effect of fish size on color change, we estimated the correlation between the distance that each fish changed along canonical axis 1 from the DFA and each fish's length. All statistical analyses were performed with JMP version 7 (SAS Institute Inc., Cary, NC).

Results

SUBSTRATE COLOR AND FISH BACKGROUND MATCHING

We observed significant variation among streams in both fish and stream substrate color (Figs. 2, 3). Variation in stream substrate color was highly significant (MANOVA $F_{12,124} = 10.2$; $P < 0.0001$) as was variation in fish color (MANCOVA $F_{10,296} = 16.6$; $P < 0.0001$). The effect of fish size (mean fish length = 65.3 mm, $SD = 13.2$, range 39–97 mm) on variation in fish color was not significant (MANCOVA $F_{2,148} = 0.06$; $P = 0.95$).

Fish color, substrate color, and stream age were significantly correlated within Glacier Bay; and thus fish and substrate color followed a clinal pattern. Older streams (closer to the mouth of the Bay) and the fish found in them were darker (greater D^* values) and more red/yellow (greater a^* and b^* values), as indicated by high positive correlation values (Table 2). Of the 12 correlations estimated, nine had P-values < 0.05 and five tests remained significant after we controlled the FDR (FDR = 0.05; Table 2).

PCA results supported the clinal nature of color variation within Glacier Bay. PC1 explained 59% and 46% of the variance

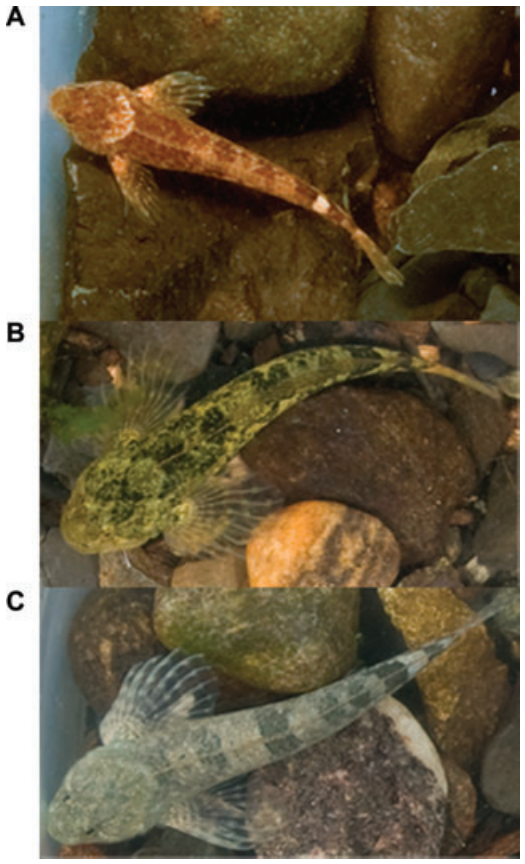

Figure 2. Color of sculpin from three Glacier Bay populations. Panel (A) shows a fish from the Salmon R. (site 2), panel (B) shows a fish from Oystercatcher Cr. (site 6), and panel (C) shows a fish from Wolf Point Cr. (site 8). Photographs were taken on a common substrate that does not reflect natural stream substrate color differences.

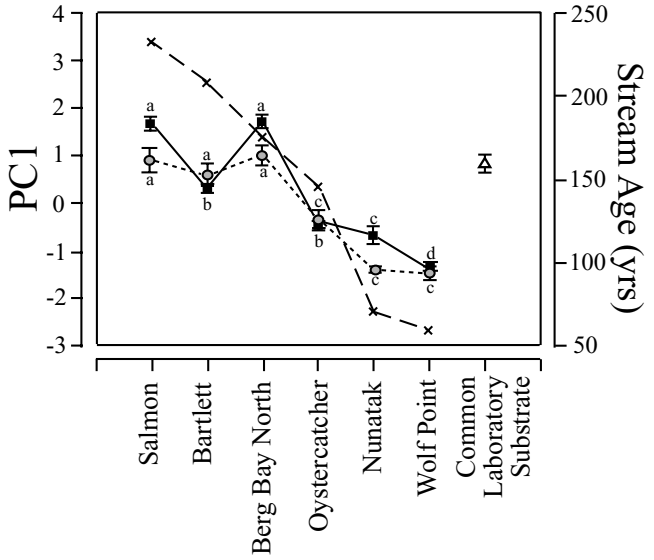

Figure 3. Substrate matching coloration of coastrange sculpin in Glacier Bay. Means (± 1 SE) of PC1 factor scores for fish color are represented by black squares, for substrate color by gray circles, and stream ages are represented by an x. PC1 explained 59% and 46% of the variation for fish and substrate color, respectively. Greater PC1 scores reflect darker, more red/yellow coloration, lower scores reflect lighter, more green/blue coloration. Stream age was estimated based on the extent of glaciation over the last 220 years and follows Table 1. The color of the common laboratory substrate used for the color change experiment is shown here (open triangle) for comparison to natural substrates. Results from Tukey's HSD tests are shown in lowercase letters and refer to statistically discernible groups for substrate color and fish color separately.

in fish color and substrate color, respectively. Loadings were generally high for all three aspects of color for both fish color and substrate color. For fish color, loading values were 0.47, 0.68, and 0.56 for D^*, a^*, and b^* values, respectively. For substrate color, loading values were 0.57, 0.66, and 0.48 for D^*, a^*, and b^* values, respectively. Mean PC1 factor scores were significantly correlated for stream substrate color, fish color, and stream age (Table 2, Fig. 3). Univariate ANOVAs of PC1 factor scores were highly significant for both fish and substrate color ($P < 0.0001$) and post-hoc tests revealed steam age-related differences in stream substrate color and fish color (Fig. 3).

POPULATION STRUCTURE

We analyzed sculpin from eight streams at seven microsatellite loci and found roughly equal levels of genetic variation in all populations (Table 1). Mean within-population expected heterozygosity (H_S) was 0.78 (range 0.76–0.80) and mean allelic richness was 9.3 (range 8.8–9.9; Table 1). We detected two significant departures from Hardy–Weinberg proportions (FDR = 0.05). Both departures occurred at *CottES19* (Bartlett River, $P = 0.0008$; Wolf Point Cr., $P = 0.0037$). F_{IS} was positive in both cases, which is consistent with a heterozygote deficit and could indicate that null alleles occur at this locus. Micro-Checker also indicated that null alleles might occur at *CottES19* in Bartlett, Oystercatcher, Nunatak, and Wolf Point Creeks. One test for gametic disequilibrium was significant (*CottES19* and *LCE29* in Oystercatcher Cr.; $P < 0.0001$; FDR = 0.05). To ensure that *CottES19* did not unduly influence our results, we performed analyses with and without this locus.

Within population genetic diversity and stream age were not significantly correlated (for H_S, $R^2 = 0.07$, $P = 0.52$; for AR, $R^2 = 0.01$, $P = 0.78$). In addition, the Bottleneck analysis provided no evidence for bottlenecks in any of the populations (Wilcoxon test, $P > 0.05$). Instead, there was a significant ($P < 0.05$) deficit of heterozygotes relative to that expected at mutation-drift equilibrium for three populations (Salmon, Bartlett, and Wolf Point Creeks), which is consistent with population expansion. Tests for

Table 2. Correlations between stream substrate color, fish color (phenotype), and stream age. Correlations are shown for scores on the Darkness axis of color variation (*D; calculated as 100 – lightness), the *a** axis of color variation, the *b** axis of color variation, and principal component axis 1 (PC1). Mean values for each of the six populations were used. Asterisks indicate significance after controlling the FDR (FDR = 0.05).**

Correlation	D^*		a^*		b^*		PC1	
	r	*P*	*r*	*P*	*r*	*P*	*r*	*P*
Substrate and phenotype	0.86	0.03	0.88	0.02*	0.78	0.07	0.93	0.007*
Substrate and stream age	0.94	0.006*	0.72	0.11	0.85	0.03	0.95	0.004*
Phenotype and stream age	0.87	0.03	0.67	0.14	0.95	0.004*	0.84	0.04

these three populations remained significant when *CottES19* was excluded, although none remained significant when we controlled the FDR (FDR = 0.05).

We also observed little evidence for genetic differentiation among populations. The overall F_{ST} was <0.001 (95% CI –0.002 to 0.001). Prior to correction for multiple tests, only one pairwise F_{ST} estimate differed significantly from zero (between Nunatak and Berg Bay North Creeks; $F_{ST} = 0.008$, $P = 0.038$; 1000 permutations), only one of seven exact tests (one for each locus) for allele frequency homogeneity across all populations was significant (*CottES19*; $P = 0.01$), and we were unable to reject the null hypothesis for 20 of 196 pairwise exact tests for genic differentiation (eight of which included *CottES19*; $P < 0.05$; 11.2 expected by chance). None of these tests remained significant after we controlled the FDR (FDR = 0.05). Structure results failed to detect genetic differentiation among Glacier Bay sculpin populations. Structure runs (with or without *CottES19*) that assumed $K = 1$ had the greatest support, which provided no compelling reason to reject the simplest hypothesis of one population group.

Table 3. Results for Matrix Correspondence Tests (MCTs). Correlations (*r*) are based on MCTs with 1,000 permutations to determine *P*-values. *ED* represents Euclidean Distance estimated from mean *L, *a**, and *b** values for either fish or substrate colors for each site. PC1 represents the absolute value of linear distances between mean PC1 factor scores for each site. Asterisk indicates significance after controlling the FDR (FDR = 0.05).**

	ED		PC1	
	r	*P*	*r*	*P*
Pairwise: phenotype and substrate	0.633	0.011*	0.752	0.002*
Pairwise: phenotype and genotype	0.009	0.984	−0.018	0.943
Pairwise: substrate and genotype	0.077	0.783	0.035	0.908
Partial: phenotype and substrate given genotype	0.634	0.011*	0.753	0.002*

CORRELATION BETWEEN SUBSTRATE COLOR, FISH COLOR, AND GENETIC DIVERGENCE

To test whether phenotypic variation was better explained by substrate color or by genetic divergence, we used MCTs. Fish color was significantly correlated with substrate color for both measures of color distances (*ED* or PC factor scores), whereas neither was correlated with genetic divergence (Table 3). Controlling for genetic divergence with a partial MCT did not influence the results, which further supported the apparent lack of an effect of genetic divergence on phenotypic divergence (Table 3).

COLOR CHANGE EXPERIMENT

At the time of capture, fish from Oystercatcher and Wolf Point Creeks closely matched the substrate color of their stream of origin (Figs. 3, 4). The laboratory substrate onto which fish were transferred was similar to the substrate color of older streams in Glacier Bay (Fig. 3). MANOVA analysis that included both populations at each time point revealed significant variation in fish color ($F_{6,392} = 91.6$; $P < 0.0001$) and all four contrasts (Wpt_0–Wpt_5; Wpt_5–Oyt_5; Wpt_0–Oyt_0; and Oyt_0–Oyt_5) were highly significant ($P < 0.0001$). In a combined DFA of fish from each population at both time points and the Wolf Point Cr., Oystercatcher Cr., and laboratory substrates, canonical axes 1 and 2 explained 96% of the variation in L^*, a^*, and b^* values (Fig. 4). Eigenvector values for Canonical Axis 1 were 0.03, 0.64, and –0.21 for L^*, a^*, and b^*, respectively. Eigenvector values for Canonical Axis 2 were –0.08, 0.09, 0.13 for L^*, a^*, and b^*, respectively. The plot of both canonical axes revealed that, following five months in this common environment, fish color for both populations moved in color space towards the laboratory substrate color (Fig. 4). In addition, mean fish color for the two populations remained significantly different after five months, but was more similar than at the beginning of the experiment (Fig. 4). This pattern was further supported by both mean squared Mahalanobis distances (15.2 between Wpt_0 and Oyt_0, 4.4 between Wpt_5 and Oyt_5) and the proportion of misclassified individuals in the DFA (4.5% between Wpt_0 and Oyt_0, 25.8% between Wpt_5 and Oyt_5).

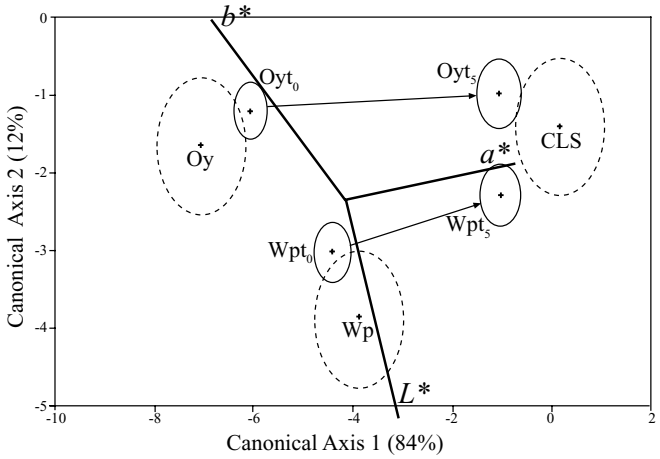

Figure 4. Discriminate function analysis of fish and substrate color at the time of capture and following five months on a common laboratory substrate. Means (+) and 95% confidence limits (ovals) are shown for each group. Dashed ovals represent 95% confidence limits for substrate colors; solid ovals are shown for fish color at either t_0 or t_5. Wp represents Wolf Point Cr., Oy represents Oystercatcher Cr., and CLS represents the common laboratory substrate. Arrows indicate the direction of color change for fish from each population. Biplot rays indicate the direction of influence of a given color axis and are scaled by a factor of three for heuristic purposes. Proportion of variance attributed to each canonical axis is shown in parentheses.

Discussion

To our knowledge, this study provides the first detailed examination of color plasticity as an important trait in colonizing populations. We observed clinal variation in substrate-matching fish color along the chronological continuum of streams. On the other hand, we observed no significant genetic subdivision within Glacier Bay and genetic differentiation was not correlated with fish color divergence. Our color change experiment revealed that morphological color change largely explained fish color differences observed in the wild.

FISH AND STREAM SUBSTRATE COLOR

Fish color and stream substrate color were significantly correlated across the chronological gradient of streams in Glacier Bay. The changes in stream color correspond to successional changes in the streams (Milner et al. 2000; Robertson and Milner 2006). As streams mature, they develop pool-riffle sequences, riparian vegetation, woody debris, and a film of algae and detritus on the substrate. Newer streams have few of these characteristics (Milner et al. 2000; Robertson and Milner 2006). By matching stream substrate colors, fish remain cryptic in each habitat. Cryptic coloration is highly adaptive as a means to avoid predation in other systems (Nachman et al. 2003; Hoekstra 2006; Rosenblum 2006). Predators of coastrange sculpin in Glacier Bay include birds, fishes, and mammals (Foerster 1930; Salyer and Lagler 1946; Roos 1959; Palmisano and Helm 1971; Krebs 1974; Scott and Crossman 1979; Polednik et al. 2004; Britton et al. 2006). All of these predators have color vision and would be more likely to see more conspicuously colored sculpin. Future studies will be necessary to test the fitness consequences of sculpin background color matching in Glacier Bay.

POPULATION SUBDIVISION

Overall, we detected little evidence of genetic subdivision within Glacier Bay. Furthermore, we did not detect an effect of colonization on within-population genetic diversity or any signature of population bottlenecks. We observed a signature of population expansion, although these results did not remain significant after correction for multiple tests. Given the short time period since colonization (14–58 generations assuming a four-year interval) the lack of genetic subdivision might be expected. However, significant differentiation exists among populations of two different species of Pacific salmon spawning in other newly deglaciated streams of Glacier Bay (C. Kondzela, unpubl. data). Significant genetic differentiation also accumulated in another salmon species following introduction ~27 generations ago in New Zealand (Kinnison et al. 2002). In addition, freshwater sculpin can exhibit marked genetic structure at small spatial scales (e.g., Nolte et al. 2006). Apparently, the amphidromous life cycle, where juveniles enter the ocean and remain planktonic for approximately 30 days before re-entering freshwater (Mason and Machidori 1976), has a large influence on the population structure of this species. Indeed, amphidromy has a large influence on the genetic structure of other fishes (McDowall 2001). The combination of a short amount of time since colonization and elevated juvenile dispersal appears to be responsible for our observations. In terms of adaptive differentiation in this system, gene flow is likely high enough to obscure the effects of selection (Lenormand 2002).

FISH COLOR CHANGE

Fish from different populations converged incompletely in color over five months in a common laboratory environment, which allows us to conclude that morphological color plasticity is at least partially responsible for observed color differences in the wild. Lack of independence of data points within our group means could have influenced these results. However, the patterns of fish color divergence at the time of capture and fish color convergence towards the laboratory environment were quite striking and it is unlikely that interpretations would differ if it had been possible to retain individual identities.

The mechanism responsible for the observed plasticity likely involves changes in the distribution and abundance (through synthesis or apoptosis) of different types of chromatophores (Kelsh

2004; Streelman et al. 2007). Combinations of five different types of chromatophores determine fish coloration: melanophores (black), xanthophores (yellow), erythrophores (red), iridophores (iridescent, blue, silver, or gold), and leucophores (dull, whitish; Kelsh 2004). Our common lab substrate was sufficient to initiate synthesis/destruction pathways and to move fish color towards the substrate color.

Incomplete color convergence of fish from the two experimental sites on the common laboratory substrate suggests that either (1) there may be genetic differences among populations at loci responsible for fish coloration or (2) environmental sensitivity could be greater at an earlier developmental stage than we examined. Rapidly evolved genetic differences could create different mean colors of each fish and morphological color change could fine-tune the color of each fish to the color of its substrate. Alternatively, greater environmental sensitivity could occur as larval fish reenter freshwater after the planktonic larval stage in the ocean (McLarney 1968). The smallest experimental fish we examined were 49 mm, which are more than one-year-old. Young-of-the-year juveniles reenter freshwater at approximately 20–25 mm in length (McLarney 1968) and could be more sensitive to color cues from the substrate at this point. Intergenerational controlled laboratory crosses will be necessary to test the prediction that fish reared from an earlier life stage would converge completely on a common substrate.

Given that fitness consequences of crypsis in coastrange sculpin are likely to be large, we hypothesize that color plasticity in this species is an example of adaptive plasticity that allows sculpin to maintain crypsis on a landscape that changes color over time. The pattern of deglaciation and corresponding landscape successional stages in Glacier Bay is repeated through time and across the landscape in a regular manner (Milner et al. 2007). After sculpin colonize a recently deglaciated stream, morphological color plasticity aids them to achieve background-matching coloration. As a stream matures and its substrate color changes (Robertson and Milner 2006), morphological plasticity would help animals maintain substrate-matching coloration.

The common laboratory substrate was similar in color to the older streams in Glacier Bay. Our experiment, therefore, also revealed that fish from younger populations are able to change to a color more similar to the fish currently found in older streams. These experimental conditions mimic the type of landscape change that occurs within rivers in Glacier Bay as they age, which again argues that morphological color change provides a flexible way to cope with conditions created by glacial recession. The type of plasticity observed in sculpin of Glacier Bay may also have been generally important for colonization of northern rivers by fishes following the retreat of major ice sheets in the last 1,0000 years.

Conclusions

Glacier Bay provides an exceptionally useful landscape to study evolutionary consequences of colonization in a context that is highly relevant to climate change. Receding glaciers in the last 220 years have created new stream habitat that is highly divergent in substrate color. Freshwater sculpin have rapidly colonized this landscape and despite a lack of neutral genetic divergence, display strong phenotypic divergence in coloration. Our results suggest that morphological color change plays an important role in background matching coloration in this species. Background matching is likely to be highly adaptive but this needs to be tested in future studies. Our study provides added emphasis for the importance of phenotypic plasticity for species that must adapt to conditions created by global climate change.

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