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Maternal genetic effects on adaptive divergence between anadromous and resident brook charr during early life history

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Abstract

The importance of directional selection relative to neutral evolution may be determined by comparing quantitative genetic variation in phenotype (Q_{ST}) to variation at neutral molecular markers (F_{ST}). Quantitative divergence between salmonid life history types is often considerable, but ontogenetic changes in the significance of major sources of genetic variance during post-hatch development suggest that selective differentiation varies by developmental stage. In this study, we tested the hypothesis that maternal genetic differentiation between anadromous and resident brook charr (*Salvelinus fontinalis* Mitchill) populations for early quantitative traits (embryonic size/growth, survival, egg number and developmental time) would be greater than neutral genetic differentiation, but that the maternal genetic basis for differentiation would be higher for pre-resorption traits than post-resorption traits. Quantitative genetic divergence between anadromous (seawater migratory) and resident Laval River (Québec) brook charr based on maternal genetic variance was high ($Q_{ST} > 0.4$) for embryonic length, yolk sac volume, embryonic growth rate and time to first response to feeding relative to neutral genetic differentiation [$F_{ST} = 0.153$ (0.071–0.214)], with anadromous females having positive genetic coefficients for all of the above characters. However, Q_{ST} was essentially zero for all traits post-resorption of the yolk sac. Our results indicate that the observed divergence between resident and anadromous brook charr has been driven by directional selection, and may therefore be adaptive. Moreover, they provide among the first evidence that the relative importance of selective differentiation may be highly context-specific, and varies by genetic contributions to phenotype by parental sex at specific points in offspring ontogeny. This in turn suggests that interpretations of Q_{ST}-F_{ST} comparisons may be improved by considering the structure of quantitative genetic architecture by age category and the sex of the parent used in estimation.

Introduction

Theoretical (Wright, 1931; Fisher, 1958) and empirical evidence (Unwin *et al.*, 2000; Hendry, 2001; Kingsolver *et al.*, 2001; Merilä & Crnokrak, 2001; Rieseberg *et al.*, 2002; Steinger *et al.*, 2002) argues the importance of natural selection in the process of population divergence. However, the effects of past selection are difficult to measure without the comprehension of neutral genetic history. Effects of selection relative to neutral processes (drift, mutation and migration) may be measured by comparing genetic differentiation between populations at neutral molecular markers (F_{ST}) to phenotypic

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differentiation (Q_{ST}) in fitness-related traits, the latter being presumably based in quantitative genetic variation (Whitlock, 1999; Merilä & Crnokrak, 2001). Adaptive differentiation may be most apparent in the evolutionary divergence of populations into different and contrasting niches (Schluter, 2000; Bernatchez, 2004).

Salmonid fishes (Osteichthyes: Salmonidae) such as salmons (*Salmo* spp.) and charrs (*Salvelinus* spp.) exploit contrasting habitats via the extremes of phenotypic variance on two principal axes: trophic morphology (i.e. pelagic/benthic forms) and anadromy/residency (seawater migratory/freshwater nonmigratory) (Gross, 1987; Dynes *et al*., 1999; Gislason *et al*., 1999; Jonsson & Jonsson, 2001; Pakkasmaa & Piironen, 2001; Boula *et al*., 2002). Morphological (Wilder, 1952; Mullan, 1958; Power, 1980) and physiological (Wilder, 1952; Boula *et al*., 2002; Morinville & Rasmussen, 2003) phenotypic contrasts between anadromous and resident fish may be particularly strong because of differential requirements for swimming efficiency and saltwater osmoregulation associated with seawater exposure. Generally, high quantitative differentiation between morphotypes relative to neutral divergence suggests that the expression of alternate morphotypes is primarily a function of local adaptation that may evolve rapidly, depending on costs and benefits relative to alternate forms (see Quinn *et al*., 2000, 2001a, b; Unwin *et al*., 2000; Hendry *et al*., 2004a, b). For anadromy and residency, energetic and stress-related migrational costs to anadromous fish are largely determined by migrational distance (see Beacham & Murray, 1993; Bohlin *et al*., 2001; Kinnison *et al*., 2003) and thus for anadromous populations, such costs should be partially predictable based on distance between the spawning site and the freshwater outflow. In this case, other fitness traits operating in energetic tradeoffs with migration should be coadapted to the migrational milieu of the specific population so as to maximize total fitness (see Kinnison *et al*., 2001, 2003).

Reproductive investment into egg/progeny size and number is a likely axis for residual adaptation in anadromous salmonids after the energetic demands of migration, since reproductive energy is greatly affected by migrational distance (Beacham & Murray, 1993; Healey, 2001; Kinnison *et al*., 2001, 2003) and since progeny size and number are themselves critical components of overall fitness (Hale, 1999; Einum & Fleming, 2000a, b, 2002; Svensson & Sinervo, 2000). However, fitness advantages associated with larger size in salmonid eggs may not be entirely uniform, but may instead vary pre- and post-hatch due to local environment at the spawning site such as gravel size and predation-foraging issues (Holtby & Healey, 1986; Quinn *et al*., 1995; Hendry *et al*., 2001; Einum & Fleming, 2002; Hendry *et al*., 2004a). Adaptive divergence between migratory and nonmigratory forms of salmonids for early size and/or developmental characteristics might be possible if the different morphotypes used alternative reproductive strategies for reasons of local adaptation (i.e. phenotype-habitat matching; Hendry *et al*., 2004b). Notably, phenotypic divergence among salmonid populations for embryonic growth and size is often quite substantial relative to estimates of neutral divergence ($Q_{ST} > 0.4$; Haugen & Vøllestad, 2000; Unwin *et al*., 2000; Koskinen *et al*., 2002).

Differential local adaptation of reproduction and early ontogeny by anadromous and resident fish would require reasonable amounts of genetic variation for these traits in order to stabilize phenotypic variance to an adapted state (see Merilä & Crnokrak, 2001). Genetic variance estimates for early (<3 months age) juvenile size traits in brook charr (*S. fontinalis*) and chinook salmon (*Oncorhynchus tshawytscha*) range from low to high depending on ontogeny, the specific trait and the source of the genetic variance (sire or dam) (Heath *et al*., 1999; Perry *et al*., 2004). Specifically, maternal and sire/animal-based genetic variance for early size traits appears to be partially segregated at the embryo-alevin boundary, with maternal genetic variance being high prior to yolk sac resorption and relatively low thereafter (see also Heath *et al*., 1999; Perry *et al*., 2004). In salmonids, genetic variance for the same character at different points in development appears to stabilize only at later ages (>1-year post-fertilization) (Fishback *et al*., 2002; Su *et al*., 2002). While some studies have considered ontogenetic variance in differentiation as a form of local adaptation over temporal scales, migration by adults or changes in selective pressure (Kindell *et al*., 1996; Gilg & Hilbish, 2000; Mulvey *et al*., 2002), questions of ontogenetic variance in adaptive divergence have not been investigated.

In this study, using the brook charr as a model organism we tested the hypotheses that maternal genetic differentiation between anadromous and resident populations for quantitative traits such as early embryonic size/growth, survival, egg number and/or developmental time would be greater than neutral genetic differentiation, and that the maternal genetic basis for differentiation would be higher for pre-resorption traits than post-resorption traits.

Materials and methods

Molecular genetic differentiation

Thirty fish were collected from each of two sites in the Laval River drainage: the Adams Brook and the Laval River mainstream. The Adams Brook system runs approximately 20 km from the Laval Bay inlet to Lake Jacques (roughly 3 km in length), then continues for an additional 10 km to the Adams Brook branch. Adams brook charr are predominantly resident types while fish in the Laval River main system are mostly anadromous (see Boula *et al*., 2002). Adams Brook individuals were line-fished directly from the brook at a single sampling point (48 °54′44″N/69 °12′55″W) in the summers of

2001 and 2002. Laval River brook charr were collected from several points in the Laval River (also using line-fishing) within approximately 10 km of the Adams Brook spawning site. These two populations are thus parapatric, but have very limited geographic separation given migrational thresholds for differentiation in this species (see Castric & Bernatchez, 2003, 2004).

Total DNA was extracted from the muscle tissues using a standard proteinase *K* phenol-chloroform protocol (Sambrook *et al.*, 1989). We estimated genetic differentiation between anadromous and resident brook charr forms within the Laval River drainage sites with nine microsatellite markers (Appendix 1). Either the forward or reverse primer was 5′-fluorescently labelled using HEX, TET or FAM dyes. The PCR was performed in a Perkin-Elmer 9600 thermocycler (Model 2.01) using 25–50 ng template DNA under two different reaction protocols. Five microsatellite loci (*SfoB52*, *SfoC28*, *SfoC88*, *SfoC113* and *SfoC129*) were amplified in a single multiplex reaction (Protocol No. 1: 2.0 units *Taq* polymerase, 2.0 μL reaction buffer (100 mm Tris–HCL (pH 9.0), 1.5 mm $MgCl_2$ 1% TritonX-100, 500 mm KCL), 15 mmol dNTPs, 20 μL total volume) and run on a single gel (Appendix 1). Three additional microsatellites (*SfoC115*, *SfoD75* and *SfoD100*) were amplified using Protocol No. 1 but run on a second gel with a final locus (*Ssa197DU*) which was amplified in a separate reaction with a different protocol (Reaction No. 2: 1.0 unit *Taq* polymerase, 1.0 μL reaction buffer (as for Reaction No. 1), 750 μmol dNTPs, final reaction volume 10 μL) (Appendix 1). A fourth locus (*SfoD105*) was included in Protocol No. 1 and scored but was not included in analysis owing to possible large-allele dropout (data not shown). The PCR profile followed the following steps: 5 min initial denaturing at 95 °C, followed by 30 cycles of 30 s at 94 °C, 30 s at annealing temperature and 30 s at 72 °C. The PCR products were denatured at 95 °C for 5 min and ice-chilled prior to loading on a 5.5% denaturing polyacrylamide gel. Gels were run for 2 h at 3000 V on an MJ Research BaseStation automated sequencer/scanner. Allelic scores and genotype tabulation was determined with Cartographer 1.2.6sg (© MJ Bioworks Inc., Sauk City, WI, USA) by reference to a Rox-labelled internal size standard present in each lane and by comparison with a standard sample of known allelic size that was run on each gel.

Three standard measures of microsatellite variation were calculated: number of alleles per locus (A), observed heterozygosity (H_o), and (unbiased) genetic diversity (H_e) using GENETIX (4.02; Belkhir *et al.*, 2000). The score test (U-test) (Rousset & Raymond, 1995) was used to determine Hardy–Weinberg Equilibrium vs. the null hypothesis of heterozygote excess or deficiency for each locus. Tests across all loci were performed using GENEPOP (3.1d; Raymond & Rousseau, 1995). The null hypothesis of no differentiation was tested at each locus between all pairs of samples via iterative estimation of Fisher's exact test (1000 iterations) in GENEPOP (3.1d) (Guo & Thompson, 1992). The Fisher method (Sokal & Rohlf, 1995) was used to obtain the probability of no differentiation for all loci simultaneously. Genetic differentiation between the resident and anadromous populations was estimated using the θ estimate of F_{ST} (F-stat 1.2; Goudet *et al.*, 1996). Bootstraps over all loci were used to identify 95% confidence intervals for the F_{ST} (1000 replicates) and probability associated with molecular differentiation via F_{ST} was determined via the identification of a new significance threshold using 1000 permutations of the original data.

Quantitative differentiation between resident and anadromous brook charr during embryo/alevin development

The progeny of 12 male and 23 female fish from the Laval River system were used in this experiment. Six male and 12 female fish originated from the second generation of a stock of fish descended from anadromous Laval River (QC) fish and being maintained at the Station Aquicole de Pointe-au-Père, Université du Québec à Rimouski (QC). The other parental individuals (6 males, 12 females) were collected in the summer of 2001 and 2002 from the resident population in the Adams Brook. For the majority of the crossings, each sire was bred to one female of each population (anadromous and resident) to create 12 sire half sib families of fish (two females per male) from mid-November, 2002 to early January, 2003 for a total of six pure anadromous families, six pure residents and 12 hybrid full sib families (Appendix). Only one full sib family was available for one of the sires since eggs from one of the females did not develop properly, resulting in 23 full sib families total (Appendix 2). The progeny of each dam were reared in separate units, but environment was rigorously controlled within the overall experimental system (12 : 12 h photoperiod at 4 °C during until after eclosion, then ambient temperatures (7–8 °C) at first external feeding onward) and local environmental effects were likely negligible compared to maternal effects on early juvenile phenotype.

Individual trait measurements

Salmonid fish hatch with substantial yolk reserves (termed the embryonic or sac-fry period) but resorb these structures shortly (~1 month) thereafter (termed the alevin period) (Pennell & Barton, 1996). A total of 40 individuals were sampled from each full sib family, 20 after 100% hatching (eclosion) by family (February 21–April 10, 2003) and 20 following 100% resorption of the yolk sac by family (April 28–May 27, 2003) (embryonic and alevin stages, respectively, Pennell & Barton, 1996). Three traits were measured in individual embryos at 100% hatch (possessing yolk sacs) using a dissecting microscope: embryonic length (length from the anterior

tip of the snout to the end of the tail, EL; mm), yolk sac length and yolk sac radius. Yolk sac volume was calculated under the rough assumption of cylindrical shape ($YSV = \pi \cdot L \cdot r^2$; mm^3; L = yolk sac length, r = yolk sac radius). Length was also measured for alevins (alevin length, AL; mm), collected post-resorption of the yolk sac. General growth rate was also estimated for the two length traits by dividing each length measurement by age in degree·days (Celsius) (°C·days) at the point of measurement (EL_{GR} = EL/ °C·days at 100% hatch by family; AL_{GR} = AL/ °C·days at 100% yolk sac resorption by family). Growth measurements were thus partially determinate by familial means for development time rather than individual measurements, but a similar approach has been used in other studies (Koskinen *et al.*, 2002). We also measured three life-history trait types (fecundity, development time and survival) at the family level. The number of eggs laid (fecundity; FEC) and effectively fertilized (fertility; FERT) was counted for each female. Development time by family was measured as °C·days until 100% eclosion (DVEC), 100% eye-up (DVOE), first stimulus response to external feeding within families (DVST) and full external alimentation of entire families (DVAL). Finally, we measured survival at eye-up (SVOE), eclosion (SVEC), stimulus response to first feeding (SVST) and full external alimentation (SVAL) as a net proportion (p) of initial n by family.

Individually measured traits (EL, EL_{GR}, YSV, AL and AL_{GR}) and familial measurements for the six family-measured traits (FEC, FERT, NFERT, DVEC, DVOE, DVST and DVAL) were tested for nonnormality prior to any analysis (PROC UNIVARIATE, SAS, 1998). Survival proportions (p) for the four-survivorship traits were arcsine-transformed prior to analysis as typical for proportional values (Sokal & Rohlf, 1995). We considered progeny age (°C·days) and dam weight (g) as potential covariates for our analyses since age-related effects were expected to occur and since maternal environmental variance for embryonic traits may be profound (Einum & Fleming, 2000a, b; Hendry *et al.*, 2001).

Trait transformation and preanalysis

The five individually measured characters, as well as FEC, FERT and NFERT were all found to be nonnormally distributed ($P < 0.01$). We therefore determined the transformation minimizing root mean squared error (RMSE) for each trait using a Box-Cox macro ('BoxCox,' M. Friendly, York University, ON,) and transformed those traits accordingly. Box-Cox analysis of the familial traits indicated that transformations of $x^{0.2}$ for all three fecundity-related characters ($FERT^{0.2}$, $FEC^{0.2}$ and $NFERT^{0.2}$) minimized RMSE. For the individual traits, optimal Box-Cox transformations were calculated as $EL^{1.4}$, $EL_{GR}^{2.0}$, $YSV^{0.2}$, $AL^{0.5}$ and $AL_{GR}^{0.7}$. The four family-measured traits for developmental time (DVEC, DVOE, DVST and DVAL) were all normal in distribution ($P > 0.09$, Shapiro–Wilk test statistic; Sokal & Rohlf, 1995) and thus were not transformed. Trait descriptions throughout the paper use transformed values unless otherwise noted. Dam weight and progeny age were included as covariates for the analysis of EL, EL_{GR}, YSV, AL and AL_{GR} (see also Perry *et al.*, 2004), with the exception of progeny age for the two growth measurements and for EL, since associations of the latter trait with age by family was not significant ($P > 0.2$; Perry *et al.*, 2004). Dam weight was significantly associated ($P < 0.05$) with DVEC, DVST, DVAL and all three fecundity measures (FEC, FERT and NFERT). Progeny age was discluded as a covariate for the familial traits since these were functions of age (developmental time) or measured at the same age point (i.e. survival traits, fertility).

REML estimation of within- and between-population genetic variance for individual traits

The extent of quantitative genetic differentiation between populations is normally measured as the ratio of phenotypic variance between populations (σ^2_{bw}) to that of the total phenotypic variance expected within the set of all populations (σ^2_{wn}), $Q_{ST} = \sigma^2_{bw}/(2\sigma^2_{wn} + \sigma^2_{bw})$, where $2\sigma^2_{wn}$ represents within-population quantitative genetic variance (see Merilä & Crnokrak, 2001). We used a set of programs [Parameter Estimation Software 3.0 (PEST), Groeneveld *et al.*, 1990; Variance Component Estimator 5.1 (VCE), Kovac *et al.*, 2002] to estimate genetic variance originating within and between populations for individually measured characters via restricted-maximum likelihood by simultaneously taking advantage of known relationships within the pedigree population of progeny animals and potential linear effects of age and dam weight on phenotypic variance. PEST was used for recoding phenotypic and genetic (pedigree) relationships into a format usable by VCE. VCE was then used to estimate genetic and phenotypic variance within and between life history types. For each trait we fit a univariate model of the form

$$\mathbf{y} = \mathbf{Xb} + \mathbf{Za} + \mathbf{e}$$

where $\mathbf{y}$ is the vector of phenotypic values for one of the individually measured traits (EL, YSV, EL_{GR}, AL or AL_{GR}), $\mathbf{X}$ is the incidence matrix of nongenetic fixed and covariate effects, $\mathbf{b}$ is the vector of coefficients for fixed effects and covariate effects, $\mathbf{Z}$ is the incidence matrix of random (genetic) effects associated with parent and the population of the parent (anadromous or resident), $\mathbf{a}$ is the vector of random (genetic) effects coefficients and $\mathbf{e}$ is the vector associated with random error. Between- and within-population genetic variance was estimated separately for each trait as a function of genetic inheritance from dams (Model 1) and sires (Model 2). Model 1 estimated variance components based on relatedness between female parents and their progeny while Model 2 used sire-offspring relationship. In Model 1, dam genetic

variance was treated as σ^2_{wn}, dam population (anadromous or resident) as between-population variance (σ^2_{bw}), and sire was fit as an additional random effect to account for other possible variance sources. In Model 2, sire genetic effects were used to estimate σ^2_{wn}, with variance from sire population treated as σ^2_{bw} and dam fit as an additional random term. Since dam weight (g) was included in all analyses, dam effects in both models would have accounted for those maternal genetic and environmental effects unrelated to dam weight. Q_{ST} was calculated for each trait in each model from σ^2_{bw} and σ^2_{wn} using the standard equation (see above). Best linear unbiased predictions (BLUP; see Mrode, 1996) of additive genetic value for dams, sires and dam and sire source populations (anadromous or resident) were made in VCE. Statistical differences between BLUP estimates of genetic value for anadromous and resident populations for juvenile traits were made using contrast statements (α_{A-R}) in PEST. All estimates of genetic variance in PEST converged within 60 or fewer iterations with the exception of EL_{GR} in the maternal model which required 260 iterations and at an absolute log-likelihood ratio (L) of 100 or greater with the exception of maternal EL ($L = |58.1|$).

The program VCE was not directly capable of bootstrapping for 95% confidence intervals for Q_{ST} analyses. Therefore, we generated standard errors and confidence intervals for Q_{ST} for each trait based on estimates of σ^2_{bw} and σ^2_{wn} and their associated standard error from VCE. Random normal distributions (μ,σ^2) of σ^2_{bw} and σ^2_{wn} were created separately for each trait and model (paternal, maternal) using SAS (1998) by generating 1000 random samples (with replacement) of the above parameters from the original variance component estimates treated as the mean (μ) and the standard error associated with these estimates derived from VCE. Q_{ST} was calculated for each pair of σ^2_{bw} and σ^2_{wn} using the normal relation $Q_{ST} = \sigma^2_{bw}/(2\sigma^2_{wn} + \sigma^2_{bw})$. The 2.5 and 97.5% quantiles of the generated distribution of Q_{ST} values by trait (SAS, 1998) were treated as the upper and lower ranges of the 95% confidence interval. Contrasts between estimated genetic value (estimated breeding value, EBV) of the resident and anadromous groups (α_{A-R}) were made using PEST (Groeneveld *et al.*, 1990).

Reproductive output, average survival and development time by parental life history type

A limited number of observations ($n = 23$) were available for the familial life history traits. However, we tentatively explored trends in associations between male and female life history type (anadromous/resident) with familial values for fecundity, survival and development time in a mixed model (PROC MIXED; SAS, 1998) of the form

$$y_{ijkm} = \mu + \alpha_i + \gamma_j + \beta_k X_k + \varepsilon_{ijkm}$$

where y_{ijkm} is the value of full sib family m for trait y of sire background i and dam background j for one of the familial traits, μ is the population mean, α_i represents the random effect of sire background i (anadromous or resident), γ_j is the fixed effect of dam background j (anadromous or resident), β_k is the regression coefficient for the association of dam weight with familial phenotype y, X_k is dam weight and ϵ_{ijkm} represents experimental error. Familial traits measured in this manner included the four survival values (SVOE, SVEC, SVST and SVAL), total egg production (fecundity; FEC), number of fertilized eggs (fertility; FERT), number of eggs nonfertilized (NFERT), and developmental time (°C·days) until 100% eclosion (DVEC), 100% eye-up (DVOE), first stimulus response within families to external feeding (DVST) and full external alimentation (DVAL). Sire and dam background were fit as random effects in this model for general correspondence with random models used above for the characterization of sire and dam effects, with the exception of FEC where sire type was not fit as an explanatory variable since this species employs external reproduction. Dam weight was included where regression of family trait value on dam weight indicated significant associations ($P < 0.05$) (PROC REG; SAS, 1998). Phenotypic differentiation between life history types was measured as α_{A-R} via contrast statements in PROC MIXED (SAS, 1998). We estimated Q_{ST} for each familial trait, where between-observational variance (residual variance, σ^2_r) was treated as σ^2_{wn} and variance associated with sires (σ^2_{sire}) and dams (σ^2_{dam}) was used as σ^2_{bw} for the calculation of maternal and paternal Q_{ST}, respectively. The Q_{ST} was then calculated as per the normal equation ($Q_{ST} = \sigma^2_{bw}/(2\sigma^2_{wn} + \sigma^2_{bw})$). Confidence intervals (95%) were generated using a bootstrapping approach with 1000 replications in SAS (1998).

Results

Neutral genetic differentiation (F_{ST}) between anadromous and resident brook charr

Of the 30 samples of each population used for genetic analysis, a minimum of 25 individuals were amplified for each locus used (Table 1). Although variable among loci, the two populations did not appear to consistently differ in allelic size ranges (A_R), average heterozygosity (H_M) or number of alleles per locus (Table 1). Allele frequencies in resident and anadromous fish were significantly different ($P < 0.05$) at all nine microsatellite loci used in the analysis (Table 1), confirming that they compose genetically distinct populations in this system. Consequently, the overall neutral genetic divergence between fish from the two forms was high ($F_{ST} = 0.153$, 95% CI = 0.071–0.214) relative to values observed among anadromous brook charr populations, which generally range between 0.05 and 0.10 (Castric & Bernatchez, 2003).

Table 1 Allelic variability at nine microsatellite loci in anadromous and resident Laval River brook charr.

Sample	*SfoB52*	*SfoC28*	*SfoC88*	*SfoC113*	*SfoC115*	*SfoC129*	*SfoD75*	*SfoD100*	*Ssa197DU*	A_M	H_M
Anadromous											
N	27	25	26	28	27	26	27	27	27		
A	4	7	3	5	3	5	6	7	3	5	
A_C	220	188	188	136	238	236	210	218	148		
F_C	0.37	0.46	0.59	0.42	0.53	0.65	0.48	0.33	0.59		
A_R	212–222	152–196	188–196	136–152	234–242	220–236	178–214	206–238	140–148		
H_E	0.71	0.68	0.68	0.70	0.60	0.48	0.69	0.76	0.51		0.64
H_O	0.70	0.52	0.52	0.57	0.44	0.53	0.85	0.70	0.44		0.57D
Resident											
N	30	30	30	30	30	30	29	30	29		
A	3	7	3	5	7	5	9	6	5	6	
A_C	222	188	188	144	238	224	206	234	148		
F_C	0.8	0.60	0.61	0.45	0.71	0.38	0.26	0.30	0.62		
A_R	202–226	172–196	188–196	136–156	222–326	220–236	182–214	206–234	140–156		
H_E	0.33	0.59	0.59	0.69	0.46	0.68	0.81	0.76	0.52		0.62
H_O	0.26	0.70	0.70	0.76	0.36	0.50	0.79	0.73	0.41		0.56D
P	<0.001	0.003	0.003	<0.001	<0.001	<0.001	<0.001	<0.001	0.77		

N, Number of samples successfully used for genetic analysis; *A*, number of alleles at each locus; A_M, mean number of alleles; A_C; most common allele (in base pairs); F_C, frequencies of the most common alleles; A_R, range of allele size; H_O, observed heterozygosity; H_E, allelic diversity at each locus; *P*, probabilities associated with genetic differentiation by locus; H_M average heterozygosity across all loci.
The symbol D indicates significant heterozygote deficit across loci (global test, Fisher's method).

Quantitative differentiation between embryonic and alevin brook charr

Maternal Q_{ST} estimates ranged from effectively 0 to 0.883. Mean quantitative differentiation based on all ten individual Q_{ST} estimates for the five traits for both sire and dam models was calculated as $Q_{ST} = 0.230 \pm 0.120$ (95% CI = −0.0411–0.501).

The extent of quantitative divergence between resident and anadromous juvenile brook charr varied by the relatedness function (maternal or paternal) used to estimate genetic variance, as well as the age category of the progeny (Table 2). Maternal Q_{ST} estimates (Model 1) for EL (0.862), YSV (0.883) and EL_{GR} (0.570) were considerably higher than the estimate of neutral genetic variance ($F_{ST} = 0.151$, 95% CI = 0.078–0.229), supporting the hypothesis that divergence between the anadromous and resident Laval River populations has been influenced by directional selection on maternal quantitative genetic variance (Table 2). BLUP contrasts between anadromous and resident phenotype in PEST indicated that the genetic effect of anadromous dams on EL, EL_{GR} and YSV was higher than that of resident dams ($\alpha_{A-R} > 0$; $P < 0.0001$; Table 2). In contrast, there was no evidence for the role of directional selection acting AL or AL_{GR} since maternal between-population genetic variance was effectively zero for both traits (Table 2).

Sire-based estimates of σ^2_{bw} and σ^2_{wn} (Model 2) approached zero for all embryonic traits in PEST ($<1e^{-10}$; not shown) and variance from sire source population scarcely exceeded 1% of total phenotypic variance for these characters in mixed modelling (PROC

Table 2 Maternal genetic estimates of within- and between-population genetic variance for embryonic length (EL), alevin length (AL), yolk sac volume (YSV), growth rate for length at eclosion (EL_{GR}), and at the alevin period (AL_{GR}) between and within anadromous and resident brook charr charr populations.

Trait	σ^2_p	σ^2_r (SE)	σ^2_{bw} (SE)	σ^2_{wn} (SE)	α^1_{A-R} (SE)	$\sigma^2_{dam}/\sigma^2_p$	*P*	Q_{ST} (CI)
EL	46.2	6.66 (0.0445)	35.3 (3.82)	3.16 (0.158)	12.6 (2.76)*	0.877	<0.0001	0.847 (0.819–0.870)
EL_{GR}	2.39	0.553 (0.0157)	1.33 (0.681)	0.502 (0.0714)	4.14 (0.633)*	0.780	<0.0001	0.570 (0.392–0.619)
YSV	0.649	0.0821 (0.00444)	0.530 (0.489)	0.0352 (0.0212)	1.57 (0.327)*	0.909	<0.0001	0.883 (0.389–1.32)
AL	0.0885	0.0623 (0.00924)	$8.46e^{-14}$ ($8.18e^{-8}$)	0.0228 (0.0261)	0.0 (0.0)	0.0	0.6605	$1.21e^{-11}$ ($-1.34e^{-5}$–$1.18e^{5}$)
AL_{GR}	0.0156	0.0128 (0.00431)	$5.82e^{-16}$ ($4.68e^{-8}$)	0.00230 (0.00722)	0.0 (0.0)	0.0	0.3567	$5.49e^{-13}$ ($-5.36e^{-5}$–$4.62e^{5}$)

Restricted maximum likelihood (REML) [PEST3.0 (Groeneveld *et al.*, 1990); VCE5.1 (Kovac *et al.* 2002)] was used for all calculations. Genetic variance and associated standard error (SE) is given for dam population (between-population genetic variance; σ^2_{bw}), dams within their source population (within-population genetic variance; σ^2_{wn}) and for phenotypic (σ^2_p) and residual variance (σ^2_r). Original Q_{ST} estimates based on the above components and their 95% confidence intervals (CI) are given by trait. All values are expressed using three significant digits. BLUP contrasts of anadromous and resident genetic value (α_{A-R}; $P < 0.05$*) were made using PEST3.0 (Groeneveld *et al.*, 1990).

MIXED; SAS, 1998) (EL: $\sigma^2_{sire}/\sigma^2_p = 4.50 \times 10^{-3}$; EL_{GR}: $\sigma^2_{sire}/\sigma^2_p = 3.00 \times 10^{-3}$; YSV: $\sigma^2_{sire}/\sigma^2_p = 0.0106$). Ratio-based estimates for paternal Q_{ST} thus overlapped considerably with zero (EL: $Q_{ST} = 0.0167$, 95% CI = −0.164–0.186; EL_{GR}: 0.675, 95% CI = −0.101–1.22; YSV: $Q_{ST} = 0.246$, 95% CI = −1.11–1.64), suggesting nonsignificance of between-population differences based on the paternal genetic componant. Nominally, paternal Q_{ST} was high for AL ($Q_{ST} = 1.00$, 95% CI = 0.999–1.002) and AL_{GR} ($Q_{ST} = 0.838$, 95% CI = −2.33–2.54) but estimates of sire and sire population variance overlapped with zero for both traits (AL: $\sigma^2_{bw} = 0.00452 \pm 0.0146$, $\sigma^2_{wn} = 1.83e^{-12} \pm 1.17e^{-6}$; AL_{GR}: $\sigma^2_{bw} = 5.31e^{-4} \pm 4.41e^{-3}$, $\sigma^2_{wn} = 5.13e^{-5} \pm 5.00e^{-3}$) indicating that actual divergence between anadromous and resident fish based on paternal genetic effects was, at most, minimal. All sire-based BLUP contrasts between anadromous and resident populations for morphological traits were so small as to be nonestimable in PEST and were therefore treated as zero.

Associations between life history type and familial traits

Female life history type was significantly associated with each reproductive character, being responsible for over 70% of total variance. Independently of size, anadromous dams produced more eggs total (FEC; $P < 0.01$) and had more surviving fertilized (FERT; $P < 0.05$) and nonfertilized eggs (NFERT; $P < 0.05$) than resident dams (Table 3; Fig. 1). Maternal Q_{ST} exceeded 0.80 for all three reproductive traits, being highest for FEC, but these estimates overlapped with zero when considering 95% CI, which appeared to be highly left-skewed from the bootstrap analysis (0–0.95, $\mu \approx 0.84$) (Table 3). Sire life history type was not significantly associated with FERT (n.s.) but NFERT was slightly higher in anadromous males than residents ($P < 0.05$; Table 3). Paternal Q_{ST} estimates were low ($Q_{ST} < 0.05$) for FERT and NFERT and not significantly different from zero.

Table 3 Quantitative differentiation (Q_{ST} (95% CI)) between resident and anadromous brook charr for (i) reproductive output including fecundity [FECT; (eggs produced)$^{0.2}$], fertility [FERT; (number of eggs fertilized)$^{0.2}$] and nonfertilized [NFERT; (eggs fertilized)$^{0.2}$], (ii) survival (arcsine (p) survivants) to eye-up (SVOE), eclosion (SVEC), first stimulus response to exogenous feeding (SVST) and 100% exogenous feeding by family (SVAL) and (iii) developmental time in °C·days to eye-up (DVOE), eclosion (DVEC), first stimulus response to exogenous feeding (DVST) and 100% exogenous feeding by family (DVAL).

Trait	Par	σ^2_p	σ^2_r	σ^2_{par}	α_{A-R}	P	σ^2/σ^2_p	Q_{ST}	CI
(i) Reproduction									
FEC	Dams	0.257	0.0379	0.219	0.819 ± 0.219	0.0015	0.929	0.873	(0.0–0.954)
	Sires			na	0.0311 ± 0.0431	0.2423	0.00344	0.0263	(0.0–0.453)
FERT	Dams	0.308	0.0274	0.274	0.705 ± 0.222	0.0045	0.889	0.834	(0.0–0.934)
	Sires			0.00605	0.0897 ± 0.0620	0.1001	0.0194	0.0994	(0.0–0.533)
NFERT	Dams	6.53	0.662	5.63	3.18 ± 1.08	0.0064	0.864	0.810	(0.0–0.944)
	Sires			0.238	0.607 ± 0.327	0.0488	0.0367	0.152	(0.0–0.414)
(ii) Survival									
SVOE	Dams	0.0310	0.0179	0.00751	−0.0930 ± 0.0494	0.0270	0.243	0.173	(0.0–0.588)
	Sires			0.00554	−0.112 ± 0.0509	0.0476	0.179	0.134	(0.0–0.548)
SVEC	Dams	0.0562	0.0491	0.00	0.00 ± 0.00*	0.8063	0.00	0.0	(0.0–0.259)
	Sires			0.00709	−0.0940 ± 0.0731	0.1298	0.126	0.0673	(0.0–0.379)
SVST	Dams	0.0550	0.0471	0.00	0.00 ± 0.00*	0.7620	0.00	0.0	(0.0–0.268)
	Sires			0.00794	−0.102 ± 0.0735	0.1124	0.144	0.0778	(0.0–0.382)
SVAL	Dams	0.0583	0.0453	0.00	0.00 ± 0.00*	0.6638	0.00	0.0	(0.0–0.247)
	Sires			0.0130	−0.141 ± 0.0778	0.0540	0.222	0.125	(0.0–0.511)
(iii) Development time									
DVOE	Dams	278	224	50.9	8.58 ± 5.31	0.0662	0.183	0.102	(0.0–0.445)
	Sires			3.01	−0.898 ± 2.28	0.2845	0.0109	0.00669	(0.0–0.345)
DVEC	Dams	267	67.0	200	18.3 ± 8.03	0.0239	0.749	0.598	(0.0–0.892)
	Sires			0.00	0.0 ± 0.00*	0.4039	0.0	0.0	(0.0–0.327)
DVST	Dams	10065	518	9324	134 ± 24.0	<0.0001	0.926	0.900	(0.760–0.981)
	Sires			224	−19.3 ± 8.68	0.0249	0.0223	0.178	(0.0–0.672)
DVAL	Dams	730	446	233	64.9 ± 21.5	0.0051	0.319	0.207	(0.163–0.899)
	Sires			51.2	−7.63 ± 6.65	0.1462	0.0701	0.0543	(0.0–0.438)

Phenotypic (σ^2_p), residual (σ^2_r) and parental (σ^2_{par}) variance is indicated. Mixed-model contrasts (α_{A-R} ± SE; PROC MIXED; SAS, 1998), significance of sire and dam model terms and proportion of phenotypic variance explained by sire and dam (Par) effects are also indicated.
*Differentiation was nonestimable due to low variance associated with the specific model term. Significance of sire and dam effects (p) was determined using type-III sums of squares in a random mixed model (PROC MIXED; SAS, 1998).
na, not applicable.

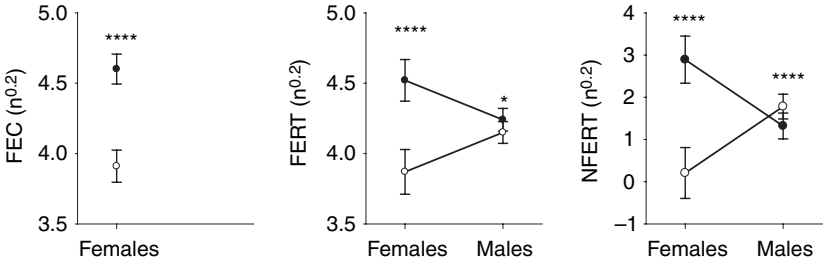

Fig. 1 Parental effects on a) number of eggs produced (fecundity, FEC), b) fertilized (fertility, FERT) and c) non-fertilized (failed fertilization, NFERT) as a function of life history. Least-squares means and associated standard error (PROC GLM; SAS, 1998) are indicated by filled circles (●) for the anadromous populations and open circles (○) for the residents. Parental sex is on the x-axis. Significant differences between means (PROC GLM; SAS, 1998) for anadromous and resident parents are given by dam and sire type for FERT and NFERT and by dam type for FECT ($p < 0.05$*; $p < 0.01$**; $p < 0.0001$****).

Maternal and paternal Q_{ST} were low for all survival measures ($Q_{ST} < 0.2$; Table 3). Parental effects on survival to 100% eye-up (SVOE) were significant ($P < 0.05$) but Q_{ST} was, as for other traits, low (0.15) and not significantly different from zero given our CI estimates (Fig. 3; Table 3). The progeny of both resident females and males had higher survival than those of anadromous parents ($\alpha_{A-R} \approx -0.10 \pm 0.05$) with population accounting for roughly 20% of phenotypic variance (Fig. 3; Table 3). SVAL was also marginally higher for families from resident males than anadromous ones ($P = 0.054$; Fig. 2; Table 3).

The progeny of anadromous dams took marginally longer to achieve 100% eye-up (DVOE) than those of residents ($P = 0.066$; Table 3; Fig. 3), accounting for 18.3% of phenotypic variation. The descendants of anadromous dams also took significantly longer to develop to eclosion (DVEC) than residents ($P < 0.05$; Fig. 3). Families from anadromous dams also took significantly longer to develop a stimulus response to feeding (DVST) ($P < 0.0001$; Fig. 3) and to achieve 100% external alimentation by family (DVAL) ($P < 0.01$; Fig. 3). The REML estimates from PROC MIXED indicated that the dam population explained only 18% of phenotypic variation in DVOE, but 75 and 93% of variation in DVEC and DVST. By full external feeding (DVAL) however, maternal variation controlled only 32% of phenotypic variation in development time. Maternal Q_{ST} estimates for DVST and DVAL were significantly higher than zero given 95% CI, although only the Q_{ST} estimate for DVST was significantly higher than F_{ST} (Table 3). Mixed modeling suggested that DVST was significantly longer in the progeny of resident sires than in anadromous types ($P < 0.05$; Fig. 3) but paternal Q_{ST} for developmental characters was low overall ($Q_{ST} < 0.2$) and all paternal estimates for development time overlapped with zero (Table 3). There were no effects of male parental life history background on DVOE (n.s.), DVEC (n.s.) or DVAL (n.s.).

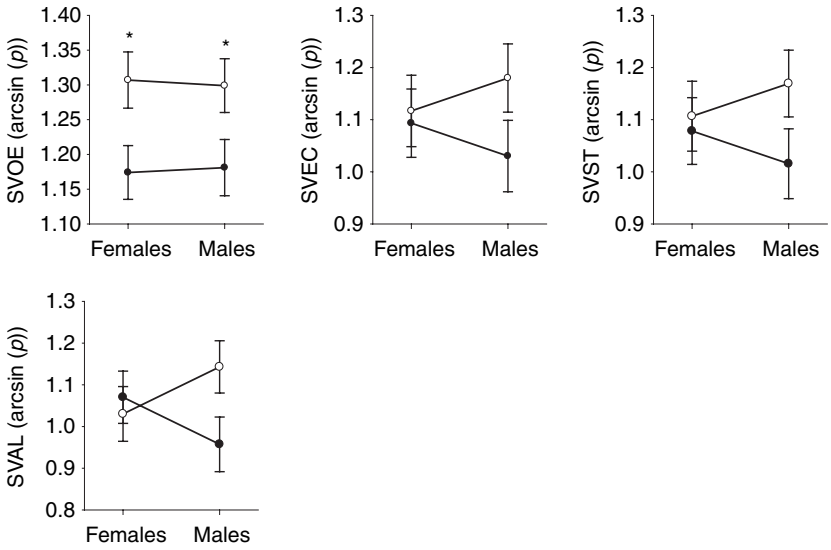

Fig. 2 Main effects of male and female (x-axis) parental population (anadromous/resident) on arcsine-transformed survival proportions to a) eye-up (SVOE), b) eclosion (SVEC), c) first stimulus response to feeding (SVST) and d) full external alimentation (SVAL). Least-squares means and associated SE (PROC GLM; SAS, 1998) are given as open circles (○) for residents and as filled circles (●) for anadromous life history. Significant phenotypic differences between life history classes are indicated using asterisks ($p < 0.05$*).

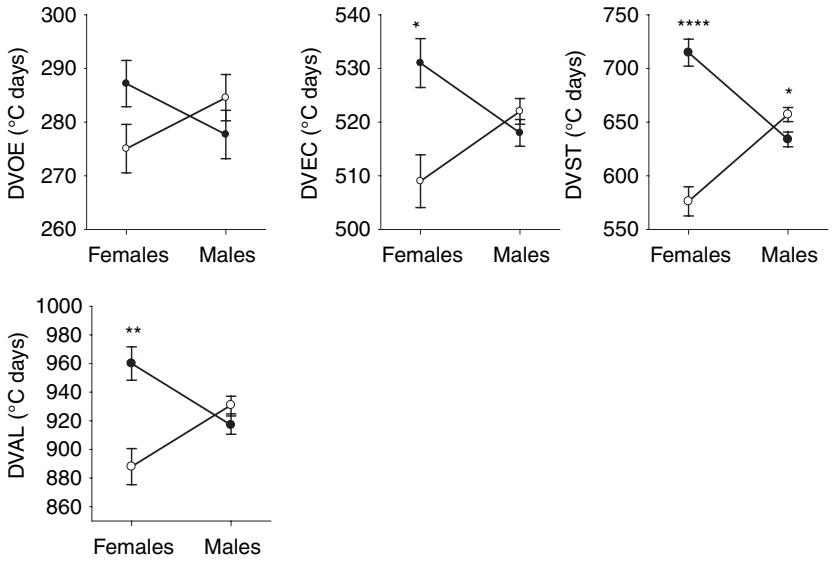

Fig. 3 Main effects of resident and anadromous parental life history on development time (°C·days) to 100% eye-up (DVOE), 100% eclosion (DVEC), first stimulus response to feeding (DVST) and 100% alimentation (DVAL) by family. Filled circles (●) indicate phenotypic means for anadromous individuals and empty circles (○) resident individuals. Significant differences between means by sire and dam populations are indicated by significance level ($p < 0.05$*; $p < 0.01$**; $p < 0.0001$****).

Discussion

Neutral genetic variation between resident and anadromous brook charr

The extent of genetic differentiation between sympatric or parapatric morphs of salmonid morphs is quite variable and may often be no higher than divergence between populations of the same morph in allopatry (see Hindar *et al.*, 1991; Cross *et al.*, 1992; Skaala, 1992; Wood & Foote, 1996; Jones *et al.*, 1997; Bernatchez *et al.*, 1999; Gislason *et al.*, 1999; Gustafson & Winans, 1999; Boula *et al.*, 2002). Our results indicated pronounced genetic divergence between resident and anadromous brook charr in the Laval River drainage despite the absence of any physical barriers to gene flow between these populations and comparatively minimal geographic separation (see Jones *et al.*, 1997; Castric & Bernatchez, 2003, 2004). This observation, coupled with the short migrational distance, suggests that there are intrinsic mechanisms in operation for the maintenance of reproductive isolation and restriction to gene flow between these two populations. We propose that such mechanisms may have evolved as a consequence of selective pressures, resulting in genetically based adaptive differences between both populations.

Adaptive differentiation in juvenile phenotype between anadromous and resident brook charr

We found that maternal quantitative genetic differentiation between embryonic anadromous and resident Laval River brook trout was also high ($Q_{ST} > 0.5$) and was greater than neutral genetic divergence in our study for specific embryonic traits including length, yolk sac volume and growth rate for length. Our findings thus indicate that divergent natural selection has played a role in driving the differentiation between these two populations at the embryonic stage, which corroborates previous studies on quantitative differentiation in embryonic (Haugen & Vøllestad, 2000; Unwin *et al.*, 2000; Koskinen *et al.*, 2002) and adult salmonids including anadromous and resident brook charr (Wilder, 1952; Mullan, 1958; Power, 1980; Boula *et al.*, 2002). Notably, quantitative population structure tends to exceed that of neutral variation (McKay & Latta, 2002; Rieseberg *et al.*, 2002; Steinger *et al.*, 2002), particularly for morphological characters (Merilä & Crnokrak, 2001). We note that since the same fish were not used for both F_{ST} and Q_{ST} analysis, there may be some bias in our results in relation to population substructure. However, the latter were composed of half sib families descended from the same parental subset, which would have necessarily been a function of within-family genetic variance rather than a representation of averaged inter-populational neutral differentiation.

Embryonic phenotype in salmonids is primarily a function of maternal genetic variance, which is considerable early in development (Ojanguren *et al.*, 1996; Bonnet *et al.*, 1999; Heath *et al.*, 1999; Perry *et al.*, 2004). High maternal genetic control at this stage is most likely dictated by the higher maternal stake in embryonic fitness via energetic investment (Bernardo, 1996) which may exceed that of males by a factor of ten (Fleming, 1998; see also Mousseau & Fox, 1998; Fleming & Reynolds, 2004). Although anadromy appears to delimit this reproductive investment (Kinnison *et al.*, 2001; Kinnison *et al.*, 2003; Hendry *et al.*, 2004b), relative embryo and yolk sac size in the progeny of our anadromous dams were higher than that of female residents. Higher investment by anadromous individuals might

represent an overcompensation for saline mortality in order to offset costs of marine migration and/or predation in transit to the spawning site, while residents adopt a more cautious tactic for reproductive investment (see Crespi & Teo, 2002) given lower instantaneous mortality rates (Rikardsen *et al.*, 1997).

High maternal genetic differentiation of embryonic phenotype, irrespective of energetic constraints, might also be necessary for local adaptation to early rearing habitat. 'Phenotype/habitat matching' as proposed by Hendry *et al.* (2001) suggests that larger females should produce more and larger eggs for reasons of ecological advantage/necessity in the selection of spawning sites (i.e. gravel size, oxygen saturation; Holtby & Healey, 1986; Quinn *et al.*, 1995; Hendry *et al.*, 2001; Einum *et al.*, 2002), although maternal-environmental covariance ($\sigma_{m,e}$) is typically assumed as zero in standard genetic variance component analysis (see Roff, 1997; Lynch & Walsh, 1998). For example, maternal-external environmental correlation could be partially imposed as a necessary adaptation to functions of passive oxygen uptake across the chorion, limiting spawning by anadromous females to superior habitat reaches and/or gravel types or conversely providing anadromous females reproductive fitness advantages for having larger eggs in these specific habitats (Quinn *et al.*, 1995; Hendry *et al.*, 2001; Einum *et al.*, 2002). Prehatch asphyxiation might result for larger eggs in poor environments from critical surface area/volume ratios (Chapman, 1988; but see also Einum & Fleming, 2000b; Einum *et al.*, 2002). Correspondingly, large anadromous females would have significant advantages in competition for such superior redd sites (Foote, 1990; Fleming & Gross, 1994) and/or be more capable of ecological risk-management behaviours for increased offspring survival (such as digging deeper redds to limit loss from scour; Steen & Quinn, 1999) to maximize returns on their inherently higher reproductive investment. In this event, female philopatry might be partially necessary to ensure appropriate coadaptation of maternal reproductive characteristics, zygotic/embryonic phenotype and early developmental traits to the local natal stream environment.

Alevin stage

Contrasting with the embryonic period, maternal Q_{ST} for post-resorption morphological traits (and for all traits on the paternal side) was effectively zero, suggesting that the effect of divergent selection on size traits between anadromous and resident brook trout is not important at the alevin stage. This may be partially reflective of low (but nonzero) maternal quantitative genetic variation at this point (Perry *et al.*, 2004). There is little known about phenotypic differentiation between the anadromous and resident brook charr during early juvenile development but morphological differentiation in alevins prior to age 1+ may be undetectable in the brook charr (Morinville, unpublished; Perry, unpublished) although not necessarily in other salmonids (see Wood & Foote, 1990; Wood *et al.*, 1999). Despite our finding of little quantitative genetic divergence between resident and anadromous alevins, phenotypic differentiation between resident and anadromous salmonids at adulthood is considerable (Wilder, 1952; Mullan, 1958; Power, 1980; Hindar *et al.*, 1991; Cross *et al.*, 1992; Bernatchez *et al.*, 1999; Dynes *et al.*, 1999; Boula *et al.*, 2002), suggesting that such differentiation is in development at some point prior to maturity. In the brook charr, this would appear to be initiated shortly after age 1+ (Thériault & Dodson, 2003; Perry *et al.*, unpublished). Our results suggest that early alevin length in anadromous and resident brook charr may be subject to stabilizing selection in stark contrast with both divergent selection operating during embryonic development and with quantitative differentiation developing from the late juvenile state up to maturation for the purposes of seawater adaptation or residency. A breakpoint in fitness functions associated with egg and early juvenile size alternate prior to and after hatching has been suggested (see Hendry *et al.*, 2001; Einum *et al.*, 2002; Hendry & Day, 2003; Einum *et al.*, 2004). For example larger post-hatch individuals, in contrast to during the embryonic period, would have higher surface area for a given volumetric size via active gill respiration and passive absorption over the epidermal surface (Rombough & Moroz, 1990) in addition to superior mobility for avoiding predation (Hale, 1999) and potential competitive advantages associated with foraging (Johnsson *et al.*, 1999). Directional selection on embryo size between resident and anadromous dams related to energetic tradeoffs of for the exploitation of advantageous spawning habitat might be relatively unimportant at this life stage. On the other hand, strong divergent differentiation on embryo size could be replaced by new stabilizing fitness surfaces for embryo size during mid-late juvenile period for the establishment of feeding territories, at which point size does again begin to play a role in inter-individual competition (i.e. Johnsson *et al.*, 1999). Stabilizing selection at this period does not appear to have been investigated specifically although negative genetic covariance between maternal and progeny genetic value for length has been detected (Perry *et al.*, 2004; see also Hutchings, 1993). In conjunction with the above, our overall results suggest that selection on size during the alevin-subadult periods is more stabilizing relative to directional differentiation at the embryonic and adult periods.

Familial traits

For traits that were measured at the familial (rather than individual) level, maternal Q_{ST} was higher than paternal differentiation for all characters with the exception of survival after eye-up. Overall however, Q_{ST} confidence

intervals for all familial phenotype were quite wide and most appeared to be highly left-skewed, overlapping with zero for all but development time to first response to feeding and age at full feeding. Few data points were available for these analyses ($n = 23$) and it seems likely the bootstrap procedure created large CI ranges via the chance selection of specific observations. The CIs for our familial life history traits may not thus be completely representative of the actual significance of differentiation. Notably, significant maternal differentiation was observed for development time to first response to feeding and to full feeding (although Q_{ST} was significantly higher than F_{ST} only for the former trait), suggesting the indirect involvement of maternal genetic variance (and selection divergence) for embryonic provisioning in the timing of the integration of external energetic input.

Conclusions

In summary, substantial maternal Q_{ST} (relative to F_{ST}) for embryonic morphology suggests that embryonic selection is overwhelmingly directional rather than stabilizing at the individual level. Our findings, in conjunction with adult phenotypic differentiation between anadromous and resident brook charr (Wilder, 1952; Mullan, 1958; Power, 1980), suggest that quantitative genetic differentiation for morphological traits (and presumably the role of selection in shaping such differentiation) between anadromous and resident brook charr might be divisible into the three major salmonid life cycle phases: embryonic (characterized by strong divergent selection for size traits based on maternal genetic variance), alevin (characterized by stabilizing selection for size traits), and late juvenile-adult life history (characterized again by divergent selection, based on individual genetic variance, for maturity, physiology and morphology). Ideally, the definition of Q_{ST} should therefore be an inclusive estimate of genetic divergence in functional characters. However, our results suggest that it may be necessary for interpretations of the statistic to consider the structure of quantitative genetic architecture by age category and the sex of the parent used in estimation. Given that additive quantitative genetic variation for early juvenile characters is likely determined by a complex suite of ontogenetically changing maternal, paternal and individually-expressed genetic variation (i.e. Hebert *et al*., 1998; Heath *et al*., 1999; Pakkasmaa & Jones, 2002; Perry *et al*., 2004; Perry *et al*., in review), it would appear that the role of directional selection in shaping patterns of phenotypic differentiation at early development is highly specific to the context of trait, period and the nature of the model.

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Appendix 1 PCR conditions and sources of primers for nine microsatellite used to identify neutral genetic differences between anadromous and resident brook charr from the Laval/Adams Brook system.

Locus name	Gel	Reaction	T_A	Dye	F (nmol)	R (nmol)
SfoB52	1	1	56	6-Fam	75	250
SfoC28	1	1	56	Tet	100	250
SfoC88	1	1	56	Hex	250	250
SfoC113	1	1	56	6-Fam	50	250
SfoC129	1	1	56	Tet	100	250
SfoD100	2	2	58	6-Fam	50	250
SfoC115	2	2	58	Tet	120	250
SfoD75	2	2	58	Tet	20	250
Ssa197DU	2	3	58	Hex	200	250

Sfo primers (B–D series) were developed by, Tim King, USGS-BRD (Leetown Science Center). *Ssa197DU* was sourced from O'Reilly *et al.* (1996).
Another locus, *SfoD105*, was included in the second multiplex (200 nmol F, 250 nmol R) but was not included in analysis due to scoring difficulties possibly based on large allele size.
F, forward primer; R, reverse primer.

Appendix 2 Juvenile brook charr families, November, 2002–January, 2003.

Sire	Dam	Dam weight (g)	Type
J043 (A)	J055 (A)	1600	AA
	J090 (R)	350	AR
J049 (A)	B014 (A)	1000	AA
	J077 (R)	230	AR
J050 (A)	J058 (A)	1600	AA
	J075 (A)	1900	AA
J052 (R)	J060 (A)	1500	RA
	J084 (R)	200	RR
J053 (A)	B052 (A)	1000	AA
	J088 (R)	250	AR
J054 (A)	J056 (A)	1800	AA
	J070 (R)	250	AR
J061 (R)	J067 (A)	1400	RA
	J082 (R)	200	RR
J065 (R)	J051 (A)	1900	RA
	J073 (R)	300	RR
J066 (A)	J079 (R)	250	AR
J068 (R)	J062 (A)	1400	RA
	J086 (R)	290	RR
J069 (R)	J072 (A)	800	RA
	J087 (R)	320	RR
J071 (R)	J059 (A)	1700	RA
	J076 (R)	250	RR

'Type' indicates AA, purestrain anadromous; RR, purestrain resident, AR, anadromous male × resident female; RA, resident male × anadromous female crosses.