

# Population size is weakly related to quantitative genetic variation and trait differentiation in a stream fish

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How population size influences quantitative genetic variation and differentiation among natural, fragmented populations remains unresolved. Small, isolated populations might occupy poor quality habitats and lose genetic variation more rapidly due to genetic drift than large populations. Genetic drift might furthermore overcome selection as population size decreases. Collectively, this might result in directional changes in additive genetic variation ( $V_A$ ) and trait differentiation ( $Q_{ST}$ ) from small to large population size. Alternatively, small populations might exhibit larger variation in  $V_A$  and  $Q_{ST}$  if habitat fragmentation increases variability in habitat types. We explored these alternatives by investigating  $V_A$  and  $Q_{ST}$  using nine fragmented populations of brook trout varying 50-fold in census size  $N$  (179–8416) and 10-fold in effective number of breeders,  $N_b$  (18–135). Across 15 traits, no evidence was found for consistent differences in  $V_A$  and  $Q_{ST}$  with population size and almost no evidence for increased variability of  $V_A$  or  $Q_{ST}$  estimates at small population size. This suggests that (i) small populations of some species may retain adaptive potential according to commonly adopted quantitative genetic measures and (ii) populations of varying sizes experience a variety of environmental conditions in nature, however extremely large studies are likely required before any firm conclusions can be made.

**KEY WORDS:** Additive genetic variation, adaptive potential, effective population size, habitat fragmentation, salmonid,  $Q_{ST}$ .

The theoretical expectation that small, fragmented populations of species will have a reduced adaptive potential relative to large populations is preeminent in evolutionary and conservation biology. It is based on the premise that (i) genetic variation is eroded more rapidly through drift and inbreeding as populations become small and isolated, and (ii) reduced genetic variation is negatively associated with adaptive potential (Lande 1988; Frankham 1996; Reed and Frankham 2003). Nevertheless, the actual relationship between genetic variation and population size in nature remains unresolved (Willi et al. 2006). Moreover, genetic drift is frequently assumed to overcome selection at small effective population size ( $N_e$ ) (i.e., via  $N_e \times s$ , where  $s$  is the selection differential). Yet rarely is it considered how habitat fragmentation might alter selective pressures in addition to the adaptive genetic characteristics of

fragmented populations as population size decreases (Willi et al. 2007; Willi and Hoffman 2012; Wood et al. 2014).

Several methodological issues might explain the disparity among previous studies regarding the relationship between population size and genetic variation in nature. Studies either compared a very small number of populations (Widen and Andersson 1993; Waldmann 2001), assumed that neutral marker diversity is a surrogate for quantitative genetic variation (see Reed and Frankham 2001), or examined genetic variation relative to census population size ( $N$ ) instead of effective population size ( $N_e$ ) (Waldmann and Andersson 1998; Meyer and Allen 1999; Podolsky 2001). The latter is important because  $N_e$ , not  $N$ , reflects the proportion of individuals contributing genetically to the next generation and influences the extent of genetic drift and inbreeding. Moreover,



$N$  and  $N_e$  are frequently assumed to be correlated (Willi et al. 2007), but  $N_e/N$  ratios vary widely among intraspecific populations, which can lead to erroneous conclusions when using  $N$  to infer the magnitude of  $N_e$  or vice versa (Palstra and Fraser 2012). Finally, empirical research on the relationship between quantitative genetic diversity and population size has been restricted to plants (Willi et al. 2006, references therein). Conclusions based on plants may not be easily extrapolated to vertebrates that exhibit substantial behavior (e.g., active dispersal, complex mate choice, inbreeding avoidance) that might alter the relationship between genetic diversity and population size.

Likewise, as population size diminishes, the relative influence of drift versus natural selection on adaptive variation and differentiation remains unclear. Between populations, this is assessed by comparing neutral marker differentiation ( $F_{ST}$ ) to quantitative trait differentiation ( $Q_{ST}$ ) (Merilä and Crnokrak 2001; Edelaar et al. 2011). When  $Q_{ST}$  deviates significantly from  $F_{ST}$ , selection is credited as the primary force causing differentiation among populations, whereas if  $Q_{ST}$  and  $F_{ST}$  do not differ, genetic drift, and selection cannot be disentangled (Merilä and Crnokrak 2001, but see Ovaskainen et al. 2011).  $Q_{ST}$  frequently exceeds  $F_{ST}$  in analyses, yielding the conclusion that directional selection is pervasive (Merilä and Crnokrak 2001). Yet there are caveats with these comparisons, including estimates of  $Q_{ST}$  based on small numbers of populations, traits or traits types (Merilä and Crnokrak 2001), and use of improper statistical methods for estimating  $Q_{ST}$  and its confidence intervals (O'Hara and Merilä 2005). Furthermore, the choice of marker for  $F_{ST}$  estimation can affect  $Q_{ST}/F_{ST}$  comparisons. For example, the high mutation rates of microsatellite loci that have often been used to estimate  $F_{ST}$  can drastically deflate  $F_{ST}$  and erroneously result in  $Q_{ST}$  being greater than  $F_{ST}$  (Edelaar and Björklund 2011).

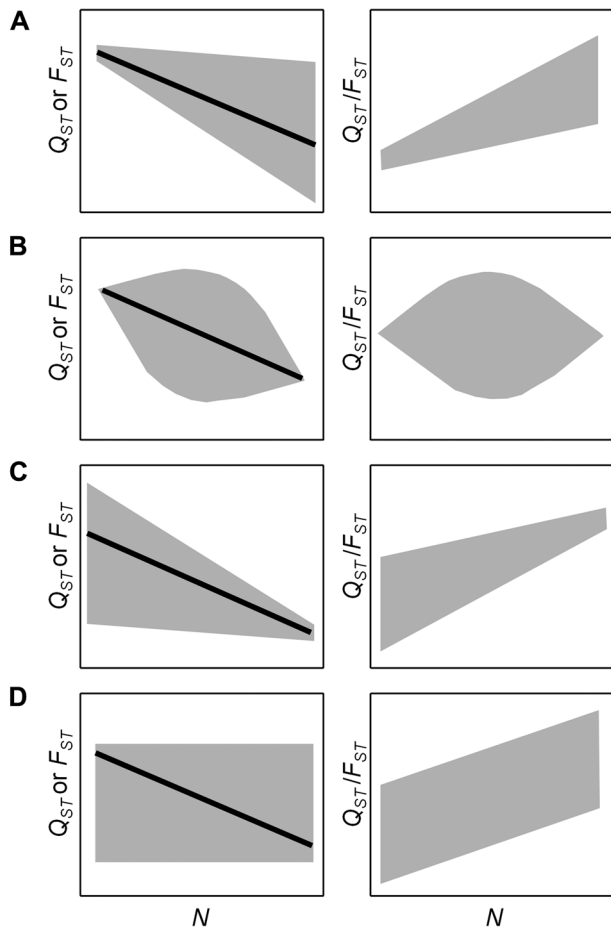
Here, we investigate two alternative hypotheses regarding the relationship between population size, quantitative genetic variation (measured as additive genetic variation,  $V_A$ ), and the relative role of drift versus selection in population differentiation ( $Q_{ST}$  vs.  $F_{ST}$ ). We compare  $V_A$  rather than narrow-sense heritability ( $h^2$ ) to population size as predictions about the role of selection and drift relate directly to  $V_A$  rather than  $h^2$  (Houle 1992; Hansen et al. 2011). The model system for this work is nine differentially abundant and fragmented populations of a stream fish (brook trout, *Salvelinus fontinalis*).

Under a first, "Directional Hypothesis" (Willi and Hoffman 2012; Wood et al. 2014) small populations are predicted to have consistently reduced  $V_A$  and adaptive potential relative to large populations. For instance, habitat fragmentation decreases population size while increasing isolation and environmental stress (e.g., Ward and Johnson 2005), and hence genetic diversity may be reduced due to the combined effects of restricted gene flow, drift, and inbreeding (e.g., Ouborg et al. 1991). Genetic drift

also imposes a directional element to the comparison of  $Q_{ST}$  and  $F_{ST}$  in relation to population size but the form of this relationship is dependent on assumptions regarding the characteristics of selective pressures acting on variously sized population pairs. For instance, drift might result in similarly high  $Q_{ST}$  and  $F_{ST}$  values among small population pairs (Willi et al. 2006) and decrease as population size increases with two possible outcomes for the ratio  $Q_{ST}/F_{ST}$ . One is that  $Q_{ST}/F_{ST}$  values might increase and also become more variable with increasing population size (Fig. 1A). This would occur if selection pressures and resulting  $Q_{ST}$  estimates are more variable, while  $F_{ST}$  decreases with increasing population size (Fig. 1A). A second outcome is  $Q_{ST}/F_{ST}$  will be similar among the smallest and largest population pairs but more variable among medium-sized pairs (Fig. 1B). This might occur if genetic drift results in  $Q_{ST} = F_{ST}$  at small population size and if large populations contain similar complements of habitat types such that  $Q_{ST}$  is consistently low and hence similar to  $F_{ST}$ .

Alternatively, under the "Variable Hypothesis" (Willi and Hoffman 2012; Wood et al. 2014), small population fragments are expected to be random samples of larger, more complex fragments. The process of habitat fragmentation might thus result in fragments that become increasingly dissimilar as they are reduced in size due to increased spatial variability in environmental conditions among fragments—and consequently, selection pressures—as fragment size and population size decreases. Hence,  $V_A$  might also be more variable among small fragments (e.g., Wood et al. 2014). In regard to  $Q_{ST}$  and  $Q_{ST}/F_{ST}$ , two outcomes are plausible under the variable hypothesis. First,  $Q_{ST}$  might be more variable among small population pairs (Fig. 1C). The ratio  $Q_{ST}/F_{ST}$  will also be variable but increase with increasing population-pair size due to the negative relationship between  $F_{ST}$  and population size (Fig. 1C). Or,  $Q_{ST}$  and the ratio  $Q_{ST}/F_{ST}$  might be equally variable among both small and large population pairs, but with  $Q_{ST}/F_{ST}$  increasing overall with increasing population size (Fig. 1D). This might be the case if fluctuating environmental conditions over long time periods result in complex, fluctuating selective pressures that ultimately yield a similar spread of  $Q_{ST}$  at all population sizes (Fig. 1D).

Our study is the first to explore, for a large number of populations of a vertebrate, the relationship between  $V_A$  and the relative effects of genetic drift and natural selection with population size (measured as adult census population size,  $N$  and the effective number of breeders,  $N_b$  a parameter which is closely associated with  $N_e$ ; Waples et al. 2013). Moreover,  $V_A$  and  $Q_{ST}$  were examined for a considerable number of traits across several trait categories including rarely examined behavior traits (see Carlson and Seamons 2008). Finally,  $F_{ST}$  was estimated using both microsatellite loci and single nucleotide polymorphisms (SNPs) to account for the potential downward bias of  $F_{ST}$  due to the polymorphic nature of microsatellites (Edelaar and Björklund 2011).



**Figure 1.** Four hypotheses for the relationship of  $Q_{ST}$ ,  $F_{ST}$ , and  $Q_{ST}/F_{ST}$  with population size. First,  $Q_{ST}$  and  $F_{ST}$  might be similar and high among small population pairs while  $Q_{ST}$  among large pairs will be either (A) more variable due to increased variability in selective pressures or (B) low and similar to  $F_{ST}$  if large populations contain similar complements of habitat types with variation in  $Q_{ST}$  being highest among medium-sized population pairs. Or,  $Q_{ST}$  might be more variable among small population pairs and  $Q_{ST}$  values among large populations will be either (C) low and similar to  $F_{ST}$  if large populations contain similar habitat types or (D) equally variable if large populations differ in selective regimes. Predictions a–d for  $Q_{ST}/F_{ST}$  are similar as for  $Q_{ST}$  with the exception that the negative relationship of  $F_{ST}$  with increasing population size results in an overall trend of increasing  $Q_{ST}/F_{ST}$  values with increasing population size. The solid line represents the mean relationship of  $F_{ST}$  with population size. The shaded areas represent the expected spread of  $Q_{ST}$  values (left column) and of  $Q_{ST}/F_{ST}$  values (right column) for each hypothesis.

## Materials and Methods

### STUDY SITE

Cape Race (CR), Newfoundland, Canada, is a region of coastal barren land traversed by a parallel series of low-order streams (0.27–8.10 km in length) that enable thorough sampling for  $N$

and  $N_b$  estimation, and which harbor resident, pristine brook trout populations. CR populations likely diverged from a common ancestor (10–12,000 ybp; Danzmann et al. 1998); all populations are genetically distinct and almost all are also isolated by virtue of terminating in a 30–50 m waterfall emptying directly into the sea (see also Wood et al. 2014). Possible exceptions in this study are the population pairs BF–WN and DY–UO for which occasional gene flow might occur (see Table S1 for population codes).

### GAMETE COLLECTION AND COMMON GARDEN EXPERIMENTAL DESIGN

Nine CR populations were monitored for spawning individuals via electrofishing in October 2011 (Table S1; for a map of CR populations see Wood et al. 2014). Breeding adults were gathered and placed in flow-through cages within the stream channel until gamete collections between 21h00 and 2h00 of the same evening (total number of females and males gathered and used in crosses per population = 16–30 and 14–29, respectively). Gametes were then transported to St. John's, Newfoundland in refrigerated coolers and air-shipped to Montreal, Quebec with a total transit time of approximately 10 hours.

Fertilization of gametes took place 10–14 hours after collection. The total fecundity of each female was subdivided into 2–7 egg lots with each lot being mixed with sperm from a different male of the same population (mean number of crosses per male = 2.5, range = 1–7). This process yielded 389 half-sib families or an average of 43.1 families per population (range = 17–64). CR females are small in size (mean length =  $138.3 \pm 28.6$  mm) and have low fecundity (mean number of eggs =  $82.8 \pm 53.9$  SD) such that mean family size was  $20.0$  eggs  $\pm 8.0$  SD (range = 3–50). Families were incubated separately within 5.2 cm diameter mesh-bottom containers placed randomly with respect to population within a single 1000 L recirculating tank and maintained at  $7.0 \pm 0.3^\circ\text{C}$  throughout the experiment. Eggs were left undisturbed until the eyed stage to reduce potential mortality following fertilization, at which point dead individuals were counted and removed daily. Dissolved oxygen and pH did not differ in different tank locations and were consistently maintained throughout the experiment ( $11.75 \pm 0.15$  SD and  $8.09 \pm 0.030$ , respectively). Across-population family mortality was generally low (mean =  $3.8$  families  $\pm 4.4$  SD) except for WC in which 14 families had zero survival. However, almost all of this mortality was in a small number of females indicating an issue with egg quality in these females. Across population family mortality without WC was  $2.5$  families  $\pm 2.2$  SD.

### TRAITS

#### Early life history

Six early life-history traits known to be related to individual fitness of salmonids were measured (Einum and Fleming 2000): (i) hatch

time, estimated as accumulated degree days from fertilization to hatch of all individuals within families; (ii) length at hatch (tip of the snout to the tip of the median rays of the tail); (iii) yolk sac volume at hatch (estimated as  $LH^2(\pi/6)$ , where  $L$  and  $H$  were the length and height of the yolk sac, respectively, following Koskinen et al. 2002); (iv) emergence length (when the yolk sac is “buttoned-up” into the body cavity); (v) yolk sac conversion efficiencies ((length at yolk absorption – length at hatch)/yolk sac volume), calculated using the family means in each population and (vi) relative family survival.

### Behavior

Three traits (pre-stimulus foraging, latency, post-stimulus foraging) relating to anti-predator behavior were assessed from 301 behavioral trials (mean number of trials per population =  $33.4 \pm 9.9$  SD) carried out from March 5th–27th, 2012. Traits were assessed using footage of individual behavioral observations captured by digital camcorders. This footage was then scored at a later date using a digital timer and hand-held tally counter. An average of 17.3 families (range 10–24, 159 total) from each CR population were evaluated; each family was represented by 3–16 individuals (depending on family size), selected randomly from holding containers and divided between one or two 30 L tanks in groups of 3–5. Prior to observations, a small amount of food was added to each tank and fish were left to acclimate for a period of 4 hours. Each observation consisted of a 5 minute pre-stimulus period during which the number of foraging attempts made by each focal fish was recorded (Brown et al. 2011). At the end of the 5-minute period, a predation attempt was simulated by introducing a predator model (a plastic bird head attached to a 45-cm plastic handle; Ferrari et al. 2010) to each tank for 5 seconds, after which the amount of time that elapsed until foraging resumed (latency) was calculated for each fish. This was followed by a second 5 minute post-stimulus period in which we recorded the number of foraging attempts. Foraging rates for the pre- and post-stimulus periods were estimated as the total number of forages attempted by each focal fish, divided by the observation time (5 minutes).

### Morphology

Landmark-based morphometrics were used to acquire data on morphology for individuals post yolk absorption. Rather than conducting a formal geometric morphometric analysis to assess body shape, we measured inter-landmark distances for 11 landmarks (Fig. S1) corresponding to seven different morphological traits that might reasonably differ among CR populations due to differences in environmental conditions such as prey regimes and flow characteristics (Taylor and McPhail 1985). An average of 6.0 individuals (range 2–14) per family per population (2107 individuals total, from 15–51 families per population) were

randomly sampled and anaesthetized non-lethally using MS-222. The number of families measured is lower than the number initially generated, as some families had an insufficient number of surviving individuals at this stage for meaningful trait data for  $V_A$  or  $Q_{ST}$  estimation. After being anaesthetized, each fish was positioned on its right side beneath a ruler with the caudal fin extended and subsequently photographed using a secured overhead digital camera. Morphological traits were then measured from digital photos imported into ImageJ (Rasband 2011).

### ADULT CENSUS POPULATION SIZE ( $N$ ) AND EFFECTIVE NUMBER OF BREEDERS ( $N_b$ )

Estimates of population size for each population in 2011 were adopted from Wood et al. (2014), based on  $N$  estimated using either the Schnabel (1938) or Peterson (1896) method and weighted harmonic  $N_b$  (three consecutive cohorts except for two in DY; Table S1) estimated using LDNe (Waples and Do 2008; see Wood et al. 2014 for details on  $N$  and  $N_b$  estimation)). Weighted harmonic  $N_b$  was strongly correlated with generational  $N_e$  for the five CR populations for which detailed life-history data were available (Wood et al. 2014; see Waples et al. 2013) and therefore  $N_b$  was used for all analyses.

### MOLECULAR GENETIC VARIATION

We used microsatellite and coding region SNP data from Wood et al. (2014) and Fraser et al. (2014) to calculate  $F_{ST}$  and its confidence intervals for comparison to  $Q_{ST}$  (13 and 163 polymorphic loci, respectively). Details on microsatellite analysis of CR populations can be found in Wood et al. (2014). Details of SNP development, validation, and sequencing are found in Sauvage et al. (2012).

Neutral genetic differentiation across populations and between population pairs at microsatellites and SNPs was quantified by estimating  $F_{ST}$  following Weir and Cockerham (1984) using tissue samples collected from wild fish sampled during the summer of 2011; associated 95% CI were estimated by bootstrapping over loci using FSTAT 2.9.3.2 (Goudet 1995). All SNPs detected to be putatively under selection using genome scans in Fraser et al. (2014) were removed before estimating  $F_{ST}$ . For microsatellite loci,  $F_{ST}$  calculated using all 13 loci or excluding loci potentially under selection for any population pairs (based on similar genome scans) generated similar results and were strongly correlated (Spearman's  $r = 0.98$   $P = <0.001$ ; results not shown). For these reasons, the inclusion of the few outlier loci among certain population pairs did not greatly influence overall  $F_{ST}$  estimates using microsatellites, and therefore all 13 loci were retained in the analyses.

## QUANTITATIVE GENETIC ANALYSIS

### Additive genetic variation and $Q_{ST}$

Additive genetic variation and  $Q_{ST}$  were estimated for each population using the offspring generated from our breeding crosses. This was achieved using pedigree data in conjunction with the animal model (Kruuk 2004). All traits were fitted with a Gaussian error distribution using Bayesian techniques implemented in the R v.3.1.0 package MCMCglmm (v.2.21; Hadfield 2010) with the exception of survival, which was modeled as a binary response variable using the family “categorical.” Variance components were estimated according to the model:

$$y = Xb + Z_1a + Z_2m + e,$$

where  $y$  is the vector of phenotypic trait values,  $b$  is the vector of fixed effects,  $a$  and  $m$  are the vectors of  $V_A$  and maternal effects ( $V_M$ ), respectively, and  $X$  and  $Z_{1-2}$  are matrices that relate the fixed and random effects to the observed trait values (Lynch and Walsh 1998; Kruuk 2004). Inverse Wishart priors were used for all traits except survival for which a prior with fixed residual variance and random effects corresponding to a marginal Cauchy distribution was specified ( $\Gamma(0.5, 0.0164)$ ; Fong et al. 2010). For morphological traits, total length was also included as a covariate to account for the potential effects of body size on morphology (Fraser et al. 2010). MCMC chains for  $V_A$  were run for 1,000,000 iterations with a burn period of 300,000 and thinning interval of 50, hence parameters and associated confidence intervals were based on sampling the posterior distribution 14,000 times. Model convergence and mixing were verified by visual examination of the posterior traces and autocorrelation values; Heidelberg and Welch stationarity tests were also conducted. Since lower limits of variance components estimated by MCMCglmm are necessarily bounded above zero, we carefully inspected the posterior distributions of  $V_A$  for evidence that the variances differed from zero; significance was indicated where posterior modes departed from zero and the 95% CIs did not converge to zero.

$V_A$  is only one of several existing measures of evolvability, therefore we also calculated the narrow sense heritability ( $h^2$ ; variance standardized) and the mean standardized additive genetic variation,  $I_A$  (Houle 1992) across populations and traits for comparison with  $V_A$ .

To estimate  $Q_{ST}$  among population-pairs an additional random effect for population was added to the models to obtain an estimate of the between population component of  $V_A$ . Here, we adopted proper priors that partitioned the total variance equally among the random effects with  $nu = 1$ ; this prior resulted in better mixing of the between-population variance component than the Inverse Wishart prior that yielded occasional extreme values in the posterior distribution, likely because of the small sample size. MCMC chains for  $Q_{ST}$  were run for 1,000,000 iterations with a

thinning interval of 500 such that estimates and confidence intervals were based on 1400 samples of the posterior distribution; results were similar using a thinning interval of 50 or 500, therefore 500 was used to reduce computation time.  $Q_{ST}$  was estimated as  $\sigma^2_{GB}/(\sigma^2_{GB} + 2\sigma^2_{GW})$ , where  $\sigma^2_{GB}$  and  $\sigma^2_{GW}$  represent the between- and within-population components of  $V_A$ , respectively (Merilä and Crnokrak 2001).

## STATISTICAL ANALYSIS

### Directional hypothesis

We used Pearson's correlations to determine whether a directional relationship existed between population size ( $N$  or  $N_b$ ) and  $V_A$  or  $V_M$  for individual traits. To provide a more robust test than correlating the point estimates of  $V_A$  and  $V_M$  alone, we combined the nine posterior probability distributions of  $V_A$  or  $V_M$  into a single data frame, constructed a corresponding data frame of population size then estimated the correlation coefficient  $r$  for each row of the data frame (14,000 estimates of  $r$ ). Then, we calculated the mode and 95% confidence intervals of the posterior distribution of  $r$  and judged the significance based on whether the confidence intervals spanned zero. We investigated the relationship of  $V_M$  with population size to determine whether there was evidence for consistent differences in maternal effects between small and large CR populations. This might occur if maternal egg provisioning is influenced by conditions within habitat fragments, which in turn might be dictated by fragment size as described by the directional or variable hypotheses.

Because  $Q_{ST}$  and  $F_{ST}$  are presented as matrices of genetic distances between pairs of populations, individual estimates are not independent of each other. Thus, simple (Mantel 1967) and partial (Smouse et al. 1986) Mantel tests were used to determine the relationship of  $F_{ST}$ ,  $Q_{ST}$ , and  $Q_{ST}/F_{ST}$  with pairwise mean  $N$  and  $N_b$ . Although the harmonic mean population size scales more closely with the effects of genetic drift (Crow and Kimura 1970), some large-small population pairs in our study had a combined population size that was extremely small when the harmonic mean was used. Therefore, we use the arithmetic mean of pairwise  $N$  and  $N_b$ . Simple Mantel tests were used to examine the correlation between  $F_{ST}$  and  $Q_{ST}/F_{ST}$  with mean population size whereas partial Mantel tests were used to determine if  $Q_{ST}$  was related to population size after controlling for  $F_{ST}$ . We also examined patterns of  $Q_{ST}$  and  $Q_{ST}/F_{ST}$  for similar-sized population pairs only using Spearman's correlations to determine if large-small population pairs might have influenced the outcome of our analyses; however, since the data points are not independent as explained above, this was for exploration purposes only.

### Variable hypothesis

To investigate whether there was increased variability in  $V_A$ ,  $V_M$ ,  $Q_{ST}$ , and the ratio of  $Q_{ST}/F_{ST}$  at small population size, White's test

was used ( $P$ -value of the corresponding test statistic =  $W$ - $p$  below) to determine whether the residual variance of each parameter exhibited heteroscedasticity in relation to  $N$  or  $N_b$ . White's test works by implementing an auxiliary regression analysis that regresses the squared residuals from the original regression model onto a set of regressors that contain the original regressors, the cross-products of the regressors, and the squared regressors (White 1980). We also examined heteroscedasticity of  $V_A$ ,  $Q_{ST}$ , and the ratio of  $Q_{ST}/F_{ST}$  in relation to small-small versus large-large population pairs only.

### *$Q_{ST}$ versus $F_{ST}$*

The most current method for comparing  $Q_{ST}$  to  $F_{ST}$  is a simulation-based resampling approach where  $Q_{ST}$  is compared to the distribution of neutral  $F_{ST}$  values (Whitlock and Guillaume 2009). This method however was designed specifically for fully nested breeding designs whereas our design is partially factorial. To implement this approach, we had to reduce the number of families in our design in such a way that it conformed to a fully nested scenario; this resulted in a drastic reduction in the number of families per population from which to estimate the  $Q_{ST}$ - $F_{ST}$  metric. Therefore, we do not report the results of the  $Q_{ST}$ - $F_{ST}$  analysis here, but instead present a qualitative exploration of  $Q_{ST}$  and  $F_{ST}$  based on comparison of point estimates and CIs.

## Results

### ADDITIVE GENETIC VARIATION

Inspection of the posterior distributions suggested that  $V_A$  was significant across populations for 10 of 15 traits except for yolk volume, yolk conversion efficiency, and the three behavioral traits where the 95% CIs for most populations were highly asymmetrical and the lower limits converged at zero. Comparisons among the nine populations showed that  $V_A$  for specific traits differed significantly between two or more of the populations in several cases (e.g., hatch time, emergence length); for many comparisons, however, CIs were either overlapping or wide such that there were few statistically significant differences in  $V_A$  among populations (Fig. 2 and Figs. S2–S4). We were unable to estimate  $V_A$  for survival to hatch in CR brook trout populations (models including  $V_A$  resulted in poor traces and high autocorrelation); most of the variance in survival appears to be attributable to maternal effects therefore we only included survival in the analysis for  $V_M$ .

#### *Additive genetic and maternal variation: Directional and variable hypotheses*

There were no consistent directional trends between point estimates of  $V_A$  and population size (Table 1, Fig. 2, and Figs. S2–S4). Relationships for 8 of 15 traits with  $N_b$  and 7 of 15 traits with  $N$  were in the opposite direction as that predicted by theory with

$V_A$  actually increasing with population size reductions. Results of Pearson's correlations however, revealed no significant correlations between  $V_A$  and population size for any of the traits examined as in every case the 95% CIs of  $r$  spanned zero. Similarly,  $I_A$  and  $h^2$  exhibited primarily negative but nonsignificant relationships with  $N$  and  $N_b$  (Table S2, Figs. S5–S7, Table S3, and Figs. S8–S10). The sole exceptions were hatch time for which  $h^2$  increased significantly with increasing population size and  $I_A$  for yolk conversion efficiency that was positively and significantly related to  $N$  and  $N_b$ .

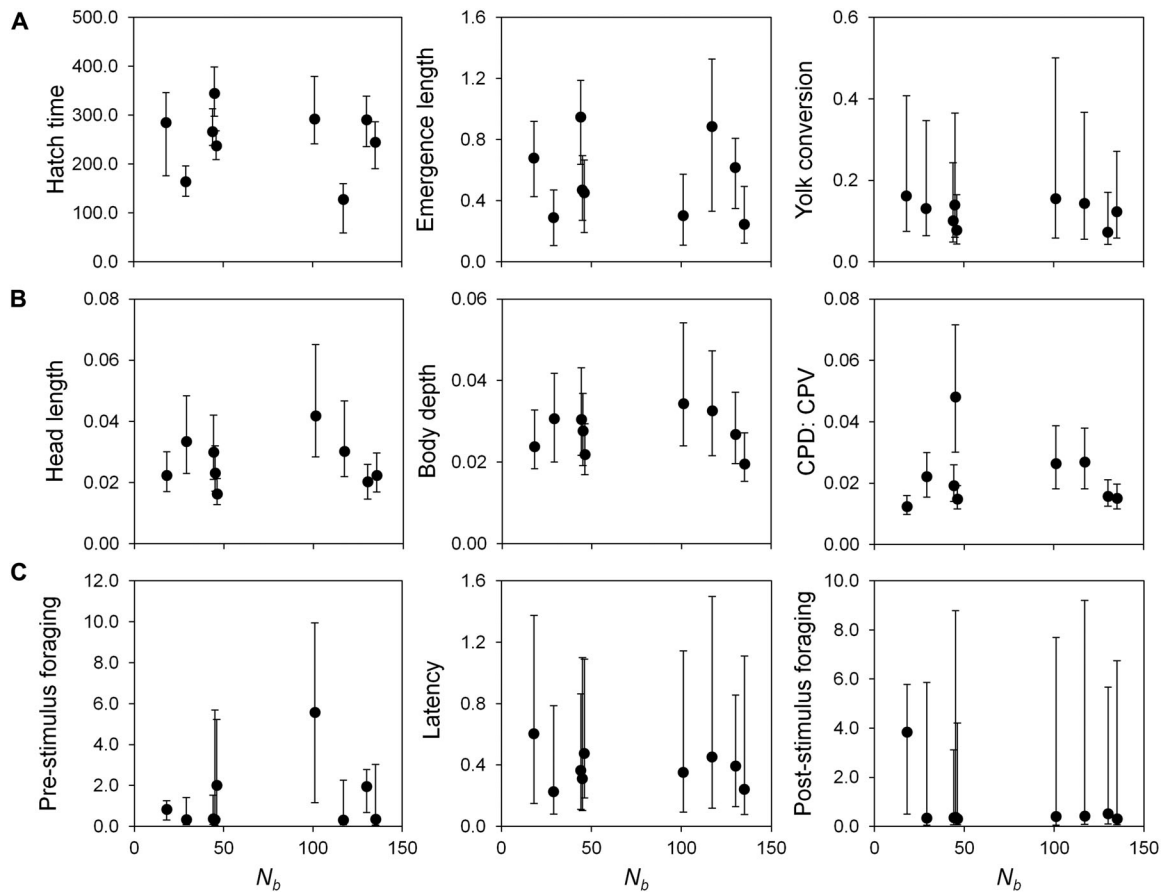
There was also little evidence for increased variation in  $V_A$  at small population size. Only 2 of 15 traits showed significant heteroscedasticity of  $V_A$  in relation to  $N_b$  (none for  $N$ ); examination of the residual plots revealed that the significant heteroscedasticity was at small population size (Table 1, Fig. 2, and Figs. S2–S4). No traits exhibited increased variation at small population size for either  $I_A$  or  $h^2$  (Table S2, Figs. S5–S7, Table S3, and Figs. S8–S10).

Most estimates of  $V_M$  did not appear to differ from zero (Figs. S11–S13), and there was also little evidence for directional differences in the point estimates of  $V_M$  with increasing population size. The only exception was hatch time for which the relationship of  $V_M$  and  $N$  was negative and significant.  $V_M$  was furthermore equally variable at all population sizes for all traits (Table 1 and Figs. S11–S13).

We tested the relationship of various quantitative genetic metrics with population size for a large variety of traits but we did not correct for multiple comparisons. Techniques that address the issue of multiple testing typically work by applying a more stringent significance threshold for individual tests; we did not feel that this correction would add anything meaningful to the interpretation of our results as the vast majority of our tests did not approach significance.

### NEUTRAL GENETIC DIFFERENTIATION

$F_{ST}$  estimates across the nine populations were large and significant with microsatellites and SNPs. Mean  $F_{ST}$  for SNPs was significantly greater than for microsatellites (0.38 vs. 0.25, Fig. 3), however the correlation between  $F_{ST}$  estimates from the two sources among all pairwise population comparisons was high (Spearman's  $r = 0.91$ ,  $P < 0.001$ ).  $F_{ST}$  decreased with increasing mean population size but the relationships were not significant ( $N_b$ ;  $r_M = -0.54$ ,  $P = 0.98$  for microsatellites, and  $r_M = -0.37$ ,  $P = 0.89$  for SNPs and  $N$ ;  $r_M = -0.38$ ,  $P = 0.91$  for microsatellites and  $r_M = -0.31$ ,  $P = 0.84$  for SNPs, Fig. 4). There was also no evidence of increased variation in  $F_{ST}$  at small population size for SNPs ( $N_b$ ;  $W$ - $p = 0.54$ , and  $N$ ;  $W$ - $p = 0.87$ ) or microsatellites ( $N_b$ ;  $W$ - $p = 0.88$ , and  $N$ ;  $W$ - $p = 0.74$ ) (Fig. 4). When considering only similar-sized population pairs,  $F_{ST}$  decreased significantly with increasing  $N_b$  ( $F_{ST}$  SNPs:  $r = -0.67$ ,  $P = 0.0043$ , and microsatellite  $F_{ST}$ :  $r = -0.74$ ,  $P = 0.0011$ ) but not with  $N$  ( $F_{ST}$



**Figure 2.**  $V_A$  versus  $N_b$  for nine traits representing (A) life history, (B) morphological, and (C) behavioral trait classes.  $V_A$  plots for all traits with both  $N_b$  and  $N$  are found in Figs. S2–S4.

SNPs:  $r = -0.44$ ,  $P = 0.094$ , and microsatellite  $F_{ST}$ :  $r = -0.47$ ,  $P = 0.068$ ), and there was no difference in the spread of  $F_{ST}$  (all  $W$ - $p > 0.16$ ).

#### $Q_{ST}$ : ALL POPULATIONS

$Q_{ST}$  estimated across all nine populations revealed significant quantitative trait differentiation for all traits analyzed (Fig. 3). Morphological traits tended to be the most differentiated among populations (mean  $Q_{ST} = 0.48$ , range 0.34–0.87) followed by life-history traits (mean  $Q_{ST} = 0.32$ , range 0.17–0.56), while behavioral traits showed the lowest levels of among population differentiation (mean  $Q_{ST} = 0.15$ , range 0.037–0.27). Among the 15 traits investigated, only two (pre-stimulus foraging, and eye diameter) had  $Q_{ST}$  values that differed from  $F_{ST}$  for both SNPs and microsatellites.  $Q_{ST}$  for pre-stimulus foraging was lower than  $F_{ST}$ , while  $Q_{ST}$  for eye diameter was greater than  $F_{ST}$ .

#### $Q_{ST}$ : PAIRWISE COMPARISONS

##### General trends

$Q_{ST}$  estimates among populations pairs were also higher for morphological traits (Mean  $Q_{ST} = 0.53$ , range 0.27–0.68) than for

life-history traits ( $Q_{ST} = 0.33$ , range 0.12–0.53) or behavioral traits ( $Q_{ST} = 0.18$ , range 0.10–0.27) (Fig. S14). Confidence intervals however, were wide and overlapping for all pairs and all traits. There was also no difference between  $Q_{ST}$  and pairwise  $F_{ST}$  estimated using either genetic marker as CIs were overlapping in all cases.

##### Pairwise $Q_{ST}$ : Directional and variable hypotheses

After correcting for  $F_{ST}$ , mean  $Q_{ST}$  for life-history traits was significantly related to mean  $N_b$  ( $r_M = 0.53$ ,  $P = 0.02$ ) but not to mean  $N$  ( $r_M = 0.34$ ,  $P = 0.14$ ). Mean  $Q_{ST}$  was not significantly related to either population size metric for behavioral traits ( $N_b$ ;  $r_M = -0.12$ ,  $P = 0.65$ , and  $N$ ;  $r_M = -0.27$ ,  $P = 0.82$ ) or for morphological traits ( $N_b$ ;  $r_M = 0.15$ ,  $P = 0.27$ , and  $N$ ;  $r_M = 0.22$ ,  $P = 0.19$ ) (Fig. 4 and Fig. S14). For traits considered individually, there was little evidence that  $Q_{ST}$  was related to mean population size as only 3 of 15 traits across the three trait classes exhibited a significant correlation with mean  $N_b$  (hatch time, yolk volume, and yolk conversion efficiency) while one trait was significantly related with mean  $N$  (emergence length; Table 2 and Figs. S15–S20). Likewise only 1 of 15 traits with mean  $N_b$  and 2 of 15 traits

**Table 1.** Pearson's correlations (Directional hypothesis) and White's test results (Variable hypothesis) for  $V_A$  versus  $N_b$  and  $N$  and Pearson's correlations for  $V_M$  versus population size for 16 traits measured using nine brook trout populations at Cape Race, Newfoundland.

Trait class	Trait	$V_A$				$V_M$			
		$N_b$		$N$		$N_b$		$N$	
		$r$	W- $p$	$r$	W- $p$	$r$	W- $p$	$r$	W- $p$
Life history	Hatch time	−0.18	0.84	0.13	0.77	−0.26	0.51	−0.43*	0.31
	Hatch length	−0.083	0.62	−0.14	0.83	0.25	0.075	0.058	0.55
	Yolk volume	−0.53	0.43	−0.45	0.24	0.32	0.94	0.38	0.90
	Emergence length	−0.090	0.74	−0.018	0.74	−0.24	0.070	−0.14	0.69
	Yolk conversion	−0.10	0.78	−0.073	0.72	0.22	0.079	0.24	0.072
	Survival	NA	NA	NA	NA	−0.16	0.35	−0.18	0.57
Morphology	Head length	0.13	0.28	0.058	0.47	0.22	0.17	0.29	0.12
	Head width	0.16	0.28	0.23	0.27	0.25	0.099	0.31	0.066
	Eye diameter	0.21	0.30	0.11	0.20	0.22	0.31	0.30	0.27
	Body depth	0.19	0.34	0.20	0.098	0.22	0.24	0.30	0.15
	ADP: CPD	0.26	0.19	0.087	0.33	0.22	0.098	0.29	0.064
	ADP: CPV	0.33	0.45	0.15	0.42	0.22	0.39	0.30	0.15
	CPD: CPV	−0.10	0.58	−0.12	0.71	0.21	0.11	0.31	0.073
Behavior	Pre-stimulus foraging	−0.16	0.21	−0.12	0.22	−0.21	0.22	−0.023	0.35
	Latency	0.091	0.034†	0.12	0.45	−0.24	0.23	−0.26	0.39
	Post-stimulus foraging	−0.11	0.047†	−0.27	0.65	−0.23	0.29	−0.12	0.25

\*95% confidence intervals did not span zero.

†Significant heteroscedasticity located at small population size.

NA =  $V_A$  for survival to hatch could not be estimated for Cape Race populations.

with mean  $N$  (yolk conversion, post-stimulus foraging, and hatch time, respectively) were significantly correlated with population size using only similar-sized population pairs (Table S4 and S5).

White's test results for  $Q_{ST}$  versus mean  $N_b$  and mean  $N$  revealed little evidence of increased variation at small population size. Mean  $Q_{ST}$  was homogeneous across population sizes for all three trait classes (life-history traits vs.  $N_b$ :  $W-p = 0.80$  and  $N$ :  $W-p = 0.66$ , behavioral traits vs.  $N_b$ :  $W-p = 0.37$  and  $N$ :  $W-p = 0.56$ , and morphological traits vs.  $N_b$ :  $W-p = 0.30$  and  $N$ :  $W-p = 0.75$ ). Across individual traits, 3 of 15 and 1 of 15 exhibited significant heteroscedasticity with mean  $N_b$  and mean  $N$ , respectively, and the same results were obtained using only similar-sized pairs (Table 2 and Table S4 and S5); examination of residual plots showed that for five of the eight cases (emergence length with mean  $N_b$ , eye diameter with both mean  $N$  and  $N_b$ , and emergence length and ADP: CPV with mean  $N_b$  using only similar-size pairs) the increased variation was at small population size.

#### RATIO OF $Q_{ST}/F_{ST}$

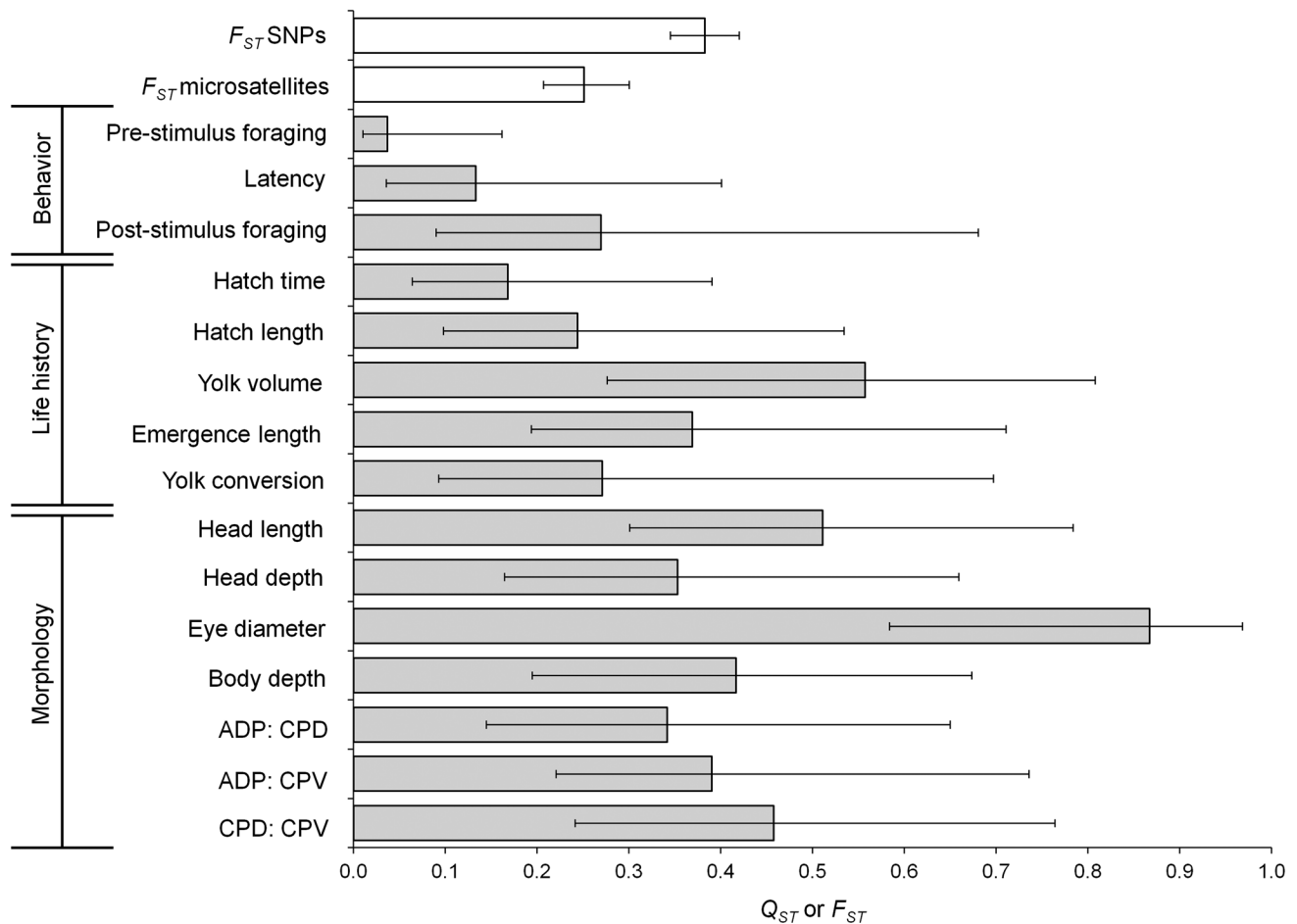
##### Directional hypothesis

For both genetic markers, the mean ratio of  $Q_{ST}/F_{ST}$  was not significantly related to population size (only SNP results reported here) for life-history traits (mean  $N_b$ :  $r_M = 0.19$ ,  $P = 0.13$ , and

mean  $N$ :  $r_M = 0.19$ ,  $P = 0.15$ ), morphological traits (mean  $N_b$ :  $r_M = 0.11$ ,  $P = 0.22$ , and mean  $N$ :  $r_M = 0.19$ ,  $P = 0.15$ ) or behavioral traits (mean  $N_b$ :  $r_M = 0.12$ ,  $P = 0.21$ , and mean  $N$ :  $r_M = 0.064$ ,  $P = 0.37$ ) (Fig. 5 and Fig. S21; see also Table 6 for microsatellite results).  $Q_{ST}/F_{ST}$  for both genetic markers was only significantly related to mean population size in one case (microsatellite based  $Q_{ST}/F_{ST}$  for eye diameter vs. mean  $N$ ; Table 3 and Figs. S22–S27) while 3 of 30 and 5 of 30 traits were significantly related to mean  $N_b$  and  $N$  using similar-sized pairs across  $F_{ST}$  estimated using both types of genetic markers (Table S4 and S5).

##### Variable hypothesis

The spread of residuals for mean  $Q_{ST}/F_{ST}$  was similar across population sizes for the three trait categories, using both genetic markers (mean  $N_b$ : all  $W-p > 0.43$ , and mean  $N$ : all  $W-p > 0.45$ ). Likewise, heteroscedasticity for individual traits did not differ with mean population size in 60 individual White's tests conducted across the two genetic marker types and two population size measures (Table 3). Contrastingly, 7 of 30 White's tests for both mean  $N_b$  and  $N$  across both genetic markers exhibited evidence of heteroscedasticity between similar-sized pairs, but in all cases the increased variation was among large population pairs (Table S4 and S5).



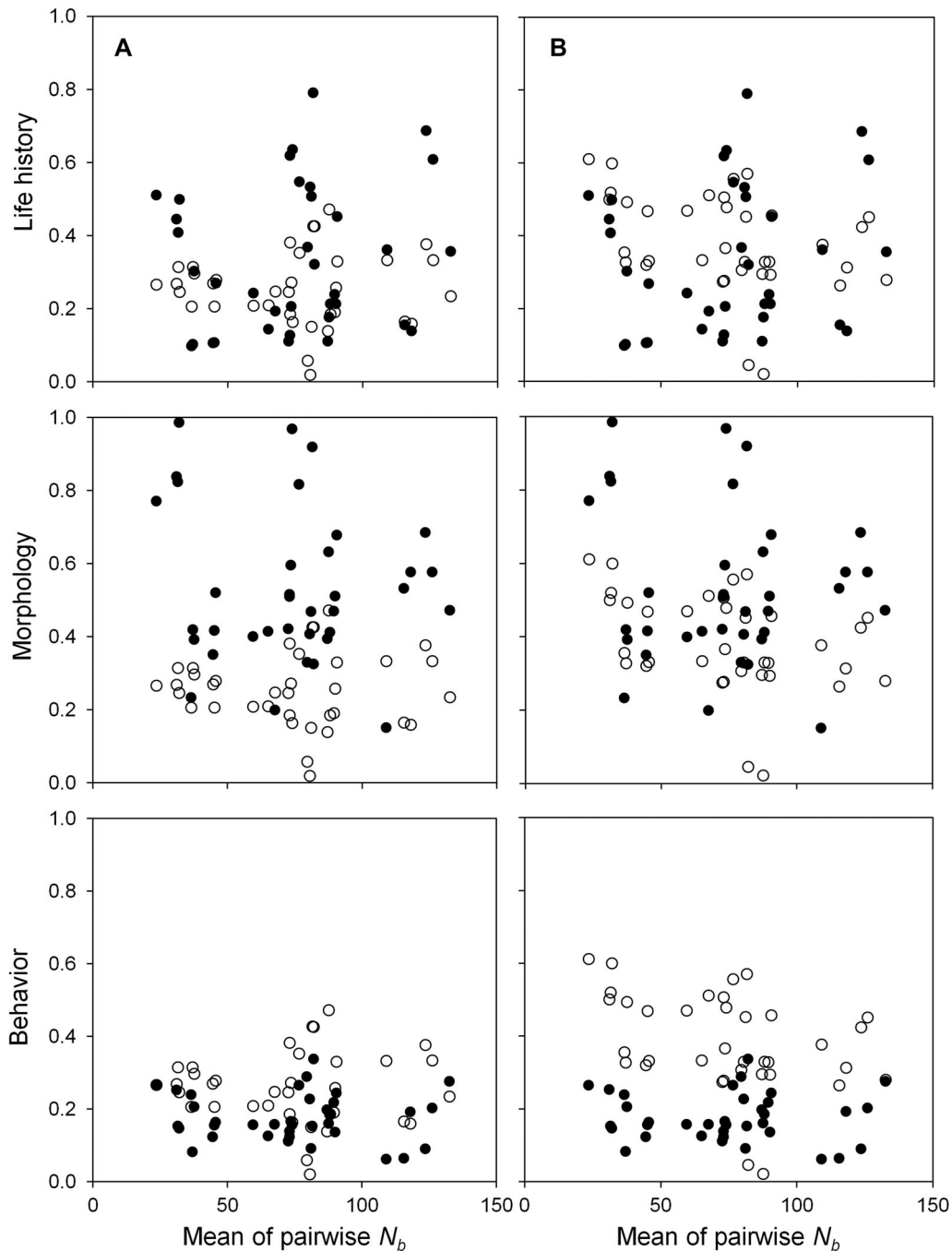
**Figure 3.**  $F_{ST}$  and  $Q_{ST}$  estimated across nine Cape Race brook trout populations. Descriptions for coded morphological traits are found in Fig. S1.

## Discussion

We found no consistent differences in quantitative genetic variation and trait differentiation in relation to population size among natural brook trout populations. These results are intriguing because (i) our study populations had a nearly 50-fold difference in  $N$  (179–8416) and 10-fold difference in  $N_b$  (18–135); (ii) 15 traits from three different trait classes were evaluated, and (iii) a relatively large number of families and populations were assessed. In regards to the Directional hypothesis, small populations did not exhibit consistently reduced  $V_A$  relative to large populations, and in fact,  $V_A$  for a number of traits increased with decreasing population size, though none of the relationships were statistically significant. Similarly, small populations did not exhibit more variability in  $V_A$  as predicted by the Variable hypothesis: only 2 of 30 tests across the 15 traits and two population size measures demonstrated significant heteroscedasticity in relation to population size. Maternal variation for the different traits was also invariant between small and large populations, suggesting that

maternal effects contribute roughly equally to the resemblance between related individuals among our study populations.

$F_{ST}$  decreased with increasing population size as expected; the correlation was not significant for either  $N$  or  $N_b$  but  $F_{ST}$  did decrease significantly with increasing population size when considering only small and large  $N_b$  population pairs. Similarly, the relationship of  $Q_{ST}$  and also  $Q_{ST}/F_{ST}$  with population size was weak and nonsignificant for most of the traits investigated although  $Q_{ST}/F_{ST}$  did tend to increase with increasing population size as expected in all but one of the initial predictions (Fig. 1A, C, and D).  $F_{ST}$  estimates for both genetic markers were not more variable at small population size and evidence for increased spread in  $Q_{ST}$  at smaller population size was rarely found.  $Q_{ST}/F_{ST}$  more often exhibited increased variation among large compared to small population pairs, but this still constituted only a small number of the total number of comparisons (14 of 60 tests). Taken together, our results support the prediction that populations of varying sizes experience a variety of environmental conditions (Fig. 1D).



**Figure 4.** Mean  $Q_{ST}$  (●) and  $F_{ST}$  (○) versus  $N_b$  across traits in each of three trait categories.  $F_{ST}$  values among trout populations pairs were estimated using (A) microsatellite loci, and (B) SNPs for each trait. Relationships for mean  $Q_{ST}$  and  $F_{ST}$  with  $N$  are found in Fig. S14 and for individual traits in Figs. S15–S20.

This study is one of the first to simultaneously investigate  $V_A$ ,  $V_M$ , and  $Q_{ST}$  versus  $F_{ST}$  for a large number of traits from several trait categories on the same populations. Although confidence intervals were often wide, morphological traits tended to have higher  $Q_{ST}$  estimates relative to  $F_{ST}$ , possibly signaling divergent selective pressures acting on morphology in Cape Race trout populations. Conversely,  $Q_{ST}$  for behavioral traits tended

to be lower than  $F_{ST}$ , suggesting that the behavioral responses favored across the populations are similar. This latter result is particularly notable given the general paucity of data regarding behavioral traits for natural populations. The nature of  $V_A$  precludes comparisons across different traits and trait classes, so we also calculated the narrow sense heritability ( $h^2$ ; variance standardized) and the mean standardized additive genetic variation,

**Table 2.** Partial Mantel test (Directional hypothesis) and White's test results (Variable hypothesis) for  $Q_{ST}$  versus mean  $N_b$  and  $N$  for 15 traits measured using nine brook trout populations at Cape Race, Newfoundland.

Trait class	Trait	$N_b$		$N$	
		$r_M$	W- $p$	$r_M$	W- $p$
Life history	Hatch time	0.46*	0.0070†	0.36	0.080
	Hatch length	0.41	0.57	0.29	0.58
	Yolk volume	0.46*	0.44	0.36	0.35
	Emergence length	0.42	0.90	0.32	0.98
	Yolk conversion	0.50*	0.52	0.24	0.29
Morphology	Head length	−0.0033	0.72	−0.18	0.96
	Head width	−0.097	0.41	−0.089	0.63
	Eye diameter	0.35	0.018†	−0.34	0.012†
	Body depth	0.18	0.42	0.029	0.84
	ADP: CPD	0.30	0.46	−0.012	0.85
	ADP: CPV	0.12	0.16	0.46	0.31
	CPD: CPV	0.28	0.055	0.15	0.12
Behavior	Pre-stimulus	−0.016	0.55	0.48	0.54
	Latency	−0.080	0.0018	0.33	0.26
	Post-stimulus foraging	−0.37	0.70	0.15	0.89

\* $<0.05$ .

†Significant heteroscedasticity located at small population size.

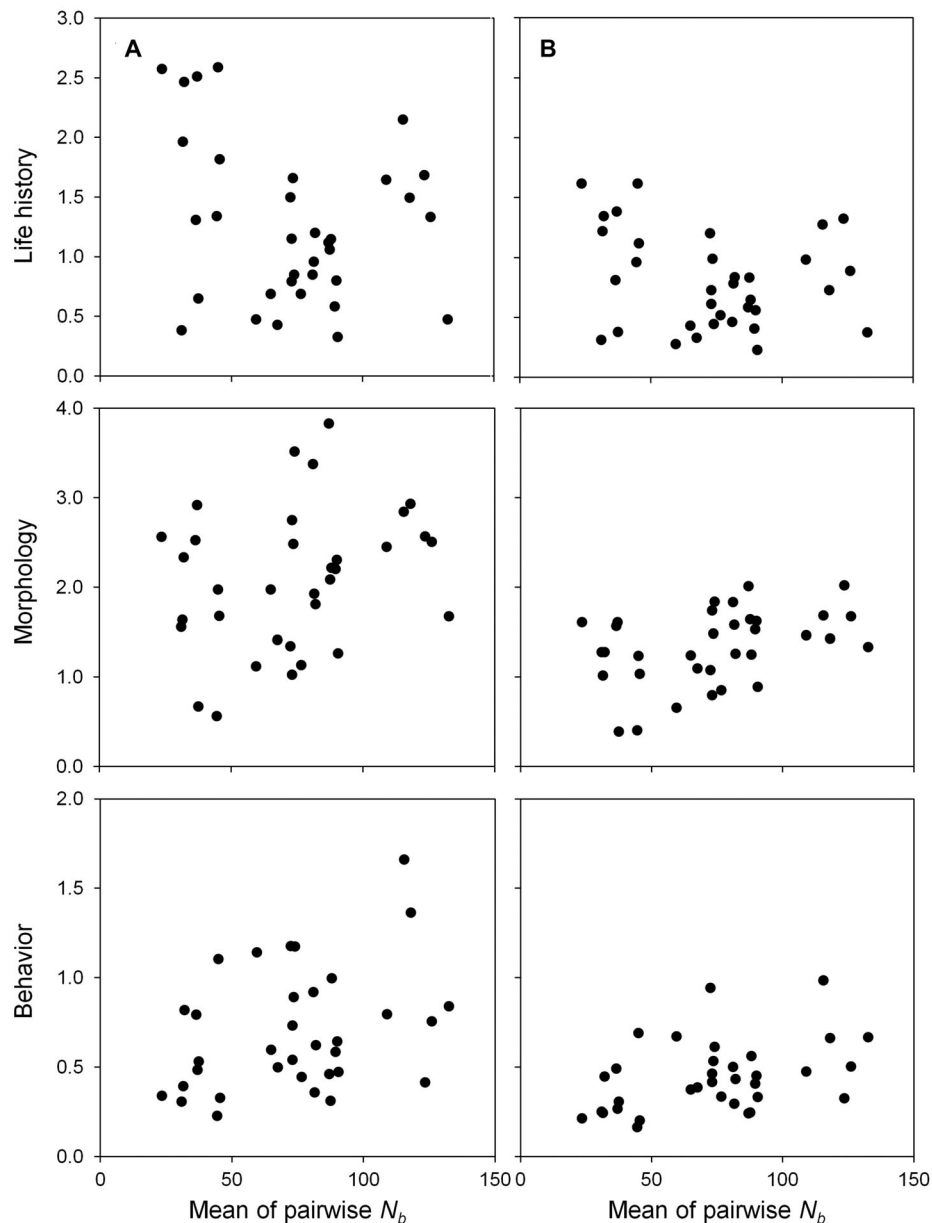
**Table 3.** Mantel test (Directional hypothesis) and White's test results (Variable hypothesis) for  $Q_{ST}/F_{ST}$  versus mean  $N_b$  and  $N$  for 15 traits measured using nine brook trout populations at Cape Race, Newfoundland.

Trait class	Trait	$F_{ST}$ microsatellites				$F_{ST}$ SNPs			
		$N_b$		$N$		$N_b$		$N$	
		$r_M$	W- $p$	$r_M$	W- $p$	$r_M$	W- $p$	$r_M$	W- $p$
Life history	Hatch time	−0.042	0.56	0.020	0.85	−0.046	0.53	−0.046	0.96
	Hatch length	0.30	0.19	0.23	0.27	0.24	0.30	0.18	0.42
	Yolk volume	0.17	0.65	0.25	0.45	0.12	0.63	0.20	0.48
	Emergence length	0.31	0.49	0.28	0.23	0.22	0.53	0.22	0.50
	Yolk conversion	0.36	0.50	0.16	0.89	0.21	0.60	0.046	0.82
Morphology	Head length	0.14	0.66	0.24	0.45	0.11	0.65	0.20	0.47
	Head width	0.12	0.65	0.20	0.45	0.088	0.64	0.17	0.47
	Eye diameter	0.30	0.68	0.39*	0.43	0.20	0.68	0.31	0.44
	Body depth	0.15	0.64	0.19	0.48	0.11	0.60	0.15	0.52
	ADP: CPD	0.082	0.67	0.22	0.45	0.069	0.67	0.20	0.45
	ADP: CPV	0.094	0.64	0.20	0.49	0.060	0.59	0.14	0.54
	CPD: CPV	0.13	0.66	0.21	0.43	0.089	0.66	0.18	0.45
Behavior	Pre-stimulus foraging	0.11	0.64	−0.089	0.75	0.073	0.66	−0.10	0.75
	Latency	0.27	0.57	0.27	0.41	0.19	0.50	0.18	0.64
	Post-stimulus foraging	0.071	0.64	0.12	0.48	0.065	0.58	0.10	0.54

\* $<0.05$ .

$I_A$  (Houle 1992) across populations and traits for each trait class. These two metrics make opposing predictions regarding the evolvability of fitness related traits; morphology traits often have higher  $h^2$  relative to life-history traits whereas the reverse is true for  $I_A$

(Hansen et al. 2011). We found that life-history traits in our study not only had greater  $I_A$  than morphology traits as expected (0.11 vs. 0.0039) but also greater  $h^2$  (0.39 vs. 0.18) than morphology traits;  $V_A$  estimated for behavioral traits did not differ from zero.



**Figure 5.** Mean  $Q_{ST}/F_{ST}$  versus mean  $N_b$  across traits in each of three trait categories.  $F_{ST}$  values among trout populations pairs were estimated using (A) microsatellite loci, and (B) SNPs for each trait. Relationships for mean  $Q_{ST}/F_{ST}$  with mean  $N$  are found in Fig. S21 and for individual traits in Figs. S22–S27.

As with  $V_A$ , there were no consistent differences in  $h^2$  and  $I_A$  with increasing population size (Figs. S5–S7 and Figs. S8–S10).

A previous study on the habitat of Cape Race trout populations found evidence to support the Variable hypothesis; there was greater spatial habitat variability among small than large populations, suggesting the former may be subject to a greater diversity of selective pressures (Wood et al. 2014). This possibility received support in a recent work in which increased adaptive differentiation was observed among small than among large populations based on signatures of balancing and diversifying selection at SNPs linked to phenotypic traits (Fraser et al.

2014). Yet, intriguingly, this did not translate into more variable  $V_A$  and  $Q_{ST}$  among small than large populations in the present study. We propose three hypotheses for the apparent disparity in the spatial habitat, genomic, and quantitative trait data on Cape Race trout populations. First, the habitat assessment was based on two years of data whereas contemporary genetic structuring among the populations is the product of a long evolutionary history. Similarly, as predicted for  $Q_{ST}$  and  $Q_{ST}/F_{ST}$ , long term fluctuating environmental conditions may have resulted in complex, fluctuating selective pressures, and similar levels of quantitative genetic variation among both small and large Cape Race

populations (Blanckenhorn et al. 1999; Siepielski et al. 2009, 2013). Second, environmental heterogeneity may induce a negative correlation between selection and  $V_A$  in small populations wherein little genetic variance is available for strong selection to act upon when conditions are harsh, but genetic variance is abundant when selection is weak under favourable conditions (Merilä et al. 2001; Wilson et al. 2006). Third, similar levels of phenotypic plasticity were observed among small and large Cape Race populations at the same life stages as in this study (Wood and Fraser 2015). If plasticity is favored to cope with increased environmental variability, this might buffer the loss of adaptive genetic variation similarly between small and large populations (Sultan 1987).

Quantitative genetic variation and differentiation were compared across Cape Race populations in relation to both  $N$  and  $N_b$  with the finding that tests of heteroscedasticity and also correlations for  $Q_{ST}$  and  $Q_{ST}/F_{ST}$  were more often significant using  $N_b$ . Although there were few significant tests overall, this result does suggest that different conclusions might be reached depending on whether  $N$  or  $N_e$  is adopted as the measure of population size.

Finally,  $F_{ST}$  estimates using SNPs were 1.53 times higher than for microsatellites. This suggests that some previous studies using microsatellite-based  $F_{ST}$  estimates and found that  $Q_{ST}$  was greater than  $F_{ST}$  might have reached incorrect conclusions. However, this does not mean that  $F_{ST}$  should always be estimated using SNPs rather than microsatellites as the appropriate choice of marker depends on mutational inputs to  $Q_{ST}$  as well (Hendry 2002) and hence merely illustrates the challenges in  $Q_{ST}/F_{ST}$  comparisons in general.

## CAVEATS

Family crosses were generated from a subset of all Cape Race populations, so one possibility is that, by chance, the small streams investigated were not representative of all regional small populations. However, habitat character means and CVs for the populations included in this study were not different from other small Cape Race populations. Moreover, the variability around the means and CVs were equal in these two groups (small populations included/excluded), suggesting that our populations represented the full range of habitat types occupied by small Cape Race populations.

Additive genetic variation and  $Q_{ST}$  were compared at early life stages. Traits at later life stages could not be investigated due to the logistical constraints of rearing large numbers of individuals. Whether similar patterns would be observed in older juveniles or adults is uncertain. However, our study included a large number of traits across several trait categories including traits that are known to be associated with fitness in salmonid fishes at a life stage that has a critical impact on recruitment (Einum and Fleming 2000).

To investigate the two alternative hypotheses, point estimates of  $V_A$  and  $Q_{ST}$  were examined in relation to population size, but it should be noted that confidence intervals calculated for  $V_A$  and pairwise  $Q_{ST}$  in this study were frequently large and overlapping across populations for all traits. Even calculating  $Q_{ST}$  using all nine populations produced confidence intervals that were as large as or larger than the point estimates of  $Q_{ST}$  themselves. This underscores the point that extremely large numbers of families and populations may be required to make firm conclusions regarding quantitative genetic characteristics of wild vertebrate populations. O'Hara and Merilä (2005) suggested >20 populations are required to achieve reasonable precision in  $Q_{ST}$  estimates, however an experiment of that magnitude would be difficult to carry out for most species. As this study is one of the largest thus far performed in a vertebrate species (see also Lind et al. 2011), it suggests that conclusions derived from studies using a smaller sample size than was included here should be interpreted with caution.

Finally, if contemporary population sizes at Cape Race do not reflect long-term population sizes, this might affect our conclusions. However, Cape Race population sizes have probably been consistent for some time because (i) the abundance of small populations is constrained by the small size of the streams they occupy (Wood et al. 2014) and (ii) neutral heterozygosity is positively correlated with population size (Fraser et al. 2014; Wood et al. 2014).

## EVOLUTIONARY AND CONSERVATION IMPLICATIONS

Our results did not support that quantitative genetic variation and trait differentiation consistently differed between small and large brook trout populations. Hence, they do not support the frequently cited assumption that the environments occupied by small populations tend to be marginal and that small populations experience disproportionate reductions in adaptive potential relative to large populations (Frankham 1996; Kawecki 2008, at least based on the quantitative genetic measures assessed). While genetic drift may indeed become more important as population size decreases, selection may also be stronger in some fragments if conditions become more extreme or variable as fragment size decreases (see also Fraser et al. 2014). Overall, these findings suggest that while the mechanisms might differ from small to large population size, these have led to a similar result in regards to  $V_A$  and  $Q_{ST}$ .

Our results also suggest that some vertebrate populations might retain the adaptive potential necessary to respond to future environmental changes even at very small population size. Reductions in fitness due to inbreeding and loss of quantitative genetic variation are expected to be disproportionately greater at  $N_e < 50$  (Franklin 1980). Five of the populations included in this study have an  $N_b$  of less than 50 and two (DY, STBC) most likely also have an  $N_e$  of less than 50; these populations have also likely been

isolated for some time and yet have retained similar levels of  $V_A$  as the larger populations. As brook trout are a colonizing species that exhibit residual tetraploidy (Allendorf and Thorgaard 1984), they might have an enhanced capacity to deal with small population size relative to other species, therefore how these results apply to other vertebrate taxa is an open question. Heritability was lower at small than large  $N$  in a recent study of plant populations (Weber and Kolb 2014); the range of  $N$  included populations smaller than in our study, but no details regarding genetic structure were presented and the smallest populations are likely highly vulnerable to demographic and environmental problems. Our findings are relevant given the paucity of similar research among salmonids, and vertebrates in general. Indeed, they suggest that demographic and environmental stochasticity rather than genetic stochasticity might pose the most immediate threat to persistence for some small vertebrate populations (e.g., Lande 1988; Caro and Laurenson 1994).

Conservation genetics theory predicts that genetic variation increases with increasing population size but our work at Cape Race has resulted in a variety of different conclusions depending on the measure of genetic variation employed. Namely, heterozygosity does indeed increase with increasing population size, while SNPs exhibit evidence of balancing selection among small populations (Fraser et al. 2014), and several metrics of quantitative genetic variation ( $V_A$ ,  $V_M$ ,  $h^2$ ,  $I_A$ ) show no differences across populations of varying size. Such disparate results raise the important question as to which (or whether) commonly available metrics adequately capture or predict the adaptive potential of populations in nature.

Finally, an exploratory power analysis suggested that a sample size of 267 populations would be needed in a typical correlation test (with a power and significance level of 0.80 and 0.05, respectively) to detect an effect size of 0.17, the average correlation we observed between  $V_A$  and population size in our study. Thus, even if weak relationships between metrics of adaptive potential and population size are common outcomes for vertebrates in nature, it would be very difficult to demonstrate conclusively. Either extremely large studies will be required or alternative approaches to address these questions may be necessary.

## ACKNOWLEDGMENTS

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## DATA ARCHIVING

Data available from the Dryad Digital Repository: <http://dx.doi.org/10.5061/dryad.rq122>.

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## Supporting Information

Additional Supporting Information may be found in the online version of this article at the publisher's website:

**Figure S1.** Landmarks used for morphometric analyses on Cape brook trout: (1) head length; (2) head depth at the posterior edge of the operculum; (3) eye diameter; (4) body depth; (5) posterior insertion of adipose fin to caudal peduncle, dorsal (ADP: CPD); (6) posterior insertion of adipose fin to caudal peduncle, ventral (ADP: CPV); (7) caudal peduncle depth (CPD: CPV); (TL) total length (used as a covariate in analyses).

**Figure S2.** Plots of  $V_A$  vs.  $N$  and  $N_b$  for five early life-history traits.

**Figure S3.** Plots of  $V_A$  vs.  $N$  and  $N_b$  for seven morphological traits.

**Figure S4.** Plots of  $V_A$  vs.  $N$  and  $N_b$  for three behavioural traits.

**Figure S5.** Plots of  $I_A$  vs.  $N$  and  $N_b$  for five early life-history traits.

**Figure S6.** Plots of  $I_A$  vs.  $N$  and  $N_b$  for seven morphological traits.

**Figure S7.** Plots of  $I_A$  vs.  $N$  and  $N_b$  for three behavioural traits.

**Figure S8.** Plots of  $h^2$  vs.  $N$  and  $N_b$  for five early life-history traits.

**Figure S9.** Plots of  $h^2$  vs.  $N$  and  $N_b$  for seven morphological traits.

**Figure S10.** Plots of  $h^2$  vs.  $N$  and  $N_b$  for three behavioural traits.

**Figure S11.** Plots of  $V_M$  vs.  $N$  and  $N_b$  for six early life-history traits.

**Figure S12.** Plots of  $V_M$  vs.  $N$  and  $N_b$  for seven morphological traits.

**Figure S13.** Plots of  $V_M$  vs.  $N$  and  $N_b$  for three behavioural traits.

**Figure S14.** Mean  $Q_{ST}$  (●) and  $F_{ST}$  (○) vs.  $N$  across traits in each of three trait categories.  $F_{ST}$  values among populations pairs was estimated using (a) microsatellite loci, and (b) SNPs for each trait.

**Figure S15.**  $Q_{ST}$  (●) and  $F_{ST}$  (○) vs.  $N_b$  for five early life traits.  $F_{ST}$  values among populations pairs was estimated using (a) microsatellite loci, and (b) SNPs for each trait.

**Figure S16.**  $Q_{ST}$  (●) and  $F_{ST}$  (○) vs.  $N_b$  for seven morphological traits.  $F_{ST}$  values among populations pairs was estimated using (a) microsatellite loci, and (b) SNPs for each trait.

**Figure S17.**  $Q_{ST}$  (●) and  $F_{ST}$  (○) vs.  $N_b$  for three behavioural traits.  $F_{ST}$  values among populations pairs was estimated using (a) microsatellite loci, and (b) SNPs for each trait.

**Figure S18.**  $Q_{ST}$  (●) and  $F_{ST}$  (○) vs.  $N$  for five early life traits.  $F_{ST}$  values among populations pairs was estimated using (a) microsatellite loci, and (b) SNPs for each trait.

**Figure S19.**  $Q_{ST}$  (●) and  $F_{ST}$  (○) vs.  $N$  for seven morphological traits.  $F_{ST}$  values among populations pairs was estimated using (a) microsatellite loci, and (b) SNPs for each trait.

**Figure S20.**  $Q_{ST}$  (●) and  $F_{ST}$  (○) vs.  $N$  for three behavioural traits.  $F_{ST}$  values among populations pairs was estimated using (a) microsatellite loci, and (b) SNPs for each trait.

**Figure S21.** Mean  $Q_{ST}/F_{ST}$  vs.  $N$  across traits in each of three trait categories.  $F_{ST}$  values among populations pairs was estimated using (a) microsatellite loci, and (b) SNPs for each trait.

**Figure S22.**  $Q_{ST}/F_{ST}$  vs.  $N_b$  for five early life traits.  $F_{ST}$  values among populations pairs was estimated using (a) microsatellite loci, and (b) SNPs for each trait.

**Figure S23.**  $Q_{ST}/F_{ST}$  vs.  $N_b$  for seven morphological traits.  $F_{ST}$  values among populations pairs was estimated using (a) microsatellite loci, and (b) SNPs for each trait.

**Figure S24.**  $Q_{ST}/F_{ST}$  vs.  $N_b$  for three behavioural traits.  $F_{ST}$  values among populations pairs was estimated using (a) microsatellite loci, and (b) SNPs for each trait.

**Figure S25.**  $Q_{ST}/F_{ST}$  vs.  $N$  for five early life traits.  $F_{ST}$  values among populations pairs was estimated using (a) microsatellite loci, and (b) SNPs for each trait.

**Figure S26.**  $Q_{ST}/F_{ST}$  vs.  $N$  for seven morphological traits.  $F_{ST}$  values among populations pairs was estimated using (a) microsatellite loci, and (b) SNPs for each trait.

**Figure S27.**  $Q_{ST}/F_{ST}$  vs.  $N$  for three behavioural traits.  $F_{ST}$  values among populations pairs was estimated using (a) microsatellite loci, and (b) SNPs for each trait.

**Table S1.** Cape Race trout population census size and  $N_b$  for 2011.  $N_b$  reported is the weighted harmonic mean of point estimates across cohorts within a population. The range of point estimates are in parentheses. See Wood et al. 2013 for the 95% CI for each individual cohort.

**Table S2.** Pearson's correlations (Directional hypothesis) and White's test results (Variable hypothesis) for  $I_A$  vs.  $N_b$  and  $N$  for 16 traits measured using nine brook trout populations at Cape Race, Newfoundland.

**Table S3.** Pearson's correlations (Directional hypothesis) and White's test results (Variable hypothesis) for  $h^2$  vs.  $N_b$  and  $N$  for 16 traits measured using nine brook trout populations at Cape Race, Newfoundland.

**Table S4.** Spearman's correlations (Directional hypothesis) and White's test results (Variable hypothesis) for  $Q_{ST}$  and  $Q_{ST}/F_{ST}$  vs. two categories of population pairs (small-small and large-large  $N_b$  population pairs) for 15 traits measured using nine brook trout populations at Cape Race, Newfoundland.

**Table S5.** Spearman's correlations (Directional hypothesis) and White's test results (Variable hypothesis) for  $Q_{ST}$  and  $Q_{ST}/F_{ST}$  vs. two categories of population pairs (small-small and large-large  $N$  population pairs) for 15 traits measured using nine brook trout populations at Cape Race, Newfoundland.

**Table S6.** Mantel test (Directional hypothesis) for mean  $Q_{ST}/F_{ST}$  vs. mean  $N_b$  and  $N$  for three trait classes using nine brook trout populations at Cape Race, Newfoundland.