

Genetic divergence between sympatric Arctic charr *Salvelinus alpinus* morphs in Gander Lake, Newfoundland: roles of migration, mutation and unequal effective population sizes

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A suite of 10 microsatellite loci was used to examine genetic divergence between two sympatric morphs of Arctic charr *Salvelinus alpinus* ('dark' and 'pale') inhabiting Gander Lake, Newfoundland. Results can be summarized as follows: (1) the morphs are strongly reproductively isolated – gene flow–migration estimates were consistently low in long and short-term evolutionary timescales of analysis; (2) intermorph divergence based on allele size (R_{ST}) was significantly larger than those based on allele state (θ) implying a cumulative effect of stepwise-like mutations; (3) historical (coalescent) and current (linkage disequilibrium) point estimates of effective population size (N_e) were consistently higher for dark than for pale *S. alpinus*. The first and second findings lend support to the hypothesis that divergence between forms may have preceded the last glacial period (ending c. 12 000 years BP). The third finding argues for significant differences in habitat quantity and quality between morphs, which were emphasized in a previous study. Overall, these analyses underscore the importance of genetic assessment and monitoring in the conservation of fish diversity, with emphasis on 'rare' or under-represented forms among temperate species pairs.

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INTRODUCTION

Resource polymorphism, or the presence of alternative forms ('morphs') within populations, is a common feature among temperate fishes inhabiting postglacial lakes (Skulason & Smith, 1995; Taylor, 1999). Natural selection is presumably the driving force behind this ubiquitous phenomenon characterized by incomplete speciation, sympatry and large differences in resource use and feeding morphology (Schluter, 1996, 2001). Indeed, variation in morphological or physiological traits, or both, involved in alimentation seems to play a prominent

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role in habitat-specific adaptive divergence among sympatric morphs of numerous fishes (Bentzen & McPhail, 1984; Taylor & Bentzen, 1993; Snorrason *et al.*, 1994; Ruzzante *et al.*, 1998; Bernatchez *et al.*, 1999; Dynes *et al.*, 1999).

Arctic charr *Salvelinus alpinus* (L.) populations provide an ideal system for studies of resource polymorphism in north temperate regions. The species is highly polymorphic and its holarctic distribution was likely interrupted during the Pleistocene's multiple glacial cycles (Behnke, 1972; Klemetsen *et al.*, 2003). After deglaciation, newly formed lakes provided empty ecological niches for the diversification of *S. alpinus* forms to take place (Knudsen *et al.*, 2006). Based on morphological and life-history traits, two or more morphs living in sympatry have been described in numerous lakes from Europe and North America, as reviewed extensively by Jonsson & Jonsson (2001). Colouration, gill rakers (number and length), mouth position and fin size are among the most common trophic adaptations (Skulason *et al.*, 1999; Jonsson & Jonsson, 2001). Overall, ecological investigations on the diversification of this species complex are abundant (reviewed by Skulason *et al.*, 1999; Jonsson & Jonsson, 2001) when compared with the paucity of molecular genetic assessments (Gislason *et al.*, 1999; Westgaard *et al.*, 2004; Wilson *et al.*, 2004). The latter are nonetheless fundamental to assess the degree of reproductive isolation between forms, thus gauging the importance of phenotypic plasticity *v.* heritable genetic changes (but see Klemetsen *et al.*, 2002 for an alternative approach). Genetic studies have also illuminated the relationship between gene flow and adaptive phenotypic divergence in other fish groups (Lu & Bernatchez, 1999; Taylor & McPhail, 2000; Hendry *et al.*, 2002, 2004; Fraser & Bernatchez, 2005a).

Novel population genetic approaches are currently attracting considerable attention because they overcome critical assumptions of traditional models, such as symmetrical gene flow (*i.e.* emigration = immigration) and equal effective population sizes (N_e) among populations, all rarely met in natural biological systems (Whitlock & McCauley, 1999). First, they permit the independent estimation of N_e and gene flow (m , migration rate), both central population parameters in evolution and conservation – N_e determines the rate of loss of neutral genetic diversity (Lande, 1988; Waples, 2005) and such losses may be mitigated through m (Ingvarsson, 2001). Second, they allow a more realistic approximation at the interplay among dispersal, genetic structure and population size (Fraser *et al.*, 2004; Hansen *et al.*, 2007; Palstra *et al.*, 2007; Palstra & Ruzzante, 2008). In addition, the widespread use of highly polymorphic microsatellite DNA has fuelled the development of studies weighing the importance of different mutation models (O'Connell *et al.*, 1997; Angers & Bernatchez, 1998; Tessier & Bernatchez, 2000) and formal statistical approaches (Hardy *et al.*, 2003) for the assessment of the relative contribution of mutation (μ) *v.* migration (m) rates in genetic differentiation (Fraser & Bernatchez, 2005b). Such advances are part of a common theme in evolutionary and conservation biology aimed at teasing apart the roles of ecological determinism (*i.e.* natural selection) *v.* historical contingency (*i.e.* chance events) in shaping fish diversity (Taylor & McPhail, 2000; Tessier & Bernatchez, 2000; Fraser & Bernatchez, 2005b).

The present study is a genetic assessment of population divergence between two sympatric morphs of *S. alpinus* in Gander Lake (GL), Newfoundland. GL is the third largest (surface area = 113.2 km²) and probably one of the deepest

lakes on the island (maximum depth, 288 m; mean depth, 105.4 m; O'Connell & Dempson, 2002). O'Connell & Dempson (2002) initially described the morphs as 'dark' and 'pale'. Dark and pale *S. alpinus* in GL differ in morphology (colour, size and meristics; O'Connell & Dempson, 2002), life-history traits (growth and life span; O'Connell *et al.*, 2005; Power *et al.*, 2005), feeding and habitat. The ubiquitous dark form dominates the littoral, pelagic and benthic zones and preys on a variety of taxa; the smaller pale form is found mainly in the deepest areas of the lake and feeds exclusively on chironomid larvae (O'Connell & Dempson, 2002; O'Connell *et al.*, 2005). Contrasting stable isotope composition and feeding preferences both argue for strong ecological segregation and trophic specialization between forms driven by divergent natural selection (Power *et al.*, 2005). The main goals in the present study concern the nature and potential origin of this polymorphism. First, the null hypothesis of no genetic differentiation is tested with a suite of 10 microsatellite DNA markers. This hypothesis is rejected as the morphs exhibit strong genetic differentiation. Second, the null hypothesis is tested that the accumulation of neutral genetic differences between the morphs is mostly because of random drift with mutations having played little or no contribution ($\mu < m$). This hypothesis is also rejected enabling inferences regarding the origin of the polymorphism. Finally, the null hypothesis is tested that there are no differences in effective population size (N_e) between dark and pale *S. alpinus*. This hypothesis is also rejected and highlights differences in habitat size and quality the morphs likely have experienced throughout their diversification.

MATERIALS AND METHODS

SAMPLING

Dark ($n = 72$) and pale ($n = 84$) morphs of *S. alpinus* were captured between 2000 and 2005 using Lundgren multimesh gillnets (Hammar & Filipsson, 1985) of bar mesh-sizes between 6.25 and 75.0 mm. Specimens were measured, sexed and their maturity stage identified. Individual biopsies from fins ('fin clips') were preserved in 95% ethanol and stored at -20°C for subsequent molecular analyses.

LABORATORY PROCEDURES

Whole genomic DNA was isolated from fin clips using the glassmilk method described by Elphinstone *et al.* (2003). Individual tissues (*c.* 10 mg each) were first digested in 200 μl of digestion buffer and 2 μl of proteinase K (10 mg ml^{-1}) (BioBasic, Markham, Canada); they were subsequently incubated overnight at 50°C and in constant motion. Thereafter, 150 μl of binding buffer, 50 μl of glassmilk and 50 μl of tissue digest were added to a filter plate (Pall, East Hills, NY, U.S.A.) placed in a vacuum manifold. Individual wells were then washed using 200 μl of wash buffer kept at -20°C to remove excess of proteins and other residues. A vacuum step of *c.* 10 min was applied to the wells until dry. DNA was finally eluted adding 100 μl of low-concentration Tris-EDTA at 65°C to each well and recovering the solution in a collection tray.

Ten microsatellite DNA markers were amplified by polymerase chain reaction (PCR): *SalF56SFU*, *SalP61SFU*, *SalJ81SFU* and *SalE38SFU* (McGowan *et al.*, 2004), *Omy301UoG* (Steinberg *et al.*, 2002), *MST-85* (Presa & Guyomard, 1996), *Sco-19* (Taylor *et al.*, 2001), *OtsG83b*, *OtsG253* (Williamson *et al.*, 2002) and *Omm1105* (Rexroad *et al.*,

2002). PCR cocktails were prepared in 10 μl volume reactions and contained 2.0–2.5 mmol l^{-1} of MgSO_4 , 200 μmol l^{-1} of each dNTP, 0.5–2.0 pmol of each (forward and reverse) primer, 1 \times Tsg polymerase buffer (BioBasic), 0.2 U of Tsg polymerase (BioBasic) and 1–2 μl of template DNA. Thermal cycles usually consisted of (1) an initial denaturing step at 95° C for 5 min, (2) 30–35 cycles of 94° C for 30 s, X° C for 30 s (X = locus-specific annealing temperature) and 72° C for 1 min and (3) a final extension step at 72° C for 5 min. For cross-species amplification, original annealing temperatures were typically decreased by 1–2° C. Specific PCR conditions and reagent concentrations are available upon request from the authors.

Amplified fragments were run in a LI-COR® Biosciences (Lincoln, NE, U.S.A.) sequencer using 6% polyacrylamide gels (Sequagel; National Diagnostics, Atlanta, GA, U.S.A.) and scored using the software SAGA™ (LI-COR®) with a molecular ladder (LI-COR) of known sizes (50–350 bp). Two researchers verified consistency of all allele scoring and sizes by eye. Allele fragments were later exported as individual genotypes into a Microsoft® (Seattle, WA, U.S.A.) Excel worksheet for statistical analyses.

STATISTICAL ANALYSES

Data were first examined with MICRO-CHECKER 2.2.3 (van Oosterhout *et al.*, 2004) to rule out potential genotyping errors and other technical issues (null alleles and stuttering). Gel images were either re-scored or specific genotypes re-amplified if errors were encountered as suggested by MICRO-CHECKER.

Statistics of genetic variation

The Microsatellite Toolkit add-in for Microsoft Excel (Park, 2001) was first employed to quantify allele frequencies and observed heterozygosities and to generate input files for additional softwares. Total number of alleles (A) per locus, allelic richness (A_R ; El Mousadik & Petit, 1996) and unbiased expected heterozygosities (H_E from Nei, 1987) were determined in FSTAT 2.9.3 (Goudet, 1995) for each morph. Differences in A_R and H_E (both unbiased measures) between morphs were tested with Student's t -test across loci after verifying for normality of the data with a Kolmogorov–Smirnov test, both implemented in SPSS for Windows® (Chicago, IL, U.S.A.).

The null hypotheses of non-significant departures from (1) Hardy–Weinberg equilibrium (HWE) within loci and (2) genotypic linkage equilibrium among all locus pairs were tested in GENEPOP 3.4 (Raymond & Rousset, 1995). An exact P -value for HWE (per locus and global) was estimated following Guo & Thompson's (1992) Markov Chain algorithm. Significant deviations from HWE were further checked for heterozygote deficiency through comparison of observed and expected (after 5000 randomizations) intra-sample inbreeding coefficients (f ; Weir & Cockerham, 1984) in FSTAT. Significance ($\alpha = 0.05$) was adjusted according to Rice (1989) using a sequential Bonferroni correction when applicable.

Measures of population differentiation

Divergence between morphs was assessed using analysis of genetic variance based on allele state (θ ; Weir & Cockerham, 1984) or allele size (R_{ST} ; Michalakis & Excoffier, 1996). To rule out a mutational component over drift to differentiation, the inequality $R_{ST} > \theta$ was tested using SPAGeDI 1.2 (Hardy & Vekemans, 2002). If differences between allele sizes are not meaningful for differentiation, theoretical R_{ST} values (pR_{ST}) and their confidence intervals should look similar to θ values (null hypothesis: $\theta = pR_{ST}$; Hardy *et al.*, 2003). If $R_{ST} > pR_{ST}$ (*i.e.* the alternative hypothesis), then differences in allele size are informative and the rate of stepwise mutation is significant when compared with drift. Hardy's *et al.* (2003) pR_{ST} performs well under different migration models (*e.g.* island, full isolation and stepping stone) and seems robust to deviations from ideal single-step mutations, particularly double-step mutations and certain multi-step mutations.

Significance of both observed θ and R_{ST} was validated through exact tests of population differentiation on allele frequencies at each locus using the GENEPOP package. Probability for the joint null hypothesis of no differentiation at any locus was calculated using Fisher's method (Ryman & Jorde, 2001; Ryman *et al.*, 2006).

Because the collection of *S. alpinus* spanned several years and locations, cryptic genetic structure of *S. alpinus* in GL was additionally investigated using STRUCTURE 2.2.2 (Pritchard *et al.*, 2000; Falush *et al.*, 2003). Three separate datasets consisting of (1) dark and pale charr genotypes pooled, (2) only dark genotypes and (3) only pale genotypes were run under the following settings: burn-in length = 50 000 repetitions; Markov Chain Monte-Carlo run = 250 000 iterations; ancestry model = admixture and allele frequency model = correlated (Falush *et al.*, 2003). Values for K were changed between 1 and 4 putative gene pools and run a minimum of three times. Convergence of population parameters such as Dirichlet's coefficient of admixture (α_{DIR}) and the symmetry of membership proportions were additionally analysed as informal pointers to the most likely value of K .

Estimation of gene flow (m) and effective population size (N_e)

Migration (m) rates and effective population sizes (N_e) were calculated considering long-term (historical) and short-term (contemporary or current) evolutionary scenarios following Fraser *et al.* (2007). The historical number of migrants were first quantified using Wright's (1951) classical island model assuming equal and constant (effective) population sizes, symmetrical migration and equilibrium of migration and drift according to $0.25(F_{ST}^{-1} - 1)$. In this case, $F_{ST} = \theta$ following Weir & Cockerham (1984). An equivalent estimate of $N_e m_{(WRIGHT)}$ using R_{ST} was also calculated assuming stepwise-like mutations (Rousset, 1996). Second, maximum likelihood coalescent-based estimates of $\Theta = 4N_e\mu$ and $N_e m_{(B\&F)}$ were obtained from MIGRATE 2.1.3 (Beerli & Felsenstein, 2001). MIGRATE relaxes most of Wright's (1951) assumptions, all of which are probably violated in natural ecosystems (Whitlock & McCauley, 1999). Simulations in MIGRATE assumed a Brownian motion approximation to Ohta & Kimura's (1973) ladder-mutation model (especially tailored to microsatellites) and included 10 short chains (100 000 trees analysed and 1000 recorded), two long chains (500 000 trees analysed and 5000 sampled) and 10 000 trees discarded per chain. An adaptive heating scheme with four 'temperatures' (1, 1.2, 1.5 and 3) was necessary to increase maximum likelihood search in the parameter space to reach stable estimates. Starting values for Θ and $N_e m_{(B\&F)}$ were initially supplied by MIGRATE from F_{ST} values (Maynard Smith, 1970); afterwards, output Θ and $N_e m_{(B\&F)}$ estimates became starting values for subsequent runs, with Θ values and $N_e m_{(B\&F)}$ from the last run being presented here. Θ values were scaled to empirical long-term $N_e(\hat{N}_{eLT})$ through published mutation rates (μ) for microsatellites ($\mu = 0.001-0.0001$; Ellegren, 2004). To derive m values, $N_e m_{(B\&F)}$ was divided by \hat{N}_{eLT} .

To obtain contemporary estimates of m or $m_{(W\&R)}$, BAYESASS 1.3 (Wilson & Rannala, 2003) was used to detect immigration within the last few generations. The method appears to be robust to deviations from migration-drift equilibrium and HWE, albeit it performs optimally in the presence of unlinked and highly differentiated loci. A total of 3×10^7 iterations with a sampling frequency of 2000 iterations were performed after a burn-in period of *c.* 1×10^6 iterations. Input delta parameters (allele frequencies, migration rate and inbreeding) were adjusted to ensure that enough parameter space was searched.

Current or short-term estimates of $N_e(\hat{N}_{eST})$ using Hill's (1981) linkage disequilibrium (LD) method among unlinked loci (Waples, 1991) were also reported to complement $m_{(W\&R)}$ values. \hat{N}_{eST} for each morph was estimated in three steps. First, empirical correlation coefficients (\hat{r}) were computed between all allele pairs at $0.5L(L - 1)$ locus-to-locus combinations ($L =$ number of loci) utilizing the LINKDOS programme (Garnier-Gere & Dillmann, 1992), with \hat{r} being a function of both Burrow's composite measure of LD and corresponding allele frequencies (Weir, 1979; Black & Krafusur, 1985). Second, the arithmetic mean of \hat{r}^2 values and harmonic mean of S (if S varies among loci) were estimated

for all locus-to-locus pairs following Waples (1991, 2006) in a worksheet of Microsoft Excel; a global mean over all pairs of loci (\bar{r}^2) was subsequently attained by weighing each pair-specific value according to the number of allele comparisons. Third, Waples' (2006) general equation linking \hat{N}_{eST} and \bar{r}^2 considering a dioecious species with random mating was adopted as follows: $\hat{N}_{eST} = [3(\bar{r}^2 - 1/n)]^{-1}$. An empirical bias correction for small sample sizes is also available (Waples, 2006), given recent findings in theoretical studies when $n < N_e$ (England *et al.*, 2006). For this dataset, each morph has $n \geq 30$ (table 2 in Waples, 2006). Additionally, 95% CIs were calculated by means of equation 12 in Waples (2006).

Last, to estimate the number of contemporary migrants for comparative reasons with the historical approach, corrected \hat{N}_{eST} values for each morph were multiplied by unidirectional $m_{(W\&R)}$ values.

RESULTS

GENETIC STATISTICS AND EQUILIBRIUM MODELS

Genetic statistics are summarized in Table I. The total number of alleles over all samples regardless of morph varied between 8 (*OtsG253*) and 46 (*SalE38SFU*). Neither A_R (*t*-test, d.f. = 9, $P > 0.05$) nor H_E (*t*-test, d.f. = 9, $P > 0.05$) differed significantly between morphs indicating similar levels of genetic variation (Table I).

Observed genotypes at each locus fitted HWE expectations in all cases except for locus *OtsG83b* in dark charr ($P < 0.01$), which after Bonferroni correction for multiple K comparisons became non-significant ($K = 20$, $P > 0.05$). A specific test for heterozygote deficit in GENEPOP on this locus and sample was not significant ($P > 0.05$). Global exact tests for heterozygote deficit were not significant within each morph (dark and pale: $P > 0.05$); comparisons of observed and expected f values further validated this result and suggested no significant departures from HWE caused by homozygote excess. Linkage equilibrium

TABLE I. Locus-specific and multilocus genetic statistics for dark and pale morphs of *Salvelinus alpinus* in Gander Lake, Newfoundland, screened for 10 microsatellite loci. Values show results as dark/pale. A , total number of alleles; A_R , allelic richness (number of alleles in 65 diploid individuals); f , intrasample inbreeding coefficient; H_E , unbiased expected heterozygosity; H_O , observed heterozygosity; n , sample size; ns, non-significant values at $\alpha = 0.05$

Locus	n	A	A_R	H_O	H_E	f
<i>SalF56SFU</i>	70/83	8/9	7.6/8.5	0.77/0.55	0.68/0.51	-0.13/-0.1
<i>SalP61SFU</i>	69/79	12/13	11.8/12.5	0.68/0.70	0.72/0.75	0.06/0.07
<i>SalJ81SFU</i>	65/82	9/7	9/6.9	0.66/0.54	0.58/0.63	-0.13/0.14
<i>SalE38SFU</i>	71/78	30/37	28.6/35.4	0.90/0.89	0.90/0.93	0.01/0.04
<i>Omy301UoG</i>	72/84	14/12	13.7/11.7	0.92/0.89	0.83/0.85	-0.10/-0.06
MST-85	72/82	23/10	22.0/9.3	0.79/0.55	0.81/0.55	0.03/0.01
<i>Sco-19</i>	71/82	11/10	10.8/9.5	0.79/0.48	0.79/0.53	-0.00/0.01
<i>OtsG83b</i>	72/82	13/21	12.8/19.8	0.90/0.91	0.89/0.91	-0.01/-0.00
<i>OtsG253</i>	72/84	5/8	5.0/7.5	0.64/0.69	0.70/0.68	0.08/-0.02
<i>Omm1105</i>	72/84	7/9	6.8/8.7	0.35/0.68	0.39/0.67	0.11/-0.01
Multilocus	70.6/82.0	13.2/13.6	12.8/12.9	0.74/0.68	0.73/0.70	-0.01 ^{ns} /0.02 ^{ns}

was equally met in all locus-to-locus comparisons ($P > 0.05$), implying that all microsatellite markers utilized in this study are likely unlinked.

LARGE INTERMORPH DIFFERENTIATION

Allele frequency distributions differed considerably between morphs and numerous private alleles were found (Fig. 1). As a result, both measures of population differentiation – θ and R_{ST} – were large and highly significant for eight of 10 loci. Only loci *OtsG83b* and *OtsG253* showed no significant differentiation (Table II).

Stepwise-like mutations seem to make an equal or larger contribution than drift to differentiation between morphs as R_{ST} were higher than pR_{ST} plus 95% CI for seven of 10 loci and for the multilocus estimator (overall $P < 0.001$; Table II). Interestingly, θ values were a decreasing linear function of mean H_E between morphs (ANOVA, $F_{1,8}$, $P < 0.05$, adjusted $R^2 = 0.41$) but not R_{ST} values. When *OtsG253* was removed from the analysis (given its negative θ -value and non-significant P -value in Table II), the relationship between θ values and mean H_E showed a better model fit as shown in Fig. 2 (adjusted $R^2 = 0.58$) and remained significant (ANOVA, $F_{1,7}$, $P < 0.05$); moreover, variance in θ was also explained by the mean A_R using an inverse linear equation (ANOVA, $F_{1,7}$, $P < 0.01$, adjusted $R^2 = 0.60$) (Fig. 2).

Results from STRUCTURE using the pooled dataset clearly support only two gene pools ($K = 2$), and no further genetic structure was found within each form (Table III). Average individual memberships were 98.5 and 98.3% for

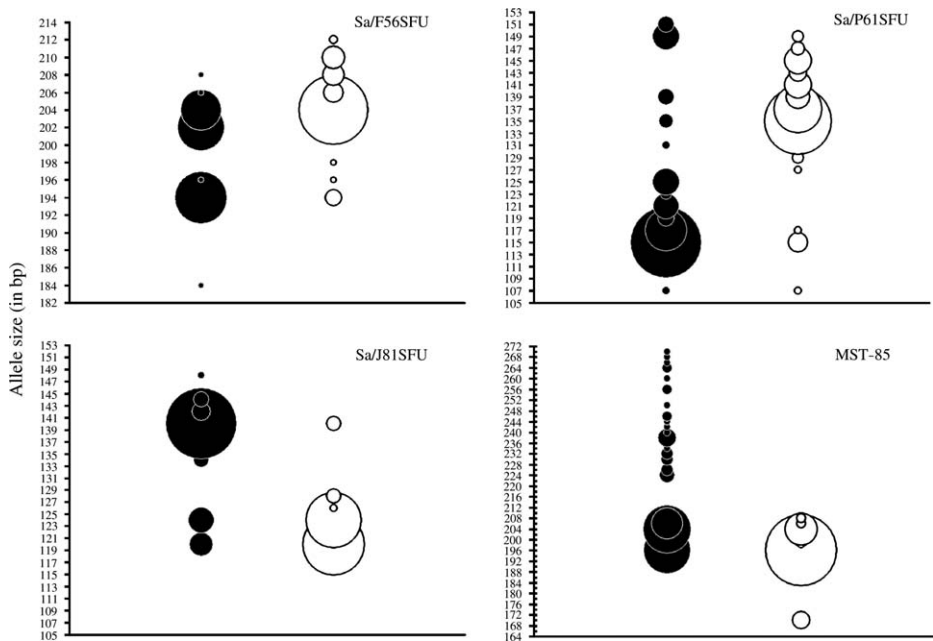


FIG. 1. Allele frequencies (bubble area) of dark (black) and pale morphs (white) of *Salvelinus alpinus* for four polymorphic microsatellite loci.

TABLE II. Observed (θ , R_{ST}) and expected (pR_{ST} plus 95% CI) population divergence between dark and pale morphs of *Salvelinus alpinus* from Gander Lake, Newfoundland screened for 10 microsatellite loci. P_1 , the significance level to reject the null hypothesis of no genetic differentiation between forms; P_2 , the level of significance to reject the null hypothesis of $R_{ST} = pR_{ST}$

Locus	θ	R_{ST}	P_1	pR_{ST} (95% CI)	P_2
<i>SalF56SFU</i>	0.265	0.472	***	0.214 (0.000–0.531)	NS
<i>SalP61SFU</i>	0.239	0.583	***	0.190 (0.000–0.625)	*
<i>SalJ81SFU</i>	0.335	0.783	***	0.274 (0.000–0.684)	**
<i>SalE38SFU</i>	0.013	0.089	***	0.014 (0.000–0.085)	*
<i>Omy301UoG</i>	0.068	0.281	***	0.062 (0.000–0.266)	*
MST-85	0.116	0.376	***	0.090 (0.000–0.290)	**
<i>Sco-19</i>	0.101	0.318	***	0.088 (0.000–0.311)	*
<i>OtsG83b</i>	0.003	0.064	NS	0.002 (0.000–0.036)	**
<i>OtsG253</i>	-0.004	-0.002	NS	-0.003 (0.000–0.008)	NS
<i>Omm1105</i>	0.203	-0.003	***	0.159 (0.000–0.345)	NS
Multilocus	0.136	0.239	***	0.068 (0.013–0.146)	***

NS, not significant ($P > 0.05$); * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

dark and pale *S. alpinus*, respectively, highlighting levels of mixed ancestry <2% for both morphs (Fig. 3).

ESTIMATES OF m AND N_e

Based on test results that $R_{ST} > \theta$, and hence $\mu \geq m$ (Hardy *et al.*, 2003), \hat{N}_{eLT} and $m_{(B\&F)}$ values assuming only $\mu = 0.001$ (rather than $\mu = 0.0001$) are reported here as independent estimates of m from Wilson & Rannala's (2003) method fell within the same order of magnitude (Table IV).

In general, estimates of $N_e m$ from all three implemented methods were congruent: $N_e m_{(WRIGHT)} = 1.58$ (symmetrical using θ); $N_e m_{(WRIGHT)} = 0.79$ (symmetrical using R_{ST}); $N_e m_{(B\&F)} = 1.13$ (dark to pale); $N_e m_{(B\&F)} = 1.14$ (pale to dark); $N_e m_{(W\&R)} = 1.55$ (dark to pale) and $N_e m_{(W\&R)} = 0.82$ (pale to dark; this last method used uncorrected \hat{N}_{eST} values from Table IV). Point \hat{N}_{eLT} and \hat{N}_{eST} values were also consistent across methods and scenarios despite low precision in contemporary 95% CI (upper values were ∞). Interestingly, estimates of N_e were always higher for dark than pale *S. alpinus*; the difference was roughly two-fold for long-term and between two-fold and 10-fold for short-term estimates (Table IV).

Long- and short-term migration rates were remarkably low, ranging from 0.00012 to 0.018 (95% CI included). Some degree of gene flow asymmetry (higher gene flow from pale to dark than *vice versa*) was manifest in long-term but not in contemporary timescales – in this latter scenario 95% CI of the estimates clearly overlap (Table IV).

DISCUSSION

The present study accomplished three main goals in explaining the nature and potential origin of *S. alpinus* polymorphism in GL, Newfoundland. First,

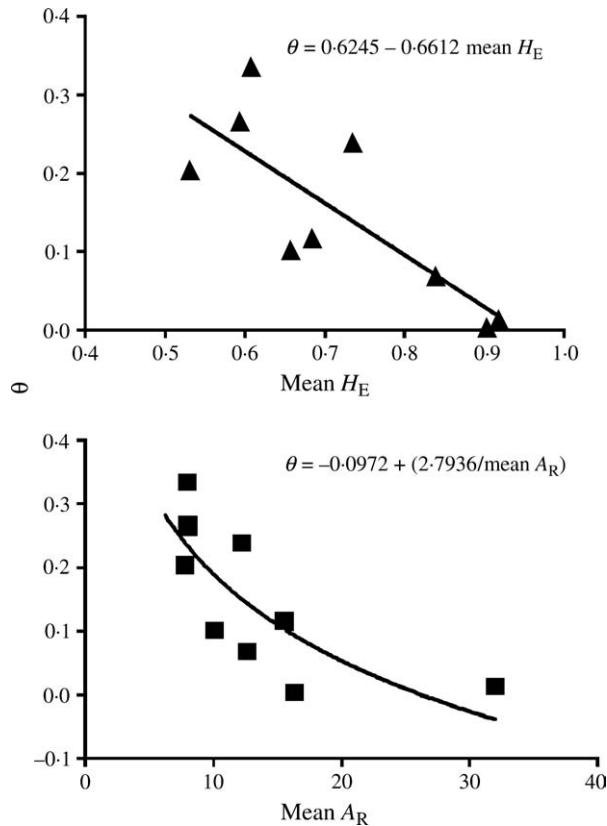


FIG. 2. Regression and curve fit analyses of θ (genetic differentiation) v. mean unbiased expected heterozygosity (H_E) (upper) and allelic richness (A_R) (lower) between morphs of *Salvelinus alpinus* screened for nine microsatellite loci (locus *OtsG253* is excluded from the analyses; see text for details).

the morphs exhibit large genetic differences and are thus strongly reproductively isolated. Second, mutations have played a significant role in the accumulation of intermorph neutral genetic differences. Third, estimates of effective population size (N_e) differed markedly across morphs regardless of the estimation method. The first two results are discussed in the context of genetic structure and divergence in this system with insights into the likely origin of the morphs. The third finding is discussed in the context of the assumptions required for the estimation of N_e with an ecological explanation for the difference in N_e estimates between morphs.

GENETIC STRUCTURE AND DIVERGENCE BETWEEN MORPHS

Genetic structure in GL *S. alpinus* was consistent with only two breeding populations that unequivocally matched dark and pale forms. Ninety-nine per cent of Bayesian individual assignment to discrete clusters indicates strong

TABLE III. Posterior probability values – $P(X|K)$ – derived from Bayesian assignment of individual multilocus genotypes (from 10 independent microsatellite loci) to K theoretical clusters (see text for the model underlying assumptions). The value of K associated with the highest $P(X|K)$ (in bold) is the most likely estimate for the number of gene pools within three datasets (dark, only dark genotypes; pale, only pale genotypes; pooled, dark and pale genotypes together) of *Salvelinus alpinus* in Gander Lake, Newfoundland

K	$P(X K)$		
	Pooled	Dark	Pale
1	1.7×10^{-200}	0.99	0.99
2	0.99	1.1×10^{-7}	5.6×10^{-9}
3	3.1×10^{-17}	1.9×10^{-12}	8.5×10^{-17}
4	2.5×10^{-46}	8.2×10^{-40}	4.1×10^{-8}

reproductive isolation between morphs. Indeed, $m_{(B\&F)}$ and $m_{(W\&R)}$ were consistently low and estimates agreed closely across timescales despite some nuances. For instance, $m_{(B\&F)}$ depends on \hat{N}_{eLT} estimates, which are in turn a function of μ ; however, $m_{(B\&F)}$ would still fall within the 95% CI for $m_{(W\&R)}$ if higher or lower values of μ are considered from the literature (see below, *Unequal Estimates of N_e between Morphs: Assumptions and Implications*). These conclusions are therefore unlikely to change as a function of μ within the range considered. In a broad context, estimates of θ and R_{ST} imply moderate to strong divergence when compared with a wide range of genetic differentiation values among sympatric forms within the *S. alpinus* complex (Gislason *et al.*, 1999; Westgaard *et al.*, 2004; Wilson *et al.*, 2004).

The present study suggests that divergence measured in allele size (R_{ST}) rather than allele state (θ) best quantifies differentiation between morphs according to Hardy's *et al.* (2003) test. Mutation is thus likely to have made an equal or greater contribution than drift to the morphs' divergence under an island model of migration ($\mu \geq m$; Hardy *et al.*, 2003). Certainly, the use of θ to measure divergence when μ is as large as m is questionable (Whitlock & McCauley, 1999), and the current data further show that θ might be a function of genetic variation at each locus (Fig. 2; Olsen *et al.*, 2004; O'Reilly *et al.*, 2004).

Do these findings shed light on the possible origin of this polymorphism? Although resource-based divergent natural selection most likely explains the rapid postglacial divergence between dark and pale *S. alpinus*, recent studies have

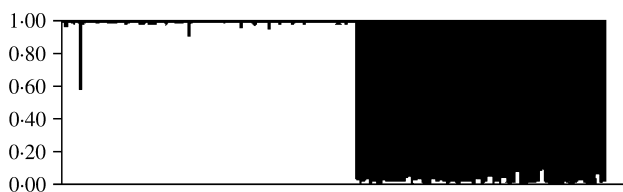


FIG. 3. Bar plots of individual membership obtained from STRUCTURE 2.2.2 assuming two gene pools ($K = 2$) and genotypes from dark (black area) and pale morphs (white area) of *Salvelinus alpinus* have been combined. Individuals with mixed ancestry are shown by a combination of white and black colours.

TABLE IV. Estimates of long-term (coalescent) and short-term (linkage disequilibrium) effective population sizes (N_e), as well as historical (coalescent) and current (Bayesian) migration rates (m) between dark and pale morphs of *Salvelinus alpinus* (arrows specify directionality of gene flow). 95% CIs are shown in parentheses

Evolutionary scenario	Model assumptions	Dark N_e (95% CI)	m (95% CI)	Pale N_e (95% CI)
Long-term historical \hat{N}_{eLT} and $m_{(B\&F)}$	$\mu = 0.001$	268 (253–285)	→	120 (115–128)
			(0.0039–0.0046) 0.0096	
Short-term contemporary \hat{N}_{eST} and $m_{(W\&R)}$	Uncorrected	352 (33–∞)	→	171 (33–∞)
	Corrected*	1011 (34–∞)	→	221 (32–∞)
			(0.00013–0.017) 0.0048	
			←	(0.00012–0.018)

*Alternative estimate using Waples' (2006) correction when $n > 30$ (see *Materials and Methods*).

documented that preglacial historical contingency may also have shaped resource polymorphisms (trophic: Taylor & McPhail, 2000; Ruzzante *et al.*, 2003; migratory: Fraser & Bernatchez, 2005b). Because stepwise-like mutations and R_{ST} best characterize genetic divergence between GL *S. alpinus* morphs, differences in size among microsatellite alleles reflect cumulative past mutation events, and therefore, time elapsed since common ancestry. Estoup & Angers (1998) argue that mutations are expected to have a substantial effect on population divergence only after 2000 generations assuming a typical rate of mutation for microsatellites ($\mu = 0.0001$), resulting in large differences between R_{ST} and θ . Generation length for dark and pale *S. alpinus* could vary roughly between 5 and 9 years based on life-history data (O'Connell *et al.*, 2005), which combined with 12 000 years of postglacial time (Shaw *et al.*, 2006) yield *c.* 1300–2400 generations elapsed since the formation of GL. It is thus likely that part of the morphs' differentiation precedes the last glacial period. Sympatric inflow and outflow migratory brook charr *Salvelinus fontinalis* (Mitchill) of Mistassini Lake in Quebec, another young postglacial lake (7000–8000 years BP), also harbour a mutational component to differentiation detected by means of Hardy's *et al.* (2003) pR_{ST} statistic, implying that such populations might have allopatric extra-lacustrine origins (Fraser & Bernatchez, 2005b). Similarly, Tessier & Bernatchez (2000) hypothesized a 'double origin' (two colonizing lineages) among landlocked and sympatric populations of Atlantic salmon *Salmo salar* L. in Lake Saint-Jean, Quebec, based on the difference in magnitude between R_{ST} and θ : R_{ST} was 1.7 times larger than θ , identical to results of Table II.

An intralacustrine and purely deterministic origin of the morphs is nonetheless difficult to rule out owing to limitations going beyond the scope of the study: (1) uncertainty in estimates of μ and (2) lack of microsatellite DNA information from other landlocked *S. alpinus* populations in Newfoundland. First, larger μ values could reduce the number of generations necessary for

stepwise-like mutations to make a significant contribution to differentiation. Simulations using \hat{N}_{eLT} , $m_{(B\&F)}$ for dark and pale *S. alpinus* and $\mu = 0.001$ as input values for EASYPOP (Balloux, 2001) showed that R_{ST} values at least 1.7 times higher than θ are still theoretically possible after 2400 generations, although they are perhaps less likely (three of 20 runs; unpubl. data). Second, a broader geographical sampling scheme would first enable quantifying divergence between allopatric 'dark' populations, placing intermorph differentiation in an evolutionary historical context, and additionally, corroborate the existence of other 'pale' forms in other deep Newfoundland lakes. However, no 'pale' *S. alpinus* have been described in structurally similar Newfoundland lakes (Red Indian Lake and Grand Lake of the western region) other than GL (O'Connell *et al.*, 2005). Either (1) a unique colonization event in GL primed by local environmental and geological conditions [ice sheets appear to have retreated earlier in eastern than western Newfoundland according to Shaw *et al.* (2006)], or (2) an unsuccessful or underrepresented sampling of those deep lakes could explain this pattern. Additionally, it is feasible that now extinct 'pale' forms inhabited those western Newfoundland lakes in the past. These arguments support both extra- and intra-lacustrine hypotheses.

UNEQUAL ESTIMATES OF N_e BETWEEN MORPHS: ASSUMPTIONS AND IMPLICATIONS

The estimation of effective population size is challenging (Wang, 2005) and models make various assumptions that deserve further consideration. Coalescent-based maximum likelihood \hat{N}_{eLT} were derived only assuming $\mu = 0.001$ based on the present results ($\mu \geq m$). This was chosen as a very conservative estimate to keep mutation and migration rates in the same order of magnitude and the uncertainty associated with mutation-drift equilibrium in this system. If larger values of μ are nonetheless utilized ($\mu = 0.005$ or $\mu = 0.010$), \hat{N}_{eLT} would decrease by half to one order of magnitude, respectively. Although even values of $\mu > 0.01$ cannot be discounted without empirical estimates, they have been the exception in comprehensive microsatellite surveys in humans (Ellegren, 2004) or particularly salmonids (Steinberg *et al.*, 2002).

Contemporary LD \hat{N}_{eST} was also computed under several conjectures. Specifically, this method assumes discrete generations, yet *S. alpinus* have overlapping year classes (O'Connell *et al.*, 2005). \hat{N}_{eST} is therefore likely to be biased down and to reflect an intermediate quantity between the effective number of breeders (N_b) in a given year and the effective population size (N_e) following Waples (2005). For semelparous Pacific salmon *Oncorhynchus* spp., N_e is related to N_b through the mean generation length (Waples, 2005) and this correction has been employed in other salmonids (steelhead *Oncorhynchus mykiss* (Walbaum): Ardren & Kapuscinski, 2003; brown trout *Salmo trutta* L., *S. salar*: Fraser *et al.*, 2007). However, the relationship between N_e and N_b in iteroparous taxa is obscure and depends on the lifetime reproductive success of individuals (Waples, 2005), information that is currently lacking for this particular biological system. A cautionary approach must, therefore, be implemented when interpreting isolated N_e values from the LD method.

Despite the caveats above, it is still possible to draw conclusions regarding historical and contemporary estimates of N_e for dark and pale forms. If N_e fluctuates within one order of magnitude, it is safe to affirm that long and short-term point values of N_e are highly concordant. This is remarkable given that they apply to contrasting temporal frameworks. Even though both measure the inbreeding N_e , \hat{N}_{eLT} is integrated over the period when microsatellite alleles coalesce to a common ancestor (Beerli & Felsenstein, 2001), while \hat{N}_{eST} reflects LD between loci found in the progeny of parents from the previous generation (Waples, 2005). Agreement of both estimators strongly suggests that N_e has remained stable over multiple generations, ruling out the possibility of recent reductions in population size (Alo & Turner, 2005).

Consistently, larger estimates of N_e for dark than for pale *S. alpinus* were observed regardless of the method employed in the estimation. In a comprehensive survey of GL fish fauna, O'Connell *et al.* (2005) reported a 1.5–2 times higher abundance (measured as catch per unit effort) for dark than pale *S. alpinus* at different depths. This can be explained in terms of contrasting habitat quality (food and predators) and quantity the morphs occupy. Abundance of the pale morph peaks in the deepest zone of GL (*c.* 280 m), while the presence of the dark morph is pervasive in almost all bottom areas of the lake, as well as the water column (O'Connell & Dempson, 2002; O'Connell *et al.*, 2005); also, the dark form appears as a generalist–opportunistic predator, while the pale form has become highly specialized (Power *et al.*, 2005). Larger coalescent- and heterozygosity-based N_e values are found within lake (a much larger habitat) rather than stream three-spine sticklebacks *Gasterosteus aculeatus* L. in Misty Lake, Vancouver, Canada (Hendry *et al.*, 2002). Similarly, migratory *S. fontinalis* reproducing in inflow rivers of Mistassini Lake in Quebec, Canada, show N_e values four times larger than an isolated population breeding in the outflow: the main inflow river (Pepeshquasati) has a comparatively larger drainage and hence more available reproductive habitat as well as fewer predators and competitors (Fraser *et al.*, 2004).

Notwithstanding the importance of N_e in conservation, estimates are scant among resource-polymorphic species, with the exception of the examples mentioned above. These results stress the need for genetic assessment and monitoring (*sensu* Schwartz *et al.*, 2007) among other temperate species pairs as N_e seems an appropriate surrogate of habitat quality and quantity as well as abundance in most cases (Frankham, 1995). For instance, an environmental dichotomy has also been described in 'profundal' and 'littoral' *S. alpinus* in Lake Fjellfrøsvatn (Klemetsen *et al.*, 2002; Westgaard *et al.*, 2004). Interestingly, disparity in N_e between morphs in this study was not reflected in their neutral genetic properties (allelic richness or expected heterozygosity). Because small populations are more likely to experience the effects of genetic drift, it is probable that the occasional gene flow mitigates its detrimental effects (Ingvarsson, 2001; Palstra & Ruzzante, 2008) especially in the less abundant pale form. Unequal estimates of N_e between GL *S. alpinus* morphs, therefore, argue for parallel genetic studies of underrepresented or 'rare' forms in this and other species complexes.

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