Morphological and genetic differentiation in anadromous smelt *Osmerus mordax* (Mitchill): disentangling the effects of geography and morphology on gene flow

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Morphological analyses were combined with genetic analyses at nine microsatellite loci to examine the determinants of gene flow at 21 spawning locations of rainbow smelt *Osmerus mordax* along the east coast of Canada. Associations between morphology, geography and gene flow were examined using a computational geometric approach and partial Mantel tests. Significant barriers to gene flow and discontinuities in morphology were observed between Newfoundland and mainland Canada, as well as within Newfoundland samples. On regional scales, contrasting patterns were present with restricted gene flow between Newfoundland populations ($F_{ST} = c.\ 0.11$) and high gene flow between mainland populations ($F_{ST} = c.\ 0.017$). Within Newfoundland populations, geographic distance was significantly associated with gene flow ($r = 0.85$, $P < 0.001$) contrasting mainland samples where gene flow was most associated with phenotypic divergence ($r = 0.33$, $P < 0.001$). At large spatial scales, weak ($r = 0.19$, $P = 0.02$) associations between gene flow and geographic distance were observed, and moderate associations were also observed between gene flow and morphology ($r = 0.28$, $P < 0.001$). The presence of significant genetic isolation by distance in Newfoundland samples and the clear discontinuity associated with the Cabot Strait suggest geography may be the primary determinant of gene flow. Interestingly, the association between genetic and morphological divergence within mainland samples and overall, supports the hypothesis that gene flow may be moderated by morphological divergence at larger spatial scales even in high gene flow environments.
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INTRODUCTION

Understanding the mechanisms regulating gene flow in wild populations is critical to successful conservation and management efforts. Gene flow is closely correlated with connectivity between demes (Slatkin, 1993; Hellberg *et al.*, 2002), and influences both the response to disturbance and the rate of

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evolution (Nosil & Crespi, 2004). The major determinants of gene flow are dispersal probability and relative fitness of immigrants and residents (Nosil & Crespi, 2004; Crispo *et al.*, 2006). Adaptive divergence may reduce gene flow through a reduction in the fitness of immigrating individuals relative to residents (Hendry *et al.*, 2002). High rates of gene flow, however, may 'erode' local adaptation, homogenizing genetic variation among demes. Accordingly, negative associations between gene flow and phenotypic divergence are common for many taxa (King & Lawson, 1995; Lu & Bernatchez, 1999; Rosenblum, 2006).

In marine environments dispersal potential may be large (100s to 1000s of km) and gene flow correspondingly high and associated with geographic distance (Uthicke & Benzie, 2003). Consistent with this generalization, ecological speciation (McKinnon *et al.*, 2004) has rarely been proposed for marine fishes, presumably because high dispersal has been thought to reduce the likelihood of habitat-associated adaptation and divergence (Mayr, 1954). Nonetheless, recent studies have suggested habitat-associated morphological divergence (Langerhans *et al.*, 2003) and ecological speciation as a mechanism for marine evolution (Taylor & Hellberg, 2003; Rocha *et al.*, 2005). Moreover, the observation of clines and barriers to gene flow in marine species supports the hypothesis that dispersal is not always the predominant force regulating gene flow (Hilbish, 1996; Bekkevold *et al.*, 2005; Jorgensen *et al.*, 2005). These studies suggest that despite seemingly high dispersal potential, ecological processes (*i.e.* trophic partitioning) may reproductively isolate demes in marine species.

Rainbow smelt *Osmerus mordax* (Mitchill) is a small pelagic fish found in coastal and freshwater systems throughout north-eastern North America (Nellbring, 1989). Anadromous smelt spawn near the head of the tide in coastal rivers and streams and the larvae develop in downstream estuaries (Bradbury *et al.*, 2004). The close association with estuarine habitat and the potential for active (*i.e.* through vertical migration) estuarine retention of larvae has lead several studies to conclude that population structure is probably associated with estuaries or retention areas (Bernatchez & Martin, 1996). Indeed, associations between morphology, genetic markers and retention areas have been observed on small geographic scales (*i.e.* mtDNA: Baby *et al.*, 1991; morphology: Frechet *et al.*, 1983). More commonly, rainbow smelt show frequent morphological adaptation and restricted gene flow associated with habitat, often between freshwater and anadromous forms (Copeman, 1977; Taylor & Bentzen, 1993*a*) as well as within freshwater populations (Schreiner *et al.*, 1984). Furthermore, instances of sympatric pairs of normal and dwarf forms are known from a number of locations throughout the species range (Taylor & Bentzen, 1993*b*; Saint-Laurent *et al.*, 2003). Lecomte & Dodson (2005) suggested that habitat-associated resource polymorphism has significantly contributed to the maintenance of distinct populations within the St Lawrence estuary. Given the tendency for morphological adaptation in rainbow smelt and other species (Bernatchez, 2003), and the potential for reduced gene flow, a reasonable hypothesis is that selection for local phenotypic adaptation may constrain gene flow reinforcing discrete populations associated with estuarine retention areas.

The objective of this study was to examine the relative roles of phenotypic divergence and geographic separation in regulating gene flow among

anadromous populations of rainbow smelt along eastern Canada. It was hypothesized, based on studies of sympatric pairs of rainbow smelt, that the presence of local selective regimes may reduce the fitness of immigrants, and constrain gene flow, promoting the evolution of distinct morphotypes. Following Hendry *et al.* (2001) and Hendry & Taylor (2004), the presence of an association between gene flow and morphological divergence was used as a test of restricted gene flow due to adaptation and was examined both at local and broad geographic scales. Correlation methods have traditionally been utilized to examine linkages between gene flow and environmental characters (Bekkevold *et al.*, 2005). These approaches, however, assess the strength of overall relationships but fail to examine any scale-dependent changes, and may be susceptible to biases associated with multicolinearity of matrices (Raufaste & Rousset, 2001). This study combined correlation approaches with a computational geometric (Manni *et al.*, 2004) technique to examine the scale-dependent determinates of gene flow in rainbow smelt. This combined approach has been successful in delineating genetic structure in other marine fishes suggestive of small-scale population structure despite high dispersal potential (Bekkevold *et al.*, 2005; Jorgensen *et al.*, 2005).

MATERIALS AND METHODS

STUDY AREA

In total, 21 locations were sampled from 2003 to 2005 (Fig. 1 and Table I) encompassing four provinces (Newfoundland, Nova Scotia, Prince Edward Island and New Brunswick). Thermal and circulation conditions vary dramatically across the study with the Labrador Current dominating coastal south-eastern Newfoundland transport (Bradbury *et al.*, 2000), and the Gulf of St Lawrence determining mainland coastal conditions. The Cabot Strait, which is *c.* 110 km across, and 500 m deep, separates Newfoundland from the mainland. Newfoundland's coastline is characterized by numerous large embayments, which are less common along the mainland coast (Fig. 1). Geographic distance between each site was measured as the shortest distance following within 5 km of the coastline using charts and Google Earth 2004. All distances were measured three times and averages used.

GENETIC ANALYSIS

Fish were collected by dip-netting, and fyke netting during the spawning period. Pectoral or caudal fin clips were taken and immediately placed in 95% ethanol. DNA was extracted following the protocol of Elphinstone *et al.* (2003), modified to work with a 96-well filter plate and automated on a robotic liquid handling system (Perkin Elmer, Boston, MA, U.S.A.). Individuals were genotyped for nine microsatellite loci using polymerase chain reaction (PCR) conditions of 5 or 10 μl volumes containing 20–100 ng DNA, 1·5 mM $MgCl_2$, 80 μM each dNTP, 0·5 U *Taq* DNA polymerase (New England Biolabs, Ipswich, MA, U.S.A.), 0·3 μM each primer (forward primers were end-labelled with Hex, or Rox dye), and 1× PCR buffer (10 mM Tris-HCl, pH 8·3; 50 mM KCl). Two temperature profiles were used for touchdown in order to allow for the possibility of multiplex PCRs. Touchdown PCR conditions were as follows: 94° C for 2 min, followed by four to five cycles of 94° C for 30 s, programme-specific touchdown annealing temperatures minus 1° C per cycle for 30 s, 72° C for 30 s, followed by 25–26 cycles where the annealing temperature, T_a, was held constant at 4° C below the starting temperature. A final extension was held at 72° C for 5 min. Reactions were run on
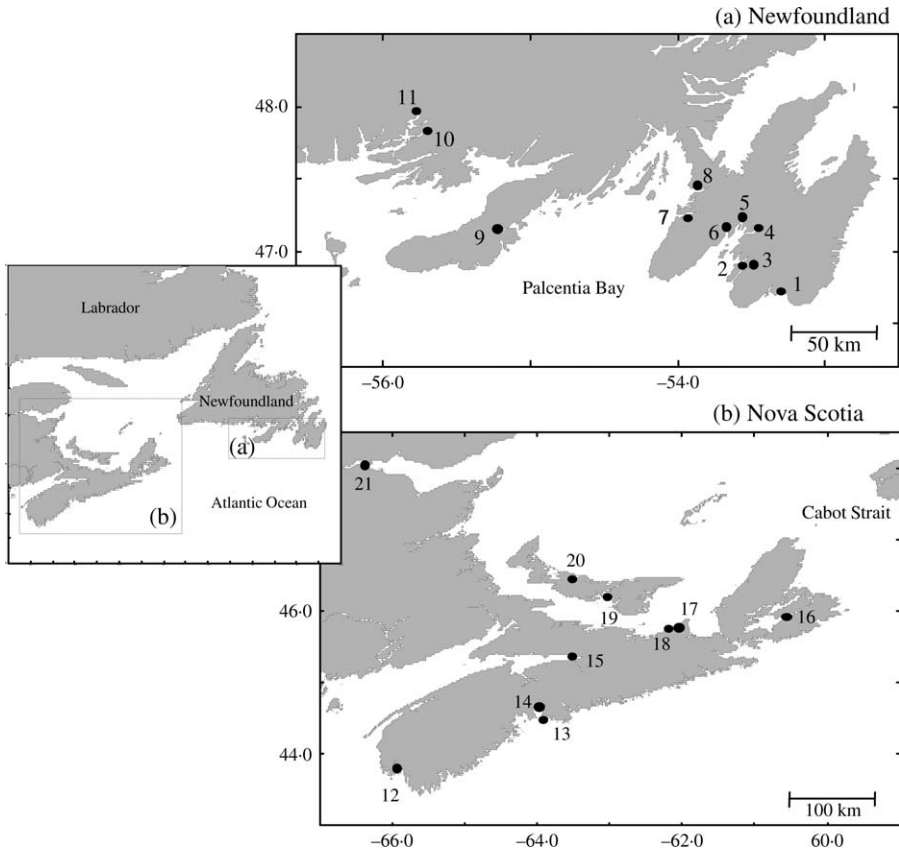
FIG. 1. Map showing sample locations for 21 populations of rainbow smelt sampled in 2003–2005. Inset shows locations with respect to eastern Canada (numbers correspond to sites listed in Table I).

Eppendorf thermocyclers and imaged on an FMBioII system (Hitachi Genetic Systems, Alameda, CA, U.S.A.) [see Coulson *et al.* (2006) for further details].

MORPHOLOGICAL MEASUREMENTS

All sampled fish were frozen to facilitate rapid collection and preservation. Within 6 months of collection 14 morphological and meristic traits were measured (see Fig. 2), including fork length (L_F; tip of snout caudal fork), eye diameter (diameter of orbit along body axis), upper jaw length (tip of jaw to end of maxilla), head depth (depth at opercular insert), head length (tip of head to posterior edge of operculum), pre-dorsal length (tip of upper jaw to anterior insert of dorsal fin), pre-pelvic length (tip of upper jaw to anterior insert of dorsal fin), pre-pectoral length (tip of upper jaw to anterior insert of dorsal fin), pelvic-pectoral fin distance (distance from pelvic insert to pectoral fin insert), gill raker count (number of rakers on first arch), length of first gill arch, length of longest raker on long arm and length of longest raker on small arm of first gill arch. All measurements were made with either Vernier callipers or based on measurements taken from digitized images using tpsDIG (Rohlf, 2005). To ensure comparability of methods 60 fish were independently measured with both approaches and no significant differences were observed between techniques ($P > 0.05$). Co-variation due to 'size' was removed following Lecomte & Dodson (2005). A 'size' variable was

TABLE I. Sampling sites, sample sizes and summary genetic statistics for 21 smelt populations sampled along eastern Canada from 2003 to 2005

| Population	Sample date	Morphology n	Genetic n	Unbiased heterozygosities	Observed heterozygosities	Number of alleles
1. Biscay Bay (NL)	April 2004	60	94	0·7033	0·6889	9·11
2. Pathend Brook(NL)	June 2005	44	94	0·7299	0·7117	10·22
3. Deerpond Brook(NL)	May 2005	40	32	0·7069	0·7085	8·22
4. Salmonier (NL)	May 2004	52	94	0·7156	0·7261	10·33
5. Colinet (NL)	June 2004	31	94	0·7191	0·7330	12·11
6. North Harbour (NL)	May 2005	22	94	0·7068	0·7354	10·67
7. Southeast Placentia (NL)	April 2005	56	94	0·6718	0·6602	9·44
8. Long Harbour (NL)	October 2004	48	79	0·6863	0·7076	7·33
9. Salt Pond (NL)	April 2004	49	92	0·7710	0·7309	10·78
10. Little River (NL)	May 2004	48	94	0·6913	0·6963	9·89
11. Conne River (NL)	May 2004	75	94	0·6828	0·6582	10·89
12. Tusket (NS)	April 2004	50	94	0·8299	0·8358	13·00
13. Indian Point (NS)	June 2004	49	50	0·8319	0·8612	11·11
14. Island View (NS)	June 2004	48	94	0·8039	0·8173	11·33
15. Portapique (NS)	May 2004	48	94	0·8573	0·8479	15·56
16. Eskasoni (NS)	February 2004	46	60	0·8470	0·8419	12·89
17. Doctors Brook (NS)	May 2004	47	55	0·8448	0·8318	12·33
18. Knoydart (NS)	May 2004	47	66	0·8538	0·8707	14·00
19. Hillsborough (PEI)	April 2004	48	94	0·8534	0·8369	14·67
20. Tyne Valley (PEI)	April 2004	48	94	0·8587	0·8572	15·89
21. Restigouche (NB)	May 2004	49	94	0·8577	0·8310	14·22
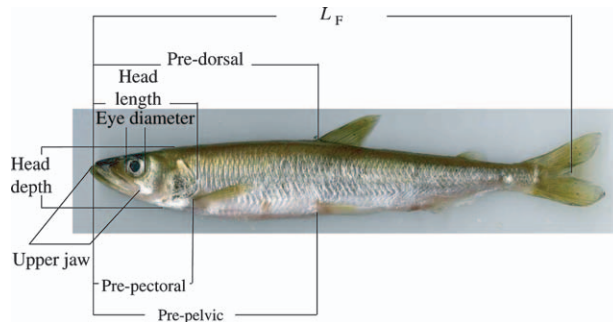
FIG. 2. Morphometric measurements used in analysis (L_F, fork length). Note that four gill raker characters are not shown.

calculated as the first factor in a factorial principle component analysis of all traits. Individual observations were plotted against this size variable and residuals were then used in further analysis.

DATA ANALYSIS

Phenotypic variation was explored two ways. First, Euclidian distances were calculated (SYSTAT v. 11) for each pair of populations based on the mean values for each trait. Second, pair-wise measurements of phenotypic (*i.e.* Q_{ST}) divergence were used as a measure of phenotypic variation between populations (Saint-Laurent *et al.*, 2003). This was defined here as P_{ST}, emphasizing that phenotypic measures were utilized and no estimates of genetic variance were made. Though phenotypic variance has been used as a surrogate for additive genetic variance elsewhere (Bernatchez, 2003; Saint-Laurent *et al.*, 2003) the distinction made here between P_{ST} and Q_{ST} seems appropriate, as environmental influences on these traits are unknown. The phenotypic variance was equal to twice the variance of individuals within populations ($2\sigma_w^2$), and the phenotypic variance between populations was used as σ_B^2. P_{ST} was calculated as: $P_{ST} = \sigma_B^2(\sigma_B^2 + 2\sigma_w^2)^{-1}$, following Spitze (1993). As ANOVAs showed significant differences among populations for all traits examined, all traits were included in preliminary analysis. Distinct ecotypes were identified using multidimensional scaling (MDS) of Euclidean distance of population mean trait values. Discriminant function analysis (SYSTAT v. 11) was used to examine the discrimination of these groups and explore linkages between morphology and large-scale habitat differences (*i.e.* Newfoundland and mainland populations).

Genetic polymorphism was quantified by examination of the number of alleles, and observed and expected heterozygosities using GENETIX (version 4.05.2; Belkhir *et al.*, 2004) Tests for Hardy–Weinberg equilibrium (HWE) and linkage disequilibrium were done using FSTAT (version 2.9.3.3; Goudet, 1995). F_{ST} values and significance were calculated using FSTAT and significance was determined with permutation tests with an $\alpha = 0.05$ (Schneider *et al.*, 2000). Genetic patterns were examined graphically using factorial correspondence analysis (GENETIX, version 4.05.2; Belkhir *et al.*, 2004), which displays correspondences for diploid genotypes three dimensionally. An analysis of molecular variance, AMOVA, was used to partition observed genetic variance into components associated with morphotypes and geographic regions and conducted using ARLEQUIN. Associations between genetic, geographic and morphological distances were explored using full and partial Mantel tests (ARLEQUIN) as well as graphically.

Discontinuities in the spatial pattern of both morphological and genetic data were examined using a computational geometric approach as implemented in the software BARRIER 2.2 (Manni *et al.*, 2004). This approach utilizes Delauney triangulation and Monmonier's algorithm to identify which connections between neighbouring populations exhibit the largest discontinuity (Bekkevold *et al.*, 2005). Barriers were

estimated using pair-wise matrices of genetic (F_{ST}) and morphological distance (*i.e.* P_{ST} and Euclidean distance) calculated above.

RESULTS

Significant morphological divergence was observed between samples, with P_{ST} values ranging from 0·13 to 0·27. MDS showed some tendency for neighbouring populations to associate (Fig. 3), however, the largest break was between Newfoundland and mainland samples. MDS plots were similar whether based on P_{ST} or Euclidean distance (Fig. 3). Assignments based on discriminant function analysis between Newfoundland and the mainland was 92% (Table II). By comparison, assignment of fish to small geographical units (Newfoundland bays) was less successful (Table II).
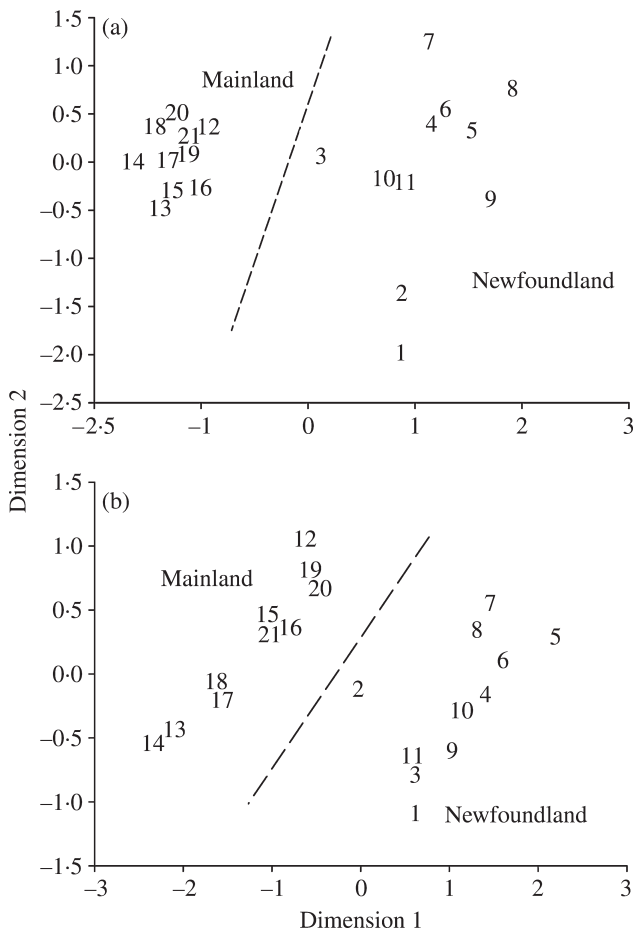

FIG. 3. Multidimensional scaling of (a) phenotypic variance (P_{ST}) and (b) Euclidean distances based on population mean trait values for 21 populations of anadromous rainbow smelt from along the east coast of Canada (Table I and Fig. 1). Analysis is based on five dimensions (first two are shown), 100 iterations and convergence of 0·0001, Kruskal stress values are 0·084 and 0·023 for P_{ST} and Euclidean distances, respectively.

TABLE II. Results of discriminant function analysis of morphometric traits on rainbow smelt from eastern Canada

Comparison	d.f.	P	Per cent correct assignment (jackknifed)
A. Between Newfoundland and mainland	1018	<0·001	92
B. Between Newfoundland bays	535	<0·001	56
C. Between sample locations	1018	<0·001	34

Summary statistics for genetic samples are included in Table I. Sample sizes varied between 32 and 94 with an average of 84 fish. Average observed heterozygosities varied between 0·67 and 0·73 for Newfoundland samples and 0·80 and 0·86 for mainland samples (Table I). No deviations from HWE were observed and data were checked for the presence of null alleles and scoring errors using MICROCHECKER (van Oosterhout *et al.*, 2004). Two loci did show weak evidence of linkage, but only within the mainland samples. Removal of either locus had no significant effect on observed trends and as they did not appear linked in Newfoundland samples they were included in further analysis.

Estimates of F_{ST} between populations ranged from 0·00 to 0·23 and were significantly higher between Newfoundland samples than between mainland samples ($P < 0·001$). Most pair-wise F_{ST} values were significant with the exception of populations, which share estuaries (*i.e.* Island View and Indian Point), though these made up a minority of the comparisons (<4%). Factorial correspondence analysis of the microsatellite data (Fig. 4) suggested the presence of two main clusters corresponding to Newfoundland and mainland samples. A division of the Newfoundland cluster into two subclusters was also discernable,
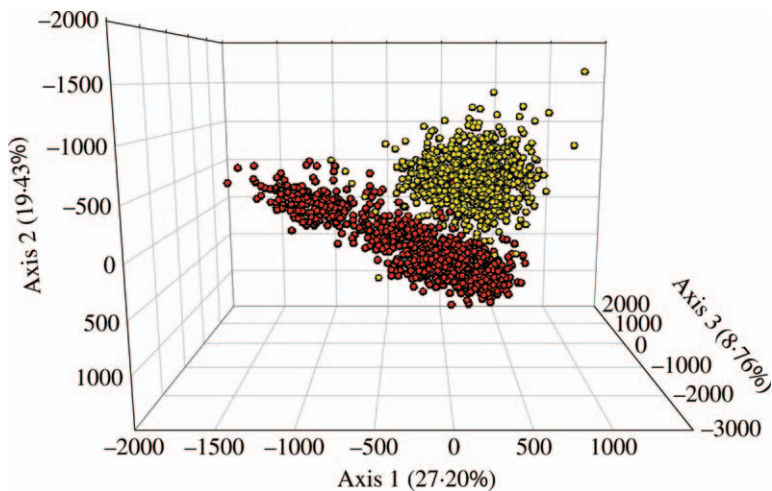

FIG. 4. Factorial correspondence analysis of multilocus genotypes based on nine microsatellite loci isolated in rainbow smelt from Newfoundland (●) and mainland eastern Canada (◉) [calculated using GENETIX (version 4.05.2; Belkhir *et al.*, 2004)].

and the subclusters corresponded to eastern and western Newfoundland populations, respectively. The AMOVA results (Table III) show significant structuring in all cases.

Partial Mantel tests examined the co-variation between geographic, genetic and morphological distance (Table IV). In the complete data set, weak association between geographic and genetic differentiation was observed [Fig. 5(a)]. Among the Newfoundland samples, geographic distance accounted for 74% of the variance ($P < 0.001$) in gene flow. Among mainland samples, geographic distance explained 6% of the variance but was not significant. Significant correlations were observed between morphological divergence (P_{ST}) and gene flow (Table IV) both in the complete data set and among mainland samples though not in Newfoundland samples (Table IV).

Significant relationships with geographic distance were observed in both P_{ST} [$r = 0.51$, $P < 0.001$; Fig. 5(b)] and Euclidean distance [$r = 0.62$, $P < 0.001$; Fig. 5(c)] at large spatial scales, although no patterns were present within regions. Both measurements of morphological divergence (Fig. 6) explained a significant proportion of the genetic variance though the strength of correlations was low ($r = c.\ 0.3$; Table IV).

BARRIER analysis of genetic data indicate two of the four suggested barriers to gene flow are associated with the Cabot Strait (I and III), and the remaining barriers divide Newfoundland samples into clusters similar to those observed in the factorial correspondence analysis [Fig. 7(a)]. BARRIER analysis of the morphological data predicted similar barriers whether P_{ST} [Fig. 7(b)] or Euclidean distance [Fig. 7(c)] were used. In the case of P_{ST}, breaks in morphology were observed between samples from Holyrood Pond Newfoundland (I), between Newfoundland and the mainland (II), and between isolated populations within Newfoundland (III and IV). Barriers indicated by the Euclidean distances of morphological data suggest breaks between Newfoundland and the mainland (I), southern Nova Scotia and the remaining mainland (II), and individual Newfoundland populations (III and IV).

DISCUSSION

The relative role of ecological processes (*i.e.* trophic partitioning; McKinnon *et al.*, 2004) and geography (*i.e.* distance; Slatkin, 1993) in regulating gene flow in marine species has been poorly understood (Mayr, 1954). Despite high dispersal potential, recent studies suggest ecological processes may be of greater importance to isolating populations than previously thought (Langerhans *et al.*, 2003; Rocha *et al.*, 2005). These results suggest that gene flow in rainbow smelt along Canada's east coast predominately results from interactions with geographic distance or isolating barriers such as the Cabot Strait. Morphological variation, however, may contribute to a lesser degree on larger geographic scales even in high gene flow environments.

THE ROLE OF GEOGRAPHY ON GENE FLOW

Two lines of evidence are presented that gene flow is strongly regulated by dispersal and geography. First, strong isolation by distance (IBD) was observed

TABLE III. AMOVA results comparing genetic variation in 21 rainbow smelt populations sampled along eastern Canada (see Fig. 1) at various spatial scales

Grouping	Source of variation	d.f.	Sum of squares	Variance component	Per cent of total	F_{CT}	F_{SC}	F_{ST}
Newfoundland and mainland (Fig. 1)	Among groups	1	261·75	0·1264	3·89	0·039***		
	Among pop w/in groups	19	755·34	0·2207	6·80		0·071***	
	Within pops	3503	10153·02	2·8984	89·31			0·107***
	Total	3523	11170·10	3·2454	100			
Newfoundland bays	Among groups	3	452·55	0·2601	8·55	0·085***		
	Among pop w/in groups	7	209·51	0·1569	5·14		0·056***	
	Within pops	1929	5081·3	2·6342	86·3			0·136***
	Total	1939	5743·3	3·0519	100			

***, $P < 0.001$.

F_{CT}, among groups; F_{SC}, among samples within groups; F_{ST}, within samples.

TABLE IV. Mantel tests (regular and partial) for matrix correlations (r) incorporating pair-wise differences in genetic (F_{ST}), geographic and morphology (P_{ST} before the/and Euclidian distance following) both within and between geographic regions (Newfoundland and mainland)

| Comparison	All data (Newfoundland and mainland)		Newfoundland only		Mainland only	
	P	r	P	r	P	r
Genetic v. geographic distance	0·020/0·020	0·19/0·19	<0·001/<0·001	0·85/0·85	0·16/0·08	0·23/0·23
Partial controlling for morphology	0·400/0·460	0·03/0·008	<0·001/<0·001	0·85/0·84	0·12/0·12	0·29/0·21
Genetic v. morphological distance	<0·001/<0·001	0·33/0·33	0·38/0·06	0·05/0·27	<0·001/<0·001	0·29/0·42
Partial controlling for geographic distance	0·050/<0·001	0·27/0·285	0·38/0·20	0·04/0·11	<0·001/<0·001	0·33/0·41
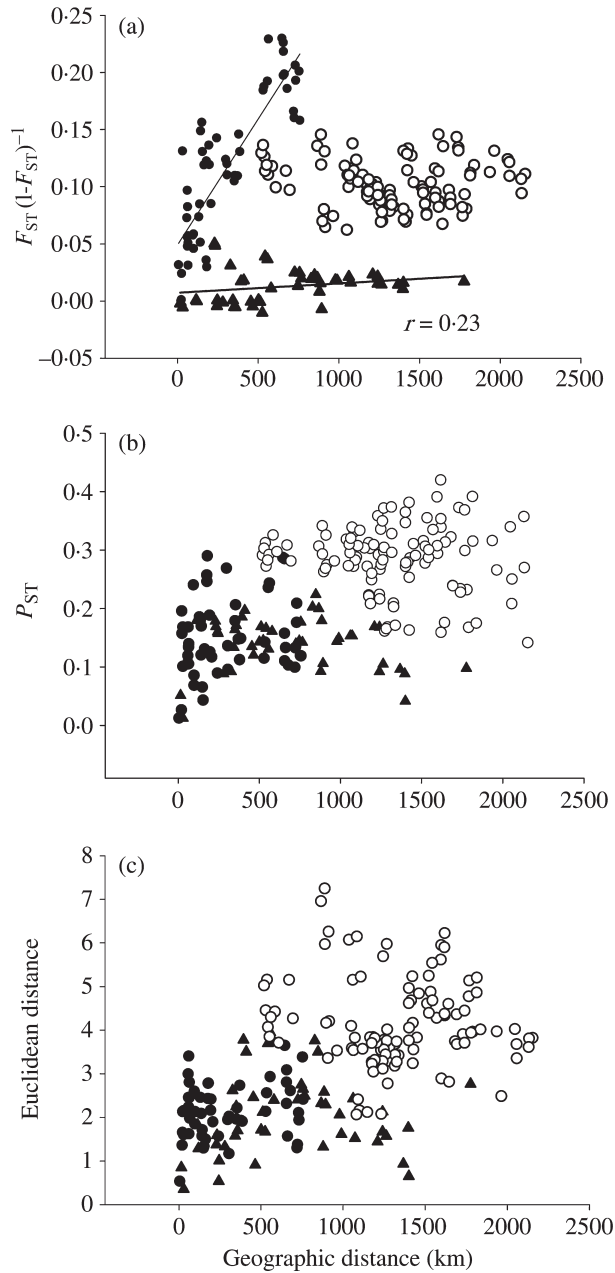
FIG. 5. Isolation by distance (IBD) of (a) genetic divergence ($r = 0.85$) and morphological divergence as quantified by (b) phenotypic variance (P_{ST}) ($r = 0.49$) and (c) Euclidean distance ($r = 0.54$) with geographic distance. Comparisons include within Newfoundland (●), within mainland samples (▲) and between Newfoundland and the mainland (○). Correlation coefficients correspond to values from Mantel tests (see Table IV).
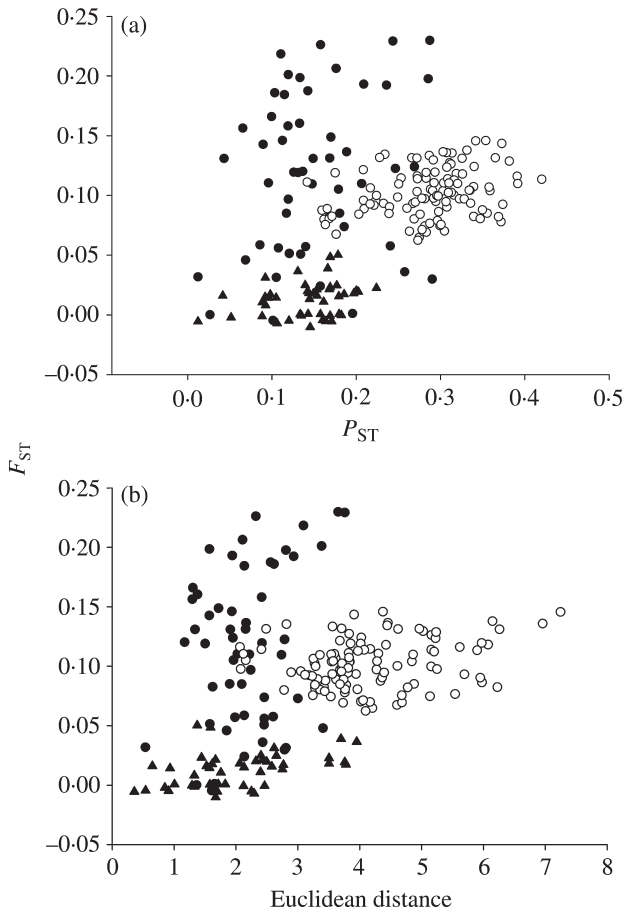
FIG. 6. Genetic divergence (F_{ST}) based on nine microsatellite loci and morphological divergence as measured using (a) phenotypic variance (P_{ST}) and (b) Euclidean distance for 21 populations of rainbow smelt from Canada's east coast.

within Newfoundland samples suggesting geography (*i.e.* distance) is the prime determinant of population structure on the island [Fig. 5(a)]. The presence of IBD within Newfoundland suggests that these populations have reached or are close to reaching equilibrium (Slatkin, 1993). It is unclear at this point why the IBD is significantly weaker among the mainland samples, but it could be related to differences in effective population sizes as suggested by the significantly higher heterozygosities observed. Alternately it could be due to restrictions of gene flow associated with phenotypic differences.

Secondly, data presented suggest that the Cabot Strait is a significant barrier to gene flow between Newfoundland and the mainland (Figs 4 and 7) as the isolation by distance relationship breaks down at larger scales and the correspondence analysis suggests two discrete clusters (Fig. 4). Moreover, two of four identified barriers to gene flow are directly associated with the Cabot Strait (Fig. 7). Similar patterns of small-scale IBD but limited large-scale association have been observed in guppies *Poecilia reticulata* (Peters) (Crispo *et al.*,
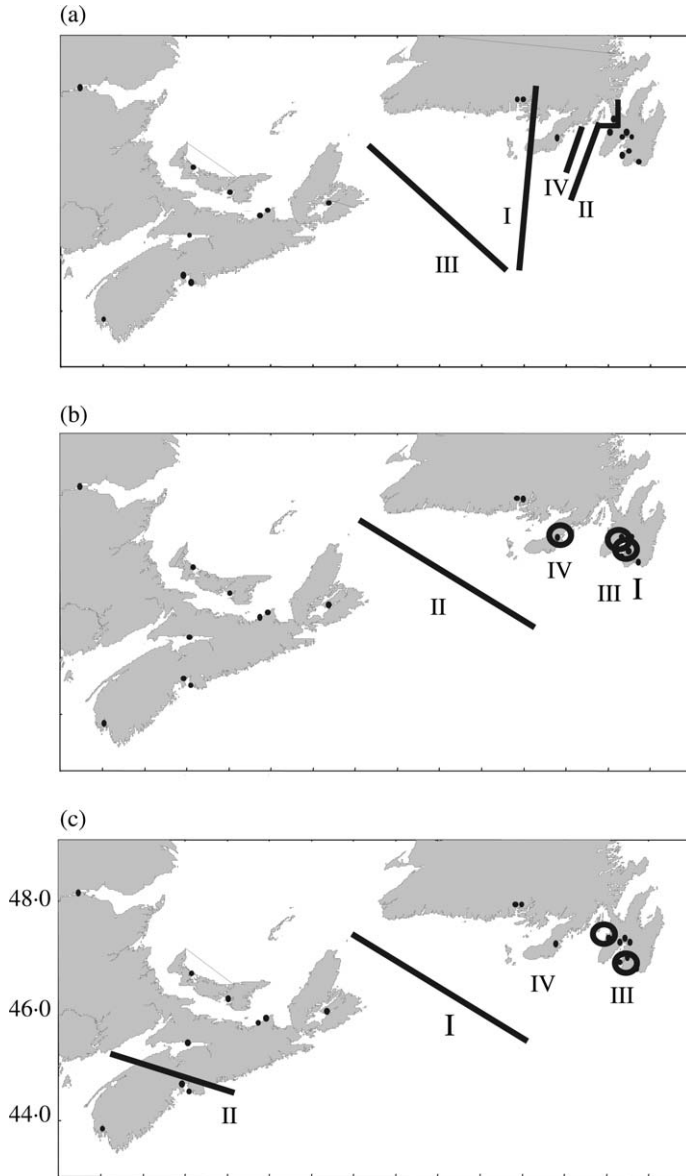
(a)

(b)

(c)

FIG. 7. Major discontinuities in (a) gene flow and morphology: (b) P_{ST} and (c) Euclidean distance suggested by BARRIER (Manni *et al.*, 2004). I–IV, order of importance; ▬, barriers between groups of locations; ○, separate individual points from all others; ●, station locations (see Fig. 1 and Table I).

2006), and brook charr *Salvelinus fontinalis* (Mitchell) (Poissant *et al.*, 2005) where IBD is strongest at smaller scales not influenced by historical barriers to gene flow. Interestingly, this break between Newfoundland and the mainland was evident in both genetic (Fig. 4) and morphometric data (Fig. 3). These differences may reflect some deeper historical separation, such as observed within the St Lawrence estuary between Acadian and Atlantic races (Lecomte &

Dodson, 2005). The limited data available from Taylor & Bentzen (1993*a*) and Bernatchez (1997) suggest colonization of the current study area from a single refugium, however, this hypothesis requires further examination.

THE ROLE OF MORPHOLOGY ON GENE FLOW

There was limited evidence of an association between phenotypic divergence and gene flow within mainland samples and overall (Table IV and Fig. 6). Significant associations were observed in both cases though correlation coefficients were moderate to low (0·3–0·4; Fig. 6) nonetheless suggesting a positive relationship. Moreover, the AMOVA between groupings (*i.e.* Newfoundland and mainland or within Newfoundland) was significant, and the explained variance was 3·89 and 8·50%, respectively. Counter to expectations, the relationship between gene flow and morphology was significant in the high gene flow (*i.e.* mainland) environment, suggesting that locally varying selective pressures are strong enough to dampen generally high levels of gene flow, resulting in local adaptation. The lack of an association between gene flow and morphology in Newfoundland suggests evolutionary independence of these populations.

Morphological divergence in rainbow smelt has most often been studied in freshwater systems, and primarily between sympatric pairs (Copeman & McAllister, 1978; Taylor & Bentzen, 1993*b*; Saint-Laurent *et al.*, 2003). In anadromous rainbow smelt, examples are fewer (Frechet *et al.*, 1983). Lecomte & Dodson (2005) documented significant morphological and genetic divergence between populations within the St Lawrence estuary. Interestingly, the patterns presented here of increasing morphological divergence with distance (Fig. 5) suggest that these large spatial scales may be necessary for the evolution of large phenotypic differences among populations of anadromous rainbow smelt. In contrast to Lecomte & Dodson (2005), the differences observed are over a larger spatial scale and probably due to the inclusion of a single glacial race (Bernatchez, 1997).

As has been suggested elsewhere (Saint-Laurent *et al.*, 2003), the interpretation of P_{ST} (*i.e.* Q_{ST}) as genetic variance without explicit knowledge of the heritability of phenotypic traits should be made cautiously given that it involves several assumptions. The distinction made here between Q_{ST} and P_{ST} avoids any confusion associated with interpreting phenotypic variance as genetic variance, yet allows direct comparison with an increasing number of studies utilizing this approach (Bernatchez, 2003; Saint-Laurent *et al.*, 2003; Roseman, 2004). Observed P_{ST} values were approximately two times higher than F_{ST} values for comparisons of the same populations. Though not as large as differences observed between freshwater dwarf and normal forms (Saint-Laurent *et al.*, 2003), the observed difference may suggest divergent selection, but requires further study (*i.e.* common-garden experiments).

GEOGRAPHY AND ADAPTATION

Several potential explanations exist for a lack of correlation between gene flow and phenotypic divergence within Newfoundland samples (Hendry

et al., 2001): the estimates of adaptive divergence are incorrect, the estimates of gene flow are incorrect or population mixing is not important to divergence. Estimates of morphological divergence presented here are primarily based on relatively fixed points, associated with osteological structures. Although estimates of heritability for these features are limited for fishes [*e.g.* 0·7 for gill-related traits from Bernatchez (2003), 0·32 for body morph from Heath *et al.* (1994) and 0·84 for number of vertebrae from Leary *et al.* (1985)], available estimates suggest significant additive genetic components. With respect to estimates of gene flow, estimates presented here are based on nine tetranucleotide loci that gave no reason to suspect genotyping error, as they were in HWE equilibrium and showed no evidence of consistent null alleles or large allele drop out, thus it seems unlikely that the estimates of F_{ST} may be subject to large error.

The remaining hypothesis then, is that gene flow is independent of morphology within Newfoundland samples. Several simulation experiments suggest that adaptive divergence should be negatively correlated with gene flow (Hendry *et al.*, 2001). Nonetheless, the suggestion that gene flow may be independent of morphological adaptation or selection has been made by several authors (Hendry *et al.*, 2001; Saint-Laurent *et al.*, 2003; Nosil & Crespi, 2004). Saint-Laurent *et al.* (2003) observed large morphological divergence between freshwater forms of rainbow smelt despite high levels of gene flow and suggested that differences resulted from strong divergent selection. Crispo *et al.* (2006) concluded that selection pressure associated with predation and habitat had little influence on gene flow, which was primarily determined by dispersal likelihood (*i.e.* distance or presence of waterfalls). As such it seems likely that gene flow in Newfoundland populations may be low enough such that these populations respond independently to selection. This is supported by the observation that at higher rates of gene flow, a significant, albeit weak, association between gene flow of phenotypic divergence was observed (*i.e.* mainland samples).

Although the evidence for an association between gene flow and phenotype for mainland populations presented here seems robust, the causal link remains uncertain. Morphological adaptation may occur despite high gene flow, or similar adaptations (as signalled by morphology) may occur under conditions of low gene flow. Nosil & Crespi (2004) demonstrate that in *Timema cristinae* walking-sticks, morphological divergence is a result of reduced gene flow not *vice versa*. Similarly, Hendry & Taylor (2004) suggest that between lake and stream three-spined stickleback *Gasterosteus aculeatus* L. pairs, gene flow has had a substantial effect on adaptive divergence, in agreement with other three-spined stickleback studies (Bourgeois *et al.*, 1994). The contrasting levels of genetic differentiation (F_{ST}) within regions (*i.e.* within Newfoundland and within the mainland) should allow further examination of the relationship between morphological divergence and gene flow. It might be predicted that reduced gene flow within Newfoundland would be accompanied by increased morphological divergence. Yet, despite an order of magnitude difference between Newfoundland and the mainland in within-region genetic differentiation, morphological divergence in the two regions was similar, supporting the hypothesis that selection and gene flow are independent within Newfoundland populations (Fig. 6).

Alternatively, selection may play a role in reducing morphological divergence. Selection might act to favour dispersal and stabilize phenotype if the suitability of spawning sites for successful reproduction is highly variable over time. Indeed, it is common for rainbow smelt spawning locations to vary dramatically in suitability and presence of fish from year to year, and spring precipitation rates often explain a large proportion of the annual recruitment variance (McKenzie, 1964). In the presence of high temporal instability, selection may maintain low genetic and adaptive divergence both as a result of increased gene flow and stabilizing selection. This possibility appears more likely for mainland populations than for Newfoundland rainbow smelt as gene flow among spawning sites within Newfoundland appeared to be much more restricted.

This study indicated that gene flow is primarily regulated by geographic features (*i.e.* distance and physical barriers) in marine species. Interestingly, the highest observed genetic distances were at small scales between Newfoundland populations and were surprisingly independent of morphological patterns contrasting observations in a relatively high gene flow environment (*i.e.* mainland populations). The association between genetic and morphological divergence between mainland populations and overall populations supports the hypothesis that gene flow may be moderated by morphological divergence at larger spatial scales even in high gene flow environments. A major break between Newfoundland and the mainland populations was observed, and the nature of the Cabot Strait as a barrier to gene flow and the phylogeographic nature of these groups require further study.

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