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Evidence for broadscale introgressive hybridization between two redfish (genus *Sebastes*) in the North-west Atlantic: a rare marine example

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Abstract

The evolutionary importance of introgressive hybridization has long been recognized by plant evolutionists, and there is now a growing recognition for its potential role in animals as well. Detailed empirical investigations of this evolutionary process, however, are still lacking in many animal groups, particularly in the marine environment. Using integrated microsatellite DNA data (eight loci analysed over 803 individuals representing 17 sampling locations) and multivariate statistical procedures (principal component, factorial correspondence and admixture proportion analyses), we: (i) provide a detailed dissection of the dynamics of introgressive hybridization between *Sebastes fasciatus* and *S. mentella*, two economically important redfishes from the North-west Atlantic; and (ii) infer the factors potentially involved in the maintenance of the hybrid zone observed in the gulf of St. Lawrence and south of Newfoundland. This study provided one of the rare examples of extensive introgressive hybridization in the ocean, and highlighted the predominant role of this process in shaping the extent of genetic diversity, interspecific differences and population structuring among redfishes from the North-west Atlantic. The extensive (average rate of introgression = 15%) but geographically circumscribed and asymmetrical pattern of introgressive hybridization, the sympatric persistence of two reproductively isolated introgressed groups, the differential patterns of linkage disequilibrium among samples, and the maintenance of genetic integrity of both species outside the defined zone of introgression despite high potential for gene flow, all implicated selection in promoting and maintaining the observed pattern of introgression.

Keywords: genetic structure, introgressive hybridization, marine fish, microsatellite, multivariate analyses, *Sebastes*

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Introduction

The potential evolutionary importance of introgressive hybridization has long been recognized by many researchers, particularly in plants (Anderson & Stebbins 1954; Harrison 1990; Hansen & Loeschcke 1994; Rieseberg 1995; Rieseberg *et al*. 1995). On the other hand, many proponents of hybrid zone concepts have considered natural hybridization as an evolutionary dead end, because hybrids could generally never do better than parental types (e.g. Barton & Hewitt 1985). For example, the 'Tension zone model' implies that endogenous selection acts against hybrids, and, in addition to the dispersal of parental genotypes into the zone, lead to the maintenance of a narrow hybrid region, with little (if any) consequence on the genetic integrity of the parental species (Barton 1979; Barton & Hewitt 1985, 1989; Hewitt 1988). Numerous

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empirical supports for this model have been reported, where hybrids were sterile, less fit or less frequent than the parental types (e.g. Dowling & Moore 1985; Potts 1986; Nürnberger *et al*. 1995; Reed & Sites 1995). Alternatively, other models have considered the possibility of higher fitness for hybrids, although only restricted in particular ecotonal habitats (e.g. 'Bounded hybrid superiority model', Moore 1977; 'Mosaic model', Harrison 1986; Howard 1986).

More recently, the view that introgressive hybridization may also represent an important and pervasive evolutionary process has gained new support from the reevaluations of previous hybrid zones studies, as well as new findings (Arnold 1992). Thus, a new perspective on hybrid zone evolution has been proposed (the 'Evolutionary Novelty model') that gave importance to both the environmentally dependent (exogenous selection) and hybrid fitness components in hybrid zone dynamics (Arnold 1997; p. 147). This concept would best explain the frequent but unexpected occurrence of broad, patchy and/or ancient hybrid zones (Arnold 1997; Martin & Cruzan 1999). For example, Martin & Cruzan (1999) supported the hypothesis that ongoing and extensive introgression resulting from high hybrid fitness, would produce a hybrid zone that expands as hybrids displace parental genotypes. Such studies on natural populations bring back the idea that hybridization may sometimes be a rich source of genetic variability, that may lead to novel adaptations.

In contrast to plants, patterns of introgressive hybridization have been much less documented in other organisms, particularly in the marine environment (but see, She *et al*. 1987; Planes & Doherty 1997; Seeb 1998; McMillan 1999; Rao & Lakshmi 1999). The apparent rarity of introgressive hybridization in the ocean is however, somewhat paradoxical, because numerous marine organisms share life history features with plants, such as high dispersal, large distribution, and low reproductive barrier via external fertilization (Palumbi 1994), which may all favour interspecific genetic exchanges (Huxel 1999). However, hybridization in the sea may be more widespread than previously thought (Campton 1987; Gardner 1997; Rao & Lakshmi 1999). Hence, the apparent rarity of introgressive hybridization in marine organisms may stem from other factors, such as limited number of studies (Rao & Lakshmi 1999), less extensive sampling of generally large and widely distributed populations, and inaccessibility of many marine taxa as compared to terrestrial organisms (Hubbs 1955). Another constraint is the difficulty in identifying cryptic species and hybrid populations (Knowlton 1993), mainly because the low divergence characterizing marine populations generally results in a lack of diagnostic characters and low resolution of genetic markers. Consequently, the dynamics of hybrid zones, and the possible role of introgressive hybridization in the ocean may have been underestimated (Dowling & Secor 1997; Seeb 1998).

In this paper, we provide evidence for broadscale extensive introgression between two redfish (genus *Sebastes*) from the North Atlantic. Redfish from the North Atlantic comprises four closely related taxa: *S. fasciatus*, *S. mentella*, *S. marinus* and *S. viviparus*. These marine, ovoviviparous and long-lived taxa have recently originated from a common ancestor of the North-east Pacific following the opening of the Bering Strait approximately three millions years ago (Briggs 1995). However, lack of mitochondrial DNA (mtDNA) diagnostic polymorphism suggests that their divergence within the Atlantic may be much more recent (Bentzen *et al*. 1998; Sundt & Johansen 1998; M. Black *et al*. in preparation). *S. fasciatus* (Acadian redfish) and *S. mentella* (deep-water redfish) are the two most important taxa in the North-west Atlantic region, and show differential ecological preferences. *S. fasciatus* occurs in shallower waters (150–300 m) with a distribution mainly restricted to the southern regions (gulf of Maine, Scotian Shelf), whereas *S. mentella* is distributed all over the North Atlantic ocean at depths varying between 350 and 500 m (Atkinson 1987; Kendall 1991) (see Fig. 1). The two species overlap in regions south of Newfoundland, and off the Scotian Shelf, but particularly in the gulf of St. Lawrence, where they are both known to extrude their larvae (Sévigny *et al*. 2000). Detailed information on the biology and life history of both species is scarce. A previous tagging study (Kelly & Barker 1961) suggested that *S. fasciatus* may be more habitat-specific, remaining in a same restricted area and depth, whereas *S. mentella* is known to undertake horizontal and vertical migrations at certain periods of the year (St-Pierre & de Lafontaine 1995).

For the last decade, the difficulty to discriminate these taxa in areas of sympatry compared to allopatry, suggested that introgression may occur between *S. fasciatus* and *S. mentella* (Rubec *et al*. 1991). This has been further supported by recent analyses of malate dehydrogenase (MDH) polymorphism (Rubec *et al*. 1991; Sévigny *et al*. 2000), and restricted fragment length polymorphism (RFLP) patterns of ribosomal DNA (rDNA) variation (Desrosiers *et al*. 1999). However, limited sampling, the use of a single polymorphic enzymatic locus, and limited resolution of rDNA precluded further investigation of the dynamics of introgressive hybridization between these two taxa.

In this context, the specific objectives of this study were to analyse microsatellite DNA polymorphism in order to: (i) provide more detailed insights into the dynamics of introgression between *S. fasciatus* and *S. mentella* in their main area of overlap, that is the gulf of St. Lawrence and the regions south of Newfoundland; and (ii) infer the factors potentially involved in the maintenance of this hybrid zone.

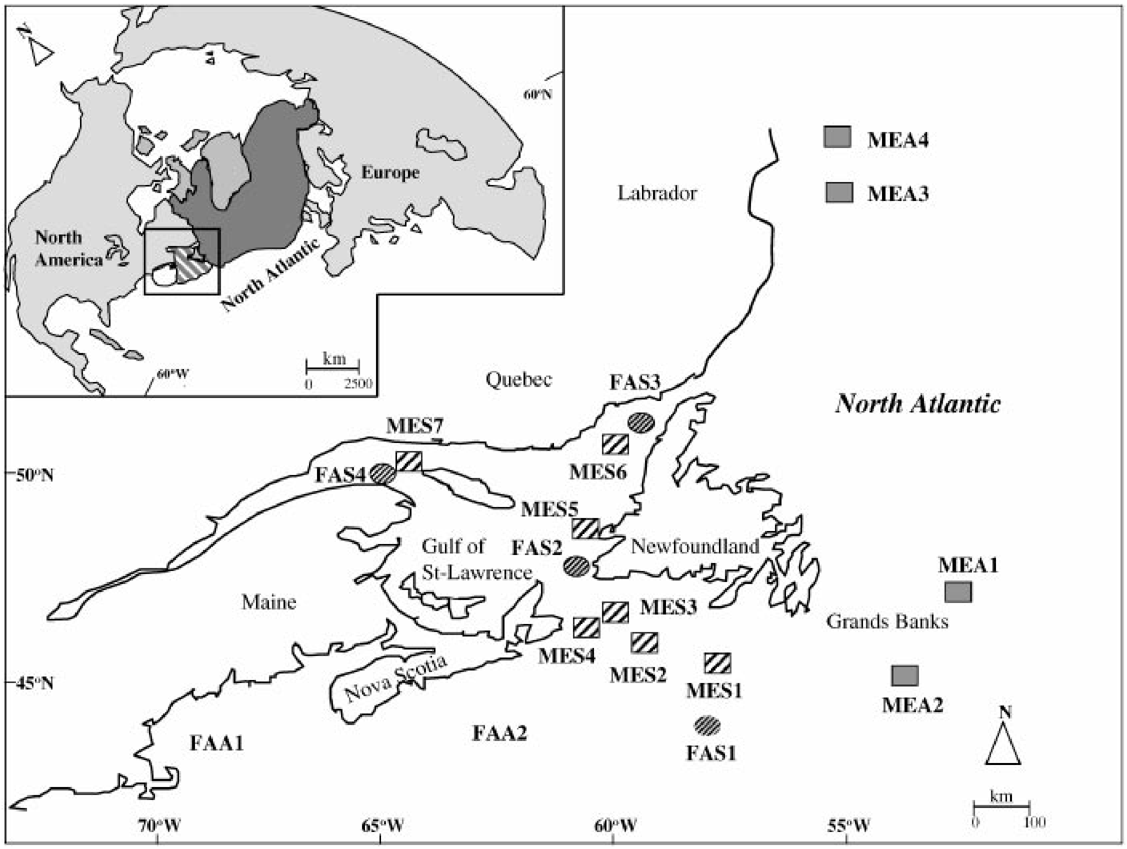

Fig. 1 Inset map: geographical distribution of *Sebastes mentella* (dark grey), *S. fasciatus* (white), zone of sympatry (striped), and study area (framed). Below, sampling localities of *S. mentella* (ME) and *S. fasciatus* (FA) samples. Grey squares: allopatric *S. mentella* (ALLOMEN); striped squares: sympatric *S. mentella* (SYMPMEN); black circles: allopatric *S. fasciatus* (ALLOFAS); striped circles: sympatric *S. fasciatus* (SYMPFAS).

Materials and methods

Sampling and genetic assays

Adult redfish (larger than 20 cm) of both *Sebastes fasciatus* and *S. mentella* were collected in a region encompassing both allopatric and sympatric distribution areas in the North-west Atlantic (Fig. 1). Sample sizes averaging 47 fish were chosen from the same fishing trawl. As no single character alone showed fixed differences between both species in the regions studied, species identification was performed by the examination of the three characters used for redfish: meristic (anal fin ray count, AFC), morphological (gas bladder musculature, GBM) and genetic (malate dehydrogenase, MDH) (Ni 1981, 1982; Payne & Ni 1982; McGlade *et al*. 1983). A total of 803 fish were collected from 1995 to 1998, including four allopatric *S. mentella* samples from the northern regions (ALLOMEN), two allopatric *S. fasciatus* from the southern regions (ALLOFAS), four *S. fasciatus* (SYMPFAS) and seven *S. mentella* (SYMPMEN) from the regions of sympatry in the gulf of St. Lawrence and south of Newfoundland (Table 1, Fig. 1). The allopatric samples are considered to represent pure samples, whereas the sympatric samples represent the putative introgressed samples that may contain a genetic admixture of the two parental taxa. DNA was extracted from muscle tissue, frozen at −80 °C or stored in 95% ethanol, using phenol–chloroform (Sambrook *et al*. 1989) or Chelex (Walsh *et al*. 1991) methods. Samples were screened for variation at eight specific microsatellites loci, as detailed in Roques *et al*. (1999a).

Descriptive statistics

We first documented the extent of intra- and inter sample genetic diversity based on allelic composition of the 17 samples. Number of alleles (A), observed heterozygosity (H_O) and unbiased gene diversity (H_E) corrected for sampling bias (He_{nb}) (Nei 1987) were calculated using the GENETIX program, version 4.0 (Belkhir *et al*. 1999). A, H_O and H_E were compared between both redfish taxa using the nonparametric *t*-test of Student using STATISTICA, version 4.5 (Statistica 1994). For those comparisons, the number of alleles was adjusted to an equal sample size of

Table 1 Sample names, geographical origins, sizes (*n*), number of alleles (A), observed (H_O) and expected (H_E) unbiaised heterozygosities, mean *f* values and probability of significance (D), probability of heterozygote deficiency (*P*), estimated proportion (in per cent) of *Sebastes fasciatus* (AFA) and *S. mentella* (AME) allelic composition in 17 redfish samples pooled in four groups: ALLOFAS and ALLOMEN (allopatry), SYMPFAS and SYMPMEN (sympatry), standard errors on estimates (SE)

Samples	Geographical origin	*n*	A	A†	H_O	H_E	*P*	*f*	D	AME	AFA	SE
ALLOFAS												
FAA1	gulf of Maine	30	94	109	0.769	0.791	0.3969	0.028	0.150	0	100	0
FAA2	Nova Scotia	35	109	121	0.748	0.757	0.0995	0.011	0.310	0	100	0
SYMPFAS												
FAS1	Newfoundland	54	108	104	0.831	0.809	0.1786	−0.027	0.891	10.18	89.82	4.44
FAS2	St. Lawrence	49	124	122	0.832	0.821	0.0554	−0.013	0.747	11.16	88.84	3.21
FAS3	St. Lawrence	48	117	116	0.843	0.827	0.0857	−0.020	0.846	13.57	86.43	3.39
FAS4	St. Lawrence	47	118	118	0.824	0.815	0.4752	−0.011	0.071	10.25	89.75	2.95
ALLOMEN												
MEA1	Grand Banks	44	150	153	0.868	0.843	0.0613	−0.029	0.915	100	0	0
MEA2	Grand Banks	47	159	159	0.833	0.873	0.0001*	0.045	0.007	100	0	0
MEA3	Labrador (U2G)	52	171	165	0.872	0.883	0.4890	0.012	0.196	100	0	0
MEA4	Labrador (U2H)	52	168	162	0.856	0.891	0.0001*	0.039	0.012	100	0	0
SYMPMEN												
MES1	South Newfoundland	48	160	159	0.84	0.873	0.0112	0.038	0.018	81.78	18.22	4.22
MES2	South Newfoundland	51	153	149	0.844	0.854	0.1496	0.007	0.356	85.68	14.32	3.92
MES3	South Newfoundland	51	153	148	0.811	0.848	0.0001*	0.044	0.007	85.62	14.38	3.99
MES4	South Newfoundland	48	142	141	0.794	0.869	0.0001*	0.087	0.0001*	77.11	22.89	5.02
MES5	St. Lawrence	49	160	157	0.914	0.895	0.6249	−0.022	0.931	82.82	17.18	3.85
MES6	St. Lawrence	48	146	145	0.86	0.882	0.0022*	0.025	0.086	78.97	21.03	4.12
MES7	St. Lawrence	50	149	146	0.847	0.848	0.0001*	0.001	0.516	82.74	17.26	4.11

*indicates significant values following sequential Bonferroni corrections ($k = 17$, $\alpha = 0.05/17 = 0.0029$).
†indicates adjusted number of alleles for an equal sample size ($n = 47$) using equation 11 in Ewens (1972).

47 per population (averaged number of individuals per sample) using equation 11 in Ewens (1972).

Deviation from Hardy–Weinberg (HW) proportions were assessed for both alternatives of deficit and excess of heterozygotes using the multisample score test available in the GENEPOP program, version 3.1 (Raymond & Rousset 1995). Probabilities of significance (*P*) were computed using the Markov chain method through 1000 iterations (Guo & Thompson 1992) as implemented in GENEPOP. The extent of HW departures was estimated in each sample using *f*, the Weir & Cockerham's (1984) estimator of F_{IS}. Significance levels were tested using the permutation procedures available in GENETIX. The null hypothesis of no linkage disequilibrium was tested in all samples, and significance values were computed for each locus pair by unbiased estimates of Fisher's exact tests using the Markov Chain method available in GENEPOP.

Heterozygote deficiency may come from either artefactual (null alleles, short allele dominance) or biological (inbreeding, population admixture, selection against heterozygotes) causes. While technical artefacts can be detected by testing whether particular loci mainly account for the deficit observed, the identification of a particular biological phenomenon remains difficult. However, a deficit of heterozygotes will occur in a subdivided population ('Wahlund effect'), or if two populations interbreed to some extent (thus producing hybrids), but mate assortively within population. In the case of hybridization, however, the expected deficit of heterozygotes in the population mixture is reduced as soon as F1 hybrids will backcross with one or both parental populations. In the absence of selection, a single generation of random mating can thus restore HW proportions in a mixture of two genetically differentiated populations. Consequently, measuring departures from HW equilibrium alone may be limited for evaluating the dynamics of hybridization and introgression. In contrast, the random association of alleles between loci will only be approached asymptotically in a randomly mating population. As a result, one may be able to detect recent introgression or the residual effects of hybridization, by estimating the amount of gametic phase disequilibrium resulting from non random association of alleles between loci. This may also potentially provide an estimate of the intensity of selection against hybrid genotypes (e.g. Scribner 1993). Consequently, we first tested whether particular loci were involved in heterozygote deficiencies to eliminate the technical artefact hypothesis. Then, we examined the possibility of older vs. more recent introgression by comparing departures from both HW and linkage equilibria.

Population differentiation

To test for genetic differentiation among the 17 samples, homogeneity tests of allele frequency distribution were performed using GENEPOP. Multilocus values of significance were obtained following Fisher's method. The extent of gene flow among samples was estimated by the unbiased F_{ST} estimator (θ) of Weir & Cockerham (1984). Disjunct allelic size distributions, in which alleles were separated by numerous base pairs, were observed for several loci (see Roques *et al*. 1999b), suggesting that they probably do not follow a strict stepwise mutational model (Di Rienzo *et al*. 1994; Angers & Bernatchez 1997). We, therefore, did not estimate the extent of genetic differentiation based on molecular variance. All probability values were adjusted for multiple test comparisons using the sequential Bonferroni adjustments (Rice 1989). The extent of divergence among the 17 samples of *S. fasciatus* and *S. mentella* was also quantified by the chord distance (D_{CE}) of Cavalli-Sforza & Edwards (1967), to test whether sympatric samples were intermediate between allopatric samples of each taxa, due to introgression. The use of D_{CE} generally leads to a higher probability of obtaining the correct tree topology either under the infinite allele model (IAM) or stepwise mutation model (SMM) assumptions (Takezaki & Nei 1996; Angers & Bernatchez 1998). Pairwise distances were used to construct a populations phenogram using the neighbour-joining (NJ) algorithm (Saitou & Nei 1987) available in PHYLIP version 3.5c (Felsenstein 1993). SEQBOOT, GENDIST, NEIGHBOUR, CONSENSE programs were successively conducted to build the tree. Confidence estimates on tree topology were estimated by the percentage of 1000 bootstraps performed resampling allelic frequencies.

Dynamics, extent and patterns of introgression

Two different multivariate analyses were used to investigate the extent and patterns of introgression between *S. fasciatus* and *S. mentella*. We first conducted a Principal Components Analysis (PCA) (Greenacre 1984) to visualize the relationships between the 17 samples based on their allelic frequency, using STATISTICA. This approach involves a linear transformation of the observed allele frequencies, where the axes (components) are chosen as to maximize the variation of the transformed data, measured along each axis. To test whether the intermediate samples observed in the PCA were composed of an admixture of individuals from the two taxa, or of individuals from mixed allelic composition, a Factorial Correspondence Analysis (FCA) was carried out using GENETIX, that allows the projection of all the individuals in a space defined by the components. The principles of the FCA have been described by Benzecri (1973), but the application of these analyses to genetic data was developed by She (1987) and Berrebi (1988). In the present study, raw genetic data were transformed following the 'allelic coding' as described by the latter authors. Briefly, the method is based on the similarity of individuals in their allelic state for each allele. A code is attributed to each individual whether it is heterozygote (1), homozygote (2) or if the given allele is absent (0). The main advantage of the FCA is that each individual can be represented using each allele as an independent variable, contrary to other multivariate analyses, that generally use a combined parameter as descriptor. Both incomplete individual multilocus genotype and low frequency alleles may create distortion and bias in the FCA projection (She 1987; Kotulas 1989). Therefore, individuals with incomplete multilocus genotype (234 out of 803), and alleles present less than five times in the whole data set ($n = 111$ out of 269) were excluded from the analysis, as suggested by Kotulas (1989).

We then estimated the contribution of the parental species to the genetic admixture of introgressed populations. We used allopatric samples of both taxa (ALLOFAS and ALLOMEN groups) as parental sources to quantify their allelic contribution (in percentages) to the 11 sympatric samples (SYMPFAS and SYMPMEN) (Table 1). Genetic admixture proportions were estimated by means of the weighted least squares (WLS) (Long 1991) using the program ADMIX (Long 1995) kindly provided by Dr Jeffrey Long (National Institute on Alcohol Abuse and Alcoholism, National Institute of Health, Bethesda). Because the use of ADMIX was limited by the maximum number of alleles that can be incorporated, we retained those ($n = 47$) for which $Z = P1 - P2 \geq 0.05$, where P1 and P2 represent the allele frequencies of parental populations 1 and 2 (JF Long, personal communication). This should result in a limited loss of information as it is generally known that low frequency alleles are poorly informative in studies of population affiliation (Smouse *et al*. 1982; Bernatchez & Duchesne 2000). This also ensures that the level of introgression is not overestimated or biased by the presence of rare alleles.

Results

Patterns of genetic variability of Sebastes fasciatus *and* S. mentella

High genetic variability was generally observed across the 17 samples, with a total number of observed alleles (A) ranging from 104 to 165 (mean = 135), and unbiased heterozygosity values ranging from 0.748 to 0.914 (mean = 0.831) (Table 1). A highly significant difference in allelic diversity ($t = -9.895$, $P < 0.00001$) was observed across all samples between *Sebastes fasciatus* and *S. mentella*, with mean number of alleles being, respectively, 115 (ranging from 94 to 124) and 155 (ranging from 146 to 171). Fewer alleles at relatively high frequencies were observed for

S. fasciatus, while *S. mentella* was characterized by numerous alleles at low frequencies (not shown), as previously observed in a smaller number of samples (Roques *et al*. 1999b). Unbiased heterozygosity values were also significantly different between taxa ($t = -6.131$, $P = 0.00019$), ranging from 0.748 to 0.843 (mean = 0.803) in *S. fasciatus*, and from 0.794 to 0.914 (mean = 0.869) in *S. mentella*.

Higher polymorphism is generally expected in sympatry, compared to allopatry, because introgression should increase genetic variability. Here, however, this was expected for *S. fasciatus*, but not necessarily for *S. mentella*, given the lower polymorphism observed in *S. fasciatus*. Indeed, we observed in *S. fasciatus* a mean unbiased heterozygosity value significantly higher for SYMPFAS (0.832) compared to ALLOFAS group (0.757) ($t = -3.69$, $P = 0.021$), although mean allelic diversity ($A = 115$) was identical for both groups. The reverse pattern was observed for *S. mentella*, with a number of alleles significantly lower for sympatric (SYMPMEN) ($A = 149$) compared to allopatric (ALLOMEN) ($A = 160$) ($t = -2.748$, $P = 0.022$) (Table 1), whereas mean unbiased heterozygosity was similar for both groups ($t = 0.461$, $P = 0.655$). This overall pattern provided a first indication of introgressive hybridization between both taxa in the zone of sympatry.

Patterns of genetic differentiation

Highly significant differences in allelic frequencies were observed for all pairwise comparisons involving samples of the two taxa (data not shown). Those differences translated into moderate estimates of θ varying from 0.074 to 0.164 with a mean value of 0.103 (Table 2). It has recently been proposed that the maximum value of θ will be greatly reduced when using highly polymorphic markers, such that, the maximum potential differentiation should roughly not exceed the average level of homozygosity (Hedrick 1999). The mean θ value between the two taxa was indeed very similar to the approximate maximum estimate of the parameter (average homozygosity = 0.117). In contrast, much smaller differences were found among samples within taxa. Within *S. fasciatus*, significant differences in allelic frequencies were observed between allopatric samples from the gulf of Maine (FAA1) and off Nova Scotia (FAA2) ($\theta = 0.0132$), but no difference was found among SYMPFAS samples (averaged $\theta = 0.0006$) (Table 2). The θ values, however, were neither significant within SYMPFAS, or between FAA1 and FAA2. Within *S. mentella*, no genetic difference was found among ALLOMEN samples, with nonsignificant values of θ ranging from -0.0006 -0.0062 (mean = 0.0056) (Table 2). For sympatric samples (SYMPMEN), no difference was observed among samples in the gulf of St. Lawrence (MES5-MES7) and the two samples from Newfoundland (MES1 and MES2) (Fig. 1, Table 1). However, significant differences were observed between the Newfoundland sample MES4, and the other samples from the same region, MES1-MES3 (mean $\theta = 0.013$). MES3 was also significantly different from MES1 and MES5. Altogether, these results suggested that samples within SYMPFAS and ALLOMEN were genetically homogeneous, whereas weak structuring may exist within the other two groups.

A general pattern in hybrid zones studies is that the exchange of genes from one species to another is expected to decrease divergence between them. Hence, *S. fasciatus* and *S. mentella* were expected to be more closely related in sympatry than in allopatry. Indeed, we found that the sympatric groups, SYMPMEN and SYMPFAS, were genetically more similar (average $\theta = 0.088$, ranging from 0.074 to 0.107), than were allopatric samples (ALLOMEN/ALLOFAS, average $\theta = 0.134$, ranging from 0.109 to 0.164) (Student t, $P < 0.001$). Sympatric *S. mentella* (SYMPMEN) samples were also less genetically differentiated from allopatric *S. fasciatus* samples than allopatric (ALLOMEN) samples were (average $\theta = 0.106$, ranging from 0.078 to 0.141) (Student t, $P = 0.0024$). Similarly, sympatric *S. fasciatus* (SYMPFAS) samples were less differentiated from allopatric *S. mentella* samples than allopatric samples (ALLOFAS) were (average $\theta = 0.111$, ranging from 0.097 to 0.135) (Student t, $P = 0.0031$). Altogether, these results further indicated the occurrence of introgression between *S. fasciatus* and *S. mentella* when found in sympatry.

Pattern, extent and level of introgression in the hybrid zone

The intermediate genetic composition of sympatric samples was illustrated by the NJ tree in which the two allopatric groups (ALLOMEN and ALLOFAS) clustered at the opposite ends of the network, and the sympatric ones (SYMPFAS and SYMPMEN) in between (Fig. 2). The distinction of the two taxa was supported by high bootstrap values (100%). Within taxon, allopatric samples formed highly supported groups (72% and 100%, respectively) relative to sympatric samples. In contrast, bootstrap values were generally low within the four groups, and particularly within SYMPMEN.

This intermediate position of sympatric samples was further supported by the PCA analysis (Fig. 3). The first component (not shown) essentially separated *S. fasciatus* and *S. mentella*, and explained most of the total variance (60%). More information on the position of samples within species was gained by the others components. The second component summarizing 26.9% of the variation, mainly accounted for the differentiation between *S. fasciatus* and *S. mentella*, but also separated sympatric from allopatric samples of *S. mentella*. Considering this axis, sympatric *S. mentella* samples were closer to *S. fasciatus* than were the allopatric ones. The third component (3.2% of the variation) differentiated allopatric from sympatric samples

Table 2 Pairwise sample differentiation estimates based on allelic variance at eight microsatellites loci in 17 redfish samples. Dotted lines circumscribe between taxa comparisons θ values

Samples	FAA1	FAA2	FAS1	FAS2	FAS3	FAS4	MEA1	MEA2	MEA3	MEA4	MES5	MES6	MES7	MES1	MES2	MES3
FAA1																
FAA2	0.0132*															
FAS1	0.0091*	0.0196														
FAS2	0.0185	0.0235	(–0.0050*)													
FAS3	0.0196	0.0274	(–0.0003*)	(–0.0058*)												
FAS4	0.0093*	0.0152	(–0.0043*)	(–0.0013*)	(0.0040*)											
MEA1	0.1355	0.1636	0.1354	0.1219	0.1178	0.1255										
MEA2	0.1154	0.1412	0.1113	0.1042	0.0991	0.1067	(–0.0039*)									
MEA3	0.1189	0.1504	0.1145	0.1101	0.1023	0.1105	(–0.0008*)	(0.0026*)								
MEA4	0.1085	0.1424	0.1125	0.1032	0.0974	0.1059	(0.0062*)	0.0014*	(–0.0006*)							
MES5	0.0830	0.1118	0.0817	0.0777	0.0741	0.0743	0.0063*	0.0153	0.0082	0.0044*						
MES6	0.0778	0.1095	0.0883	0.0846	0.0819	0.0792	0.0103	0.0154	0.0096	0.009	(0.0002*)					
MES7	0.1009	0.1313	0.1015	0.0977	0.0941	0.0927	0.0109	0.0167	0.0156	0.0181	(0.0011*)	(–0.0005*)				
MES1	0.0794	0.1135	0.0829	0.0829	0.0794	0.0766	0.0052*	0.0149	0.0125	0.0103	(0.0020*)	(0.0020*)	(–0.0030*)			
MES2	0.1035	0.1349	0.1016	0.1005	0.0962	0.0933	0.0077	0.0181	0.0117	0.0146	(0.0003*)	(0.0017*)	(–0.0046*)	(0.0000*)		
MES3	0.1065	0.1410	0.1035	0.107	0.1027	0.0987	0.0078*	0.0224	0.0158	0.0163	0.0093	0.0043*	0.0038*	0.0101	0.0045*	
MES4	0.0817	0.1143	0.0836	0.0865	0.0839	0.0793	0.0169	0.0274	0.0193	0.0213	0.0095	(0.0036*)	0.0057*	0.0098	–0.0094	0.0186

( ), indicates non significant allelic frequency heterogenity following the method of Fisher ($\alpha = 0.001$).

* indicates non significant θ estimates following Bonferroni corrections ($k = 120$, $\alpha = 0.05/120 = 0.00042$).

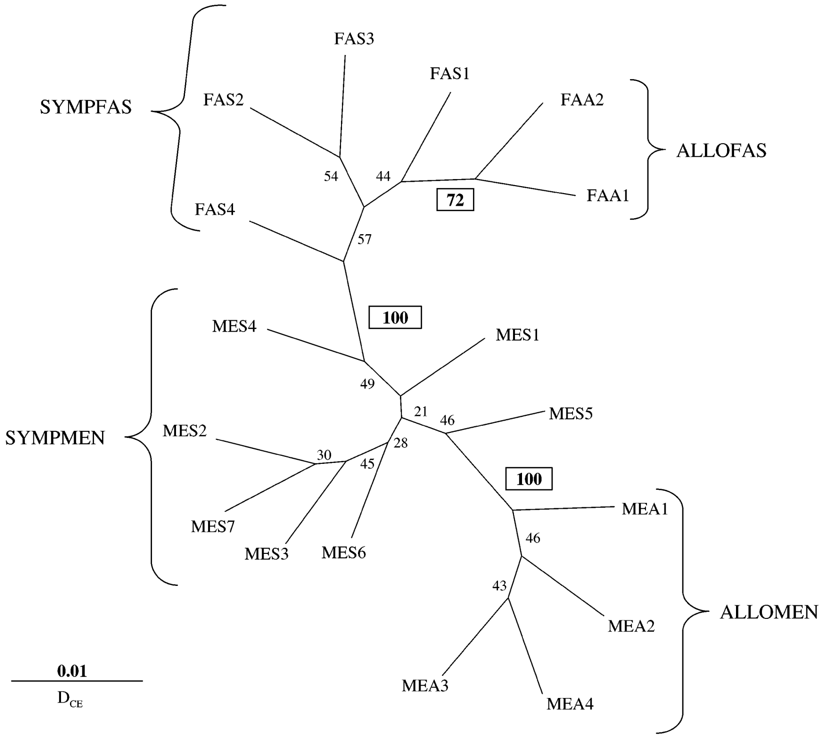

Fig. 2 Neighbour-joining tree illustrating the relationships among 17 redfish samples based on pairwise D_{CE} genetic distances. Bootstrap values indicate the degree of support for each branch after 1000 resampling over loci.

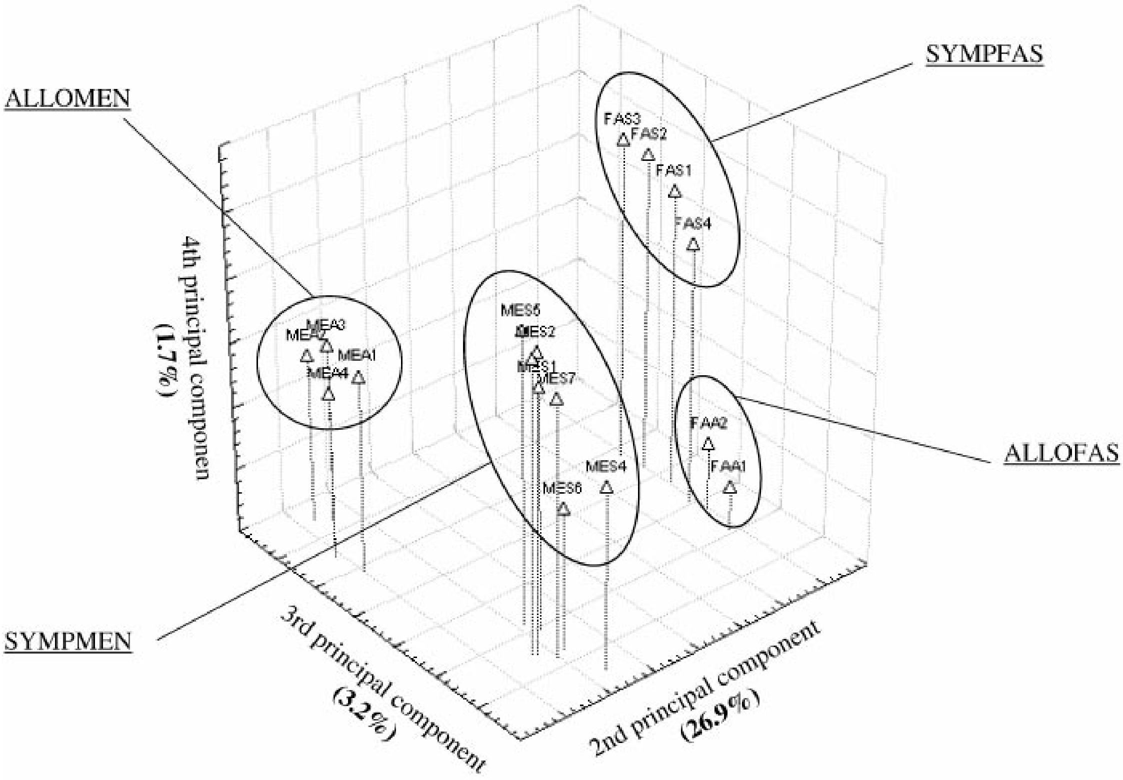

Fig. 3 Diagram of the Principal Components Analysis (PCA) showing the 17 redfish samples in a three multidimensional space defined by the second, third and fourth components. Their respective contributions to the grouping are shown in percentage (%). The four groups correspond to the sympatric and allopatric *Sebastes mentella* and *S. fasciatus* samples described in Table 1.

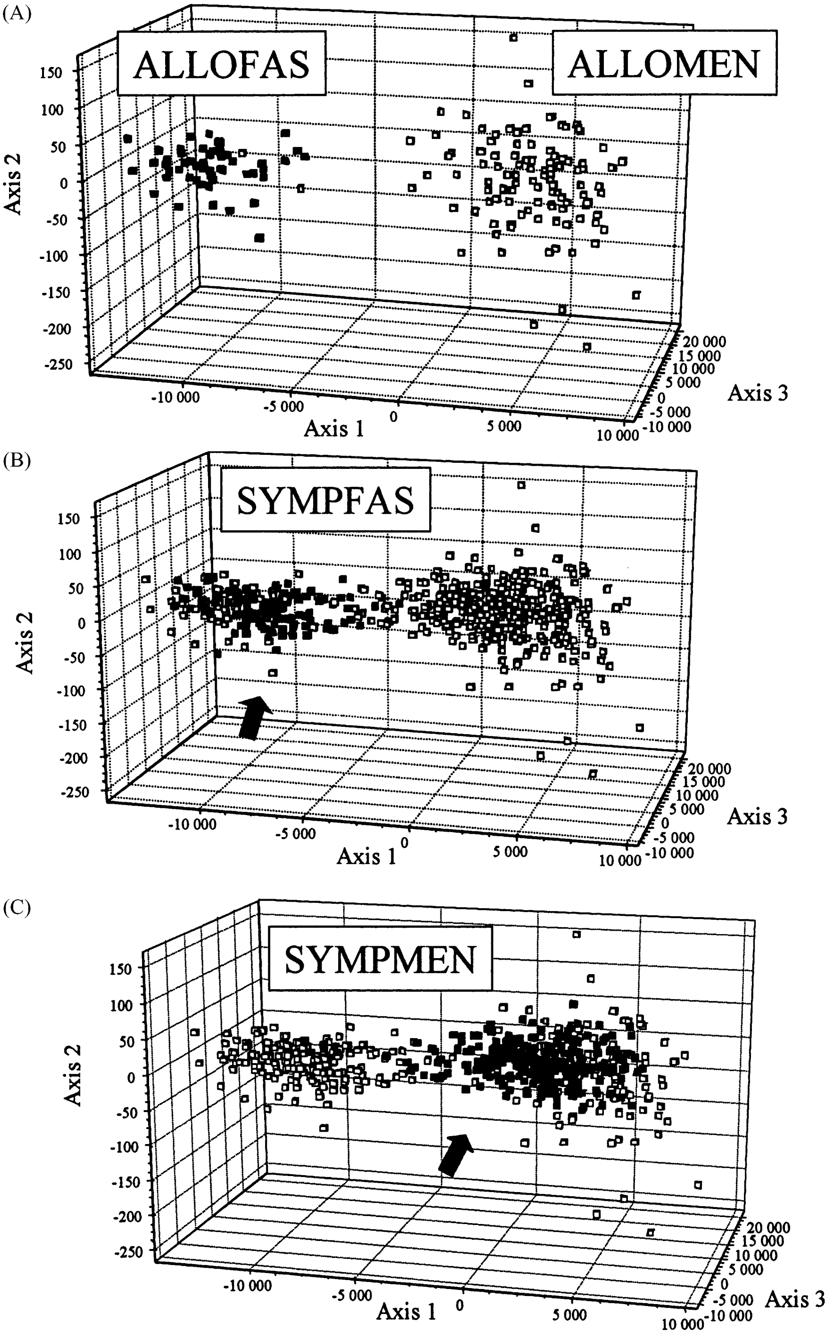

Fig. 4 Diagram of the Factorial Correspondence Analysis (FCA) showing redfish individuals in a multidimensional space. (A) Allopatric samples of *Sebastes fasciatus* (in white) and *S. mentella* (in dark). (B) Sympatric *S. fasciatus* samples (in dark). (C) Sympatric *S. mentella* samples (in dark).

within each taxon (ALLOMEN from SYMPMEN, and ALLOFAS from SYMPFAS), and also accounted for the intermediate position of SYMPFAS between the two allopatric groups. The fourth component represented a small percentage of the total variance (1.7%), but accounted for the difference between the allopatric groups of both taxa, and also brought SYMPFAS and SYMPMEN samples together, relative to allopatric samples.

The factorial correspondence analysis, based on 569 individuals, revealed that sympatric samples comprised individuals possessing alleles of both *S. fasciatus* and *S. mentella*, rather than pure individuals from the two taxa. Figure 4(A) represented the projections in the FCA space of all the allopatric individuals (ALLOMEN and ALLOFAS). The first axis accounted for 66.5% of the total variance and totally separated the two groups, except for two individuals of *S. mentella* found in *S. fasciatus*, possibly due to a priori misclassification. Figure 4(B,C) represented the same allopatric individuals, to which sympatric *S. fasciatus* and *S. mentella* individuals were added. This showed that sympatric individuals of a given species tended to position closer to the cluster of individuals of the other species, than did allopatric individuals. They also created a continuum between the two species groups, further supporting an admixed allelic composition in individual fish of both *S. fasciatus* and *S. mentella* when found in sympatry. It is also worth noting that most sympatric individuals positioned in or closer to the allopatric groups, whereas few others were found at mid distance between the extreme. This suggested that most introgressed individuals are genetically more similar to one or the other 'parental' type, whereas few 'hybrid swarms' are composed of more intermediate allelic composition between both. The examination of mean coordinates of each group of individuals along the first axis further revealed that the cloud of SYMPMEN tended to be closer to the ALLOFAS one (Δd = 1164), than was SYMPFAS from ALLOMEN (Δd = 1359), which suggested that *S. mentella* may be more introgressed by *S. fasciatus* than the reciprocal.

The respective admixture proportions of *S. fasciatus* and *S. mentella* allelic composition in the sympatric samples further indicated a relatively high level of introgression between *S. fasciatus* and *S. mentella*, when found in sympatry (mean = 15.6%, see Table 1). This analysis also supported the results of PCA and FCA, suggesting that introgression was bidirectional, but more importantly towards *S. mentella*. Thus, higher introgression values were found for sympatric *S. mentella*, ranging from 14.32 to 22.89% (mean = 17.89%), than for sympatric *S. fasciatus*, which ranged from 10.18 to 13.57% (mean = 11.29%) (Student *t*, $P = 0.004$).

Departures from Hardy–Weinberg equilibrium

The exact tests of Hardy–Weinberg equilibrium revealed highly significant deficits of heterozygotes in six out of the 17 samples, following sequential Bonferroni corrections (k = 17, $\alpha = 0.05/17 = 0.0029$) (Table 1). This was unlikely imputable to a particular locus, as four out of the eight loci analysed were significantly deviating from HW proportions (k = 8, $\alpha = 0.05/8 = 0.00625$). A trend towards more marked heterozygote deficiency was found in the sympatric *S. mentella* group, as four of the six deficits were observed in SYMPMEN (Table 1). In contrast, a single sample (FAS2) deviated from HW proportions within *S. fasciatus*. The highest deficit value ($f = 0.087$) was observed in the MES4 sample. The results of population differentiation also showed that this sample was the most genetically distinct *S. mentella* sample (Table 2). It was also the most introgressed sample with 22.89% of specific *S. fasciatus* alleles (Table 1). These observations are suggestive of ongoing and more pronounced introgressive hybridization in this location.

Allelic composition and linkage disequilibrium in sympatric S. fasciatus *samples*

The detailed analysis of frequencies of selected alleles in the 17 samples further confirmed the dynamics of the hybrid zone in the gulf of St. Lawrence and the regions south Newfoundland (Fig. 5). Figure 5(A,B) qualitatively illustrated the extent of introgression of *S. mentella* alleles into *S. fasciatus* samples, while Fig. 5(C,D) illustrated that from *S. fasciatus* into *S. mentella.* Figure 5(A,B) showed that alleles found in *S. mentella* samples (allopatric and sympatric) were also present in all the sympatric *S. fasciatus* samples (SYMPFAS), while they were absent or at very low frequency in allopatric ones (ALLOFAS). Similarly to *S. fasciatus*, Fig. 5(C,D) illustrated that *S. fasciatus* alleles introgressed in *S. mentella*. These qualitative observations corroborated previous results showing a higher level of introgression in *S. mentella* than found in *S. fasciatus*.

The examination of genetic linkage among the 17 samples and the eight loci analysed revealed 12 (2.5% of 476 pairwise comparisons) significant departures from equilibrium (k = 28, $\alpha = 0.05/28 = 0.00178$). Disequilibria occurred significantly more often in sympatric *S. fasciatus* samples than in the other three groups ($\chi^2 = 6.73$, $P = 0.006$), with seven out of 12 observed significant departures (58%) which were partitioned as follows: FAS1 (4), FAS2 (1) and FAS3 (2). Other significant values were found in *S. mentella*, both in allopatry and sympatry [ALLOMEN: MEA2 (1), MEA3 (1), MEA4 (1); SYMPMEN: MES3 (1) and MES7 (1)].

Discussion

Patterns of diversity between and within taxa

The overall high genetic variability quantified in this study corroborates observations of microsatellite analyses in other marine organisms (Ruzzante *et al*. 1998; Bagley *et al*. 1999; Lundy *et al*. 1999; Shaw *et al*. 1999). Significantly higher polymorphism, however, was found in *Sebastes mentella* relative to *S. fasciatus*. Rocha-Olivares *et al*. (1999) proposed that the high variability generally observed in redfish may be due to their exceptional longevity, high degree of generational overlap, and low temporal variability in effective population size. Large geographical range of distribution, along with high dispersal capabilities are also regularly invoked to explain the high genetic variability of

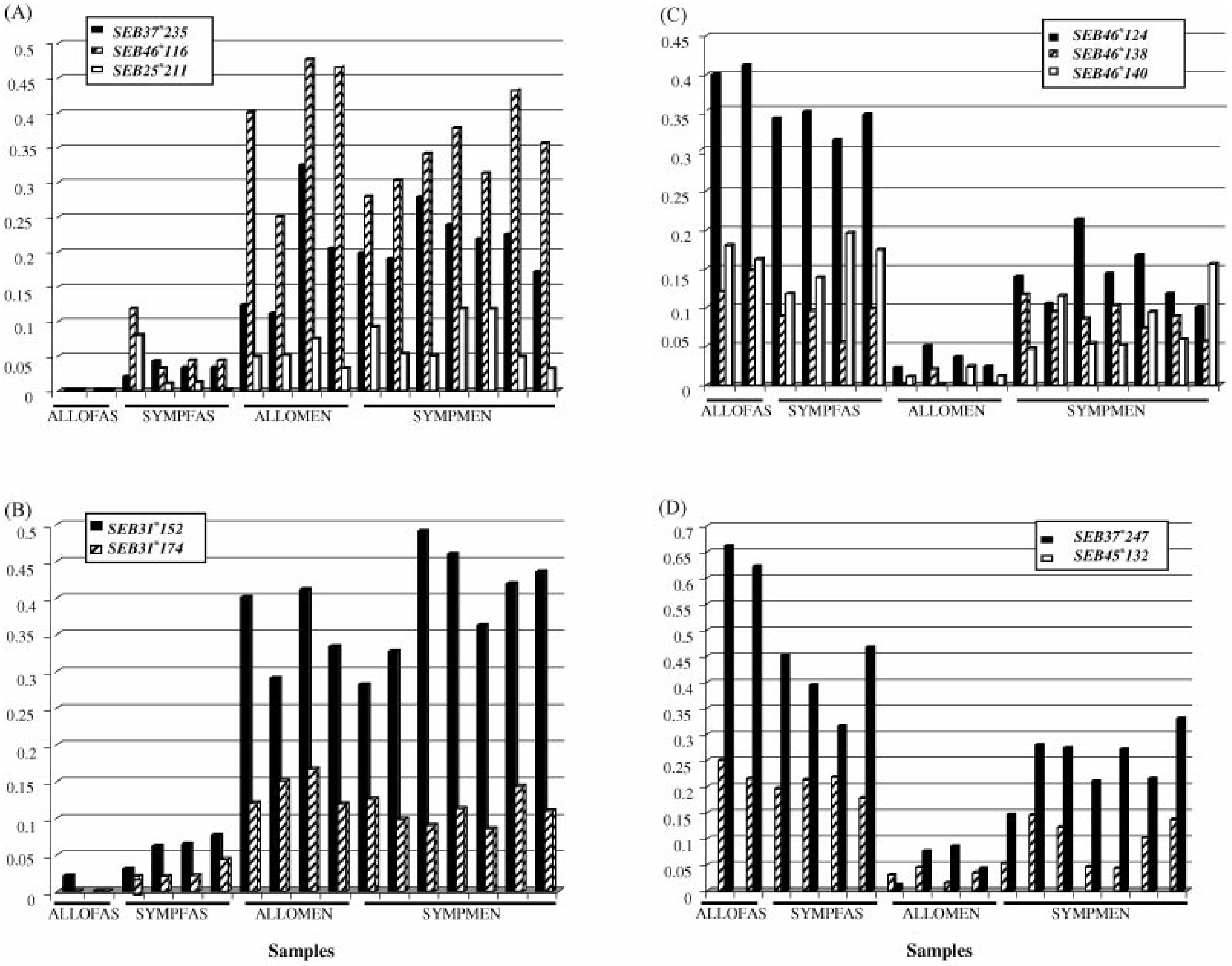

Fig. 5 Frequencies of selected microsatellite alleles among the 17 redfish samples, illustrating the introgression of *Sebastes mentella* alleles into *S. fasciatus* sympatric samples (A and B), and the introgression of the *S. fasciatus* alleles *into S. mentella* sympatric samples (C and D).

marine organisms (Smith & Fujio 1982). Concomitant with these assumptions, S. *mentella* is distributed over a much larger area than *S. fasciatus* throughout the North Atlantic, the latter taxon being restricted to a relatively small geographical range in the south-western regions.

The patterns of population structure observed in both *S. fasciatus* and *S. mentella* were also congruent with weak genetic structuring usually reported for marine organisms (Gyllensten 1985; Palumbi 1994, 1996). Differential patterns of population structure, however, were observed between *S. fasciatus* and *S. mentella*, which may be partly explained by their respective life histories. For instance, lower population structure is expected for pelagic and largely distributed marine species compared to more benthic ones or those more geographically restricted (Avise *et al*. 1987; Doherty *et al*. 1995). As mentioned above, *S. mentella* is more frequently encountered in the pelagic zone, in deeper waters, while *S. fasciatus* is more associated with the epibenthic zone of shallower waters (Litvinenko 1980).

Most importantly, our results highlighted the predominant role of introgressive hybridization in shaping the extent of genetic diversity, interspecific differences and population structuring among *S. mentella* and *S. fasciatus*. Hence, introgressive hybridization first resulted in reduced genetic divergence between both taxa in the main zone of sympatry relative to what was observed elsewhere. Second, all samples of the 'sympatric group' in each taxon were genetically distinct from their allopatric congeners, whereas almost no population structuring was observed among samples of the latter group. Third, higher polymorphism was observed in sympatric samples of *S. fasciatus*, compared to allopatric ones, as a direct consequence of introgression from *S. mentella*. The observed level of introgression between both redfish taxa was relatively important, averaging 15% over all samples. Strict comparisons with previous studies in other marine organisms remain difficult however, given their scarcity and the differential use of genetic markers and/or measures of introgression. Based on the percentage of allozymic conspecific alleles, the estimated level of introgression (average = 7%) observed between three Pacific redfishes, *S. auriculatus*, *S. caurinus* and *S. maliger*, was twice lower than observed in this study (Seeb 1998), whereas the level of introgression observed between two flatfishes, *S. aegyptiaca* and *S. senegalensis* (average = approximately 14%) was comparable to that observed between *S. mentella* and *S. fasciatus* (She *et al*. 1987).

Dynamics of introgressive hybridization

It had been proposed in several previous studies that introgressive hybridization might occur between *S. fasciatus* and *S. mentella* when found in sympatry (McGlade *et al*. 1983; Rubec *et al*. 1991; Desrosiers *et al*. 1999; Sévigny *et al*. 2000). This large-scale microsatellite analysis, in combination with multivariate analytical approaches, however, allowed a much finer dissection of the dynamics of introgressive hybridization between these taxa. Our data first showed that hybridization between *S. fasciatus* and *S. mentella* led to bidirectional introgression, which resulted in the coexistence of two introgressed populations of *fasciatus* and *mentella* which remained reproductively isolated in the main zone of sympatry, despite partial gene flow between them. These hybrid populations do not represent hybrid swarms, since most fish were relatively similar to either one or the other parental species, rather than being of intermediate genetic composition. This was mainly illustrated by the presence of four distinct clusters in the PCA, and by the bimodal distributions of introgressed individuals in the FCA. This pattern is particular, as hybridization generally results in a unique genetic admixture localized in a geographical transect (Tension zone), although not exclusively. Hence, Goodman *et al*. (1999) observed a similar pattern to that observed in redfish between the red and sika deer (genus *Cervus*). They proposed that hybridization in this case consisted of the 'leakage' of alleles from one gene pool to another, rather than the fusion of the two parental types, and was suggestive of rare hybridization, and/or of numerous backcrosses between F1 and one or the other parental type. Similarly, hybrids between the flatfishes *Solea aegyptiaca* and *S. senegalensis* were most often genetically closer to one or the other parent (She *et al*. 1987).

While bidirectional, the pattern of introgression quantified here was asymmetrically more important towards *S. mentella*, indicating that hybridization did not affect both taxa in the same way. Several causes, such as selective (asymmetrical selection) or ecological factors (abundance, reproductive periods) have been proposed to explain this phenomenon, although none of these potential factors have been rigorously quantified (Rao & Lakshmi 1999). Seeb (1998) proposed that differential abundance between hybridizing taxa may explain the asymmetrical pattern of introgression observed between three pacific redfish species. In the present study, a combination of several selective and ecological factors may also be potentially invoked to infer the asymmetrical pattern of introgression observed between *S. mentella* and *S. fasciatus* (see below). The relative importance of these factors, however, remains hypothetical, given the limited knowledge on the life history and ecology of both taxa, and the difficulty of rigorously quantifying selective effects in these species, as in any marine pelagic organisms.

Opportunities for introgressive hybridization

Several reasons may be invoked for the occurrence and circumscribed localization of introgressive hybridization between *S. fasciatus* and *S. mentella.* One possible scenario is that the hybrid zone may have formed following secondary contact between both taxa, as generally inferred in most studies of hybrid zones (Barton & Hewitt 1989). Barsukov (1972) proposed that North Atlantic redfishes were all derived from a common Pacific ancestor that colonized the North Atlantic following the opening of the Bering strait about 3 million years ago (Briggs 1995). According to this scenario, vicariant events (although not defined by Briggs 1995) eventually broke the distribution range of this ancestral species, and the isolated populations evolved as *S. marinus* and *S. mentella* in the north-eastern regions, and as *S. fasciatus* in the South-west. Subsequently, *S. mentella* extended their range in the West Atlantic, where they came into contact with *S. fasciatus.* Incomplete and/or relaxed reproductive barriers between *S. fasciatus* and *S. mentella* would have then favoured their hybridization in sympatry. For instance, St-Pierre & de Lafontaine (1995) suggested that the reproductive characteristics of *S. fasciatus* and *S. mentella* in the gulf of St. Lawrence were very similar, and may not represent sufficiently strong barriers to maintain their genetic integrity in sympatry. Thus, several reproductive characteristics of both taxa, such as age at sexual maturity and mating period are almost synchron and could facilitate interspecific mating. In addition, reproductive isolation through size assortive mating for these ovoviviparous redfish could potentially be relaxed because of size and morphological convergence in sympatry. Thus, a study of size at maturity of the two taxa in the North-west Atlantic showed a clinal pattern, with average size decreasing from Baffin to the gulf of St. Lawrence for *S. mentella*, and from Newfoundland to the gulf of Maine for *S. fasciatus* (Ni & Sandeman 1984). Consequently, size at maturity was more similar for both species in the zone of sympatry than elsewhere. Similarly, a greater overlap of morphological and meristic characters used for redfish identification was observed in the gulf of St. Lawrence compared to the Grand Banks regions (Rubec *et al*. 1991).

A second possible explanation for the observed pattern and localization of introgressive hybridization between *S. fasciatus* and *S. mentella* could reside in the intermittent periods of their spatial overlaps, which is often invoked to explain increased probability of hybridization in fish (Hubbs 1955; Huxel 1999; Rao & Lakshmi 1999). These species tend to occupy their preferential depth range in sympatry for most of the time, with *S. mentella* associated with deeper waters, relative to *S. fasciatus*. Spatial overlap, however, may be more pronounced at certain periods of the year. For instance, it has been reported that both taxa agglomerate in the fall, at the period of reproduction in the western Newfoundland (D. Power, personal communication). Besides, we observed in this region higher levels of introgression for both *S. mentella* and *S. fasciatus* (see Table 1: MES4, 22.9%; MES6, 21.03%; FAS3, 13.57%). Also, *S. mentella* may perform nocturnal vertical feeding migrations (Kenchington 1991). The extension of *S. mentella* distribution to upper layers at certain periods could thus increase the probability of encounter and eventually, reproduction with *S. fasciatus*.

Numerical imbalance between hybridizing species has also been invoked as one of the potent factors conducive to hybridization in fishes (Hubbs 1955; Rao & Lakshmi 1999). For instance, Avise & Saunders (1984) proposed that the absence of conspecific partners and stimuli for females of a rarer species may be an important factor in increasing the likelihood of interspecific hybridization. In this study, although a great geographical and temporal variability in abundance has generally been reported in the main zone of sympatry between these two redfish species, *S. mentella* generally dominates numerically (Payne & Ni 1982; Ni & Sandeman 1984).

Studies of population structure and hybrid zones have also indicated that natural hybridization is often found in tracts of intermediate habitats, or where conditions favouring both taxa are found in proximity and at the ecological limits of their distribution range (Dowling & Secor 1997; Huxel 1999). In this context, another potential cause for the occurrence of hybridization between redfish taxa is that the oceanographic conditions found in the main zone of sympatry are peculiar for these taxa. The hydrodynamics of the gulf of St. Lawrence and the Laurentian Channel is characterized by circulation patterns of freshwater in the surface layers and deep marine waters, and may be considered as an unstable environment, compared to the typical marine waters found throughout the redfish distribution elsewhere (Koutitonsky & Bugden 1991). The geographical location of the main zone of sympatry (see Fig. 1), as well as the predominance of introgressed individuals in this region, also support the idea that this zone is a point of contact of the two taxa at the ecological limit of their distribution range (Huxel 1999). In addition, sampling within this area indicated that *S. fasciatus* and *S. mentella* were also present together at depths between 300 and 400 m, that corresponds to the extreme limit of their usual depth preferences, being, respectively, the shallowest for *S. mentella* (distribution between 350 and 500 m) and the deepest for *S. fasciatus* (distribution between 150 and 300 m). This suggests that the main zone of sympatry between these two species may also include an ecotone of intermediate vertical habitat.

Maintenance of the hybrid zone: a role for selection?

The extensive asymmetrical introgressive hybridization restricted in the large area associated with the Laurentian channel, the persistence in sympatry of two introgressed groups, and the maintenance of genetic integrity of both species outside this region, all indicate that introgression between *S. mentella* and *S. fasciatus* is not random, and that selection may be involved in maintaining the observed genetic pattern. Several recent studies argued that ecological tolerance may be important in promoting introgressive hybridization (Cruzan *et al*. 1993; Crespin & Berrebi 1999; Hatfield & Schluter 1999). For instance, a recent study in charrs (*Salvelinus*) suggested the introgression of mtDNA genes from the more cold-adapted species (*S. alpinus*) into the genome of the more temperate one (*S. fontinalis*) may provide physiological advantages to cold water temperatures in high altitude lakes (Bernatchez *et al*. 1995; Glémet *et al*. 1998). This raises the possibility that genetic exchanges between *S. mentella* and *S. fasciatus* in intermediate habitats relative to their main ecological preferendum could be beneficial for both species, although this remains to be empirically demonstrated.

An additional indication for the role of selection in shaping the observed pattern of introgressive hybridization was that some classes of hybrid genotypes were more frequent than others. Thus, relatively few individuals with approximately intermediate allelic composition groups were observed in the FCA, compared to the predominance of fish carrying alleles of one or the other parental types. The predominance of hybrids more like one or the other parental species is typical of hybrid zones in a wide variety of taxa, and suggests that mixed matings between one or the other parental species and hybrid individuals may occur more often than expected by random reproduction (Harrison & Bogdanowicz 1997; Burke *et al*. 1998; Goodman *et al*. 1999). Furthermore, the idea that hybrids with nearly intermediate allelic combinations are generally less fit than others has already been proposed by several authors (Cruzan *et al*. 1993; Cruzan & Arnold 1994; Arnold & Hodges 1995). We, therefore, propose that selection may act in the main zone of sympatry between *S. mentella* and *S. fasciatus* in favouring the differential survival of certain hybrids relative to either other hybrids or pure parental genotypes, depending on their genotypic composition. Martin & Cruzan (1999) have also recently proposed that the expansion of the *Piriqueta* complex hybrid zone across the central Florida may come from the higher fitness of some hybrid genotypes that are displacing parental ones.

As mentioned above, the occurrence of relatively fewer individuals of a given species in an area of large abundance of a second one should increase the probability of introgression towards the least abundant species (Arnold *et al*. 1993). Hence, the higher level of introgression found in *S. mentella* compared to *S. fasciatus* does not corroborate this prediction, since this latter species is the least abundant in the studied area and consequently, should be most prone to introgression (Payne & Ni 1982; Ni & Sandeman 1984). Consequently, this may also be suggestive of the role of selection in shaping the observed pattern of asymmetrical introgression. This is further corroborated by the analyses of linkage disequilibrium. Thus, significantly more pairwise disequilibria were detected in the *S. fasciatus* samples of the sympatric group (SYMPFAS) relative to any others. Assuming that the pattern of introgressive hybridization observed at microsatellites partly reflects that of functionally more important genes (e.g. because of genetic linkage), this suggests that selection in the zone of sympatry may be acting more importantly against the incorporation of *S. mentella* alleles into *S. fasciatus* than the reciprocal.

Finally, an additional indication for the role of selection in shaping the observed pattern of introgression lies in the relatively sharp geographical discontinuity in the distribution of introgressed and nonintrogressed fish for both taxa, despite obvious potential for dispersal and genetic exchange. Thus, gene flow is high enough to almost completely hamper the development of population structure over very large geographical areas, either within or outside the zone of introgression. For instance, *S. mentella* samples, MEA2 and MEA4, were almost genetically identical (θ estimate = 0.0014) although separated by nearly 2000 km. In contrast, the amount of genetic divergence between samples of *S. mentella*, respectively, belonging to the allopatric and sympatric group (e.g. MEA2 and MES1) was relatively large and highly significant (θ estimate = 0.015) despite their relative geographical proximity and lack of obvious barriers to dispersal.

Conclusion

This study provided one of the rare examples of extensive introgressive hybridization in the ocean, and highlighted the predominant role of this process in shaping the extent of genetic diversity, interspecific differences and population structuring among redfishes from the Northwest Atlantic. The extensive but geographically delimited pattern of asymmetrical introgressive hybridization, the sympatric persistence of two introgressed groups, and the maintenance of genetic integrity of both species outside the defined zone of introgression despite high potential for gene flow, all indicated that selection may be involved in promoting and maintaining the observed pattern of introgression. These observations demand more detailed genetic investigations of the genetic architecture of barriers to gene flow and eventually, of the quantitative trait loci affecting phenotypic differences in redfish (Rieseberg *et al*. 2000). Such studies, along with similar investigations in other species groups, could significantly contribute to our understanding of the evolutionary importance of introgressive hybridization in the ocean.

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This study is part of S. Roques' PhD thesis on the population genetics and evolutionary history of the north Atlantic redfish (*genus Sebastes*) species complex. The major interests of L. Bernatchez are in the understanding of the patterns and processes of molecular and organismal evolution, as well as their significance to conservation. Major interests of J.-M. Sévigny are in molecular ecology and conservation of exploited marine species.