Molecular Ecology (1999) **8**, 1703–1717

Potential of microsatellites for individual assignment: the North Atlantic redfish (genus *Sebastes*) species complex as a case study

SÉVERINE ROQUES, PIERRE DUCHESNE and LOUIS BERNATCHEZ
GIROQ, Département de biologie, Université Laval, Ste-Foy, Québec, Canada, G1K7P4

Abstract

We used the four redfish taxa (genus *Sebastes*) from the North Atlantic to evaluate the potential of multilocus genotype information obtained from microsatellites in assigning individuals at two different levels of group divergence. We first tested the hypothesis that microsatellites can diagnostically discriminate individual redfish from different groups. Second, we compared two different methods to quantify the effect of number of loci and likelihood stringency levels on the power of microsatellites for redfish group membership. The potential of microsatellites to discriminate individuals from different taxa was illustrated by a shared allele distance tree in which four major clusters corresponding to each taxa were defined. Concomitant with this strong discrimination, microsatellites also proved to be powerful in reclassifying specimens to the taxon of origin, using either an empirical or simulated method of estimating assignment success. By testing for the effect of both the number of loci and the level of stringency on the assignment success, we found that 95% of all specimens were still correctly reclassified with only four loci at the most commonly used criterion of log0. In contrast, the results obtained at the population level within taxa highlighted several problems of assignment that may occur at low levels of divergence. Namely, a drastic decrease of success with increasing stringency illustrated the lack of power of our set of loci. Strong discrepancy was observed between results obtained from the empirical and simulated methods. Finally, the highest assignment success was obtained when reducing the number of loci used, an observation previously reported in studies of human populations.

Keywords: assignment, genetic structure, individual classification, microsatellite, redfish, *Sebastes*

Received 2 February 1999; revision received 8 May 1999; accepted 3 June 1999

Introduction

Microsatellite loci are increasingly replacing or complementing other markers for numerous applications in evolutionary and conservation genetics (Jarne & Lagoda 1996). They are of particular interest in studies requiring fine-scale resolution for which other markers may have reached their limits of applications (Estoup & Angers 1998). Namely, their high level of polymorphism provides the potential to define unique multilocus genotypes. This, coupled with their relative ease of application and reliability of allelic determination compared with other methods, makes microsatellites particularly useful in studies requiring individual identification.

To date, most individual-based applications have concerned kinship, parentage analysis, mating systems and reproductive success (Queller *et al*. 1993; Kellogg *et al*. 1995; San Cristobal & Chevalet 1995; Estoup & Angers 1998; Jones *et al*. 1998). Less attention has been paid to using individual genotype information to determine the population membership of a single individual. This may be relevant to more precisely quantify gene flow (Waser & Strobeck 1998; Bossart *et al*. 1998), the degree of genetic differentiation among populations (Bowcock *et al*. 1994; Paetkau 1998; Paetkau *et al*. 1995) and to establish relationships among individuals within and among populations or higher taxonomic groupings (Estoup & Angers

Correspondence: L. Bernatchez. Fax: +1-418-656-2043; E-mail: Louis.Bernatchez@bio.ulaval.ca

1998; Estoup *et al*. 1995; Cornuet *et al*. 1996; Ellegren *et al*. 1996). An extension of these approaches is to detect an admixture of populations in a sample of individuals of unknown origins (Paetkau *et al*. 1995; Smouse & Chevillon 1998). However, in order to optimize the use of microsatellites for such applications, it is important to determine how factors such as allelic diversity and the extent of population differentiation will affect the success of population assignment (Shriver *et al*. 1997; Estoup & Angers 1998; Smouse & Chevillon 1998).

North Atlantic redfish (genus *Sebastes*) is a marine, viviparous fish, living at great depths in the North Atlantic ocean. It consists of a complex of four taxa: *S. mentella*, *S. marinus*, *S. fasciatus* and *S. viviparus* that can be found in sympatry and thus contribute to mixed fisheries. The discrimination of those taxa and the understanding of their evolutionary relationships have been hampered by their morphological similarity and variable extent of overlap in criteria used to define them, depending on regions (Barsukov 1968, 1972; Ni 1982; Power & Ni 1985). Low levels of inter- and intraspecific genetic variation have also generally been observed with proteic markers (Nedreaas & Naevdal 1991; Rubec *et al*. 1991). Allele frequencies may differ among taxa but these alone do not allow species identification (Nefyodov 1971; Payne & Ni 1982; Johansen *et al*. 1993). Recent studies of the D-loop region of the mitochondrial DNA have not proved to be more informative (Bentzen *et al*. 1998; M. Black, personal communication). Consequently, a combination of several morphological and proteic characters must still be used to confidently distinguish these taxa at adult stage. These characters, however, are not easily used at early (young) life-history stages. Consequently, the problems met at the 'species' level in redfish resemble those encountered at the population level in other taxonomic groups, not including the potential of population substructuring within each of the four taxa (Nedreaas *et al*. 1994).

In this context, the two specific objectives of this study were: (i) to provide a first quantification of the amount of divergence among *Sebastes* species based on microsatellites; and (ii) to test the hypothesis that microsatellites can discriminate individual redfish based on their multilocus genotypes. In doing so, we also compared two different methods to estimate the power of microsatellites for redfish group membership at different levels of divergence.

Materials and methods

Samples

Two geographical samples of each of the four taxa, *S. fasciatus*, *S. mentella*, *S. marinus* and *S. viviparus* were collected in the North Atlantic (Table 1). Samples were identified using meristic, morphological and genetic characters usually used for redfish (genus *Sebastes*) identification (Barsukov 1968, 1972; Ni 1981, 1982; Power & Ni 1985). DNA was extracted from muscle tissue stored in 95% ethanol, using the Chelex method (Walsh *et al*. 1991). Samples were screened for variation at eight specific microsatellites loci as described in Roques *et al*. (1999).

Descriptive statistics

We first documented the extent of intra- and intersample genetic diversity based on allelic composition of each sample. The intrasample genetic diversity was estimated by the number of alleles (A) per locus, gene diversity (H_E) and observed heterozygosity (H_O) (Table 1). Locus independence, Hardy–Weinberg equilibrium and allelic frequency heterogeneity were tested by probability tests computed using the Markov chain method (1000 iterations, Guo & Thompson 1992) available in GENEPOP, version 3.1 (Raymond & Rousset 1995). Deviations from Hardy–Weinberg equilibrium were tested using both alternative hypotheses of deficit and excess of heterozygotes for each locus and globally across loci and populations (Fisher's method). Statistical significance levels were adjusted for multiple comparisons using sequential Bonferroni adjustments (Rice 1989).

The extent of genetic divergence among samples was estimated by θ, an unbiased estimator of F_{ST} (Weir & Cockerham 1984) using GENEPOP, and Φ_{ST}, which integrates allelic size differences as a parameter of population differentiation (Michalakis & Excoffier 1996) using the program ARLEQUIN, version 1.1 (Schneider *et al*. 1997). θ and Φ_{ST} were first calculated between each pair of samples, and a hierarchical gene diversity analysis was also performed using ARLEQUIN to quantify the extent of genetic variance imputable to intertaxon differences relative to intersample within taxon.

Individual-based analyses

The relationships among the 260 redfish analysed based on their multilocus genotype was achieved by first calculating shared allele distances (D_{AS}) as described by Chakraborty & Jin (1993). This distance matrix was then used to build a neighbour-joining (NJ) tree, using a program provided by Jean-Marie Cornuet (Laboratoire de Modélisation et Biologie Evolutive, INRA, Montpellier). We used the index of classification (Ic, Estoup *et al*. 1995) to quantify how effectively individuals of a given group clustered together.

Species and population assignment

Two different approaches were used to quantify the assignment success at different levels of divergence in

Table 1 Gene diversity (H_E), observed heterozygosity (H_O), number of alleles (A), significant exact probability (P) for Hardy–Weinberg departures proportions (after corrections for null alleles), for each locus/population and overall (global tests)

Populations*		SEB31	SEB30	SEB45	SEB46	SEB37	SEB25	SEB9	SEB33	P
Famai	A	5	17	7	10	14	14	8	19	
(n = 60)	H_E	0.586	0.882	0.500	0.800	0.867	0.902	0.799	0.939	
	H_O	0.667	0.793	0.544	0.789	0.886	0.833	0.759	0.931	0.146
Fane1	A	2	25	10	11	17	16	8	20	
(n = 70)	H_E	0.507	0.920	0.589	0.778	0.900	0.787	0.691	0.881	
	H_O	0.486	0.857	0.485	0.882	0.886	0.800	0.647	0.943	0.102
Mene1	A	14	22	15	10	15	15	9	24	
(n = 58)	H_E	0.835	0.930	0.878	0.832	0.886	0.893	0.772	0.958	
	H_O	0.893	0.793	0.714	0.741	0.720	0.828	0.964	0.893	0.029
Metne	A	15	23	14	11	22	18	11	28	
(n = 96)	H_E	0.780	0.889	0.827	0.821	0.923	0.909	0.835	0.960	
	H_O	0.783	0.652	0.745	0.800	0.886	0.813	0.761	0.917	< 0.00001*
Mano1	A	12	17	12	13	12	19	9	21	
(n = 60)	H_E	0.854	0.693	0.821	0.912	0.823	0.886	0.786	0.948	
	H_O	0.767	0.600	0.733	0.867	0.759	0.964	0.759	0.929	0.220
Mano2	A	8	20	12	14	13	15	9	25	
(n = 56)	H_E	0.797	0.791	0.824	0.898	0.804	0.894	0.777	0.963	
	H_O	0.821	0.786	0.750	0.964	0.679	0.929	0.786	0.857	0.244
Vino1	A	6	26	11	20	17	4	5	23	
(n = 60)	H_E	0.612	0.960	0.761	0.918	0.933	0.606	0.628	0.956	
	H_O	0.926	0.897	0.667	0.667	0.611	0.714	0.483	1.000	0.025
Vino2	A	5	22	12	22	23	5	6	20	
(n = 60)	H_E	0.599	0.949	0.645	0.914	0.955	0.614	0.715	0.940	
	H_O	0.759	0.862	0.586	0.967	0.467	0.767	1.000	0.931	0.982
Total	A	23	56	27	33	36	23	14	44	
(n = 530)	H_E*	0.883	0.960	0.869	0.915	0.947	0.911	0.860	0.967	
	P	0.595	0.402	< 0.00001*	0.061	0.636	0.011	0.113	0.163	

†*S. fasciatus*, Famai (Gulf of Maine; 67°32′N, 43°15′W); Fane1(Nova Scotia; 62°20′N, 43°38′W); *S. mentella*, Mene1(Nova Scotia; 60°44′N, 43°39′W); Metne (Newfoundland; 59°16′N, 46°42′W); *S. marinus*, Mano1 (Norway; 63°32′N, 8°15′W); Mano2 (Norway; 64°30′N, 9°13′E); *S. viviparus*, Vino1 and Vino2 (both samples from Norway).

*Significant deficit in heterozygotes following adjustments for multiple tests with the sequential Bonferroni method (α = 0.05, k = 8).

redfish. We first conducted an empirical method that consisted in reclassifying individuals of known population origin, as described in Paetkau *et al*. (1995). This consisted of assigning an individual to the group in which its multilocus genotype has the highest probability of occurring, assuming reliable allelic representation, Hardy–Weinberg equilibrium and locus independence (Paetkau *et al*. 1995). The proportion of correct classifications was used as an estimate of the probability of assignment success (P_s). In this method, the reassigned specimens are also the ones from which the population allelic distribution estimates were obtained (Paetkau *et al*. 1995). This leads to an upward bias of the probability of successful assignment. To eliminate this bias, the contribution of the specimen to be classified is removed from the estimation of the allelic distributions (Waser & Stroebeck 1998).

We also used a Monte-Carlo simulation approach to randomly generate 1000 artificial multilocus genotypes from each population, as used in Shriver *et al*. (1997). In this method, the probability of generating a given genotype is precisely the theoretical probability computed from the allelic frequencies of the population, assuming random mating. As for the empirical method, the proportion of correct classifications was used as an estimate of the probability of assignment success (P_s). We refer to this method as the simulated method.

Each of the two procedures above was extended following the log-likelihood method detailed by Shriver *et al*. (1997) to define assignment success at different thresholds of stringency. To assign an individual, we first calculate its probability of occurrence within each population. The log-likelihood statistic is the logarithm (base 10) of the ratio of the largest probability over the next largest. If the log-likelihood is greater than a predefined acceptance threshold, the specimen is assigned to the population corresponding to the largest probability of occurrence. Four acceptance thresholds were applied

(log3, log2, log1, log0), log1 to log3 meaning that a multilocus genotype has to be 10, 100, or 1000 times more likely in one group than any other to be assigned. Log0 simply requires that a genotype is more likely in one group than another, and corresponds to the criterion used in the method of Paetkau *et al*. (1995). Increasing the stringency of the criterion reduces the probability of misassignment at the expense of increasing the undetermined proportion of specimens.

Finally, we investigated the effect of using different numbers of loci on assignment success. In order to find the best set for each number of loci, all possible combinations of 1–8 loci have been computed according to the empirical method. A total of 8 (one locus), 28 (2 loci), 56 (3 loci), 70 (4 loci), 56 (5 loci), 28 (6 loci), 8 (7 loci), and 1 (8 loci), totalling 255 estimates, have been calculated. Then, we were able to select the combination with the highest assignment success for each number of loci.

All the procedures described above were performed with programs written with the algebric computer system MapleV, version 5.

Results

Polymorphism, locus independence and Hardy–Weinberg equilibrium (HWE)

All loci showed high variability with the total number of alleles varying from 14 to 56 (average = 33), and a mean gene diversity per locus varying between 0.860 (SEB9) and 0.967 (SEB33) (mean = 0.914) (Table 1). High polymorphism was also generally observed within each sample.

Exact tests for locus independence revealed no significant locus comparison at the 5% level. Global tests (Fisher's method) for HWE across loci revealed significant heterozygote deficiency within all eight samples. Global tests across populations showed that these departures were mainly caused by heterozygote deficiencies at loci SEB37 and SEB30 found in most samples, suggesting the presence of null alleles at these loci. A null allele test was thus performed using the EM algorithm of Dempster *et al*. (1977) implemented in GENEPOP. This method first assigns a frequency to the null allele suspected for a given locus, considering deviations from Hardy–Weinberg expectations, and provide the allelic combination of the null allele with the other alleles that minimizes departures from HWE. The data set was corrected for the null allele before performing statistical analysis. As it is assumed that the null allele is the same in all samples, this correction will generate a conservative bias in assignment tests. Global tests for Hardy–Weinberg equilibrium were conducted again for all samples, and only the Metn1 sample (*Sebastes mentella*) remained in significant heterozygote deficiency, suggesting a deviation from HWE expectations caused by other factors (Table 1). Such disequilibrium may partially bias the results of the assignment tests at population level, but tests were not performed for *S. mentella* (see below). Its effect is probably of minor significance at the intertaxon level, given the major differences observed in allelic composition (see below).

Genetic differentiation

Highly significant differences ($P \leq 0.001$) in allelic frequencies were observed at all loci for all pairwise comparisons of taxa (Table 2). Only 20% of all alleles over all loci were shared among them. Differences in allelic frequencies among taxa translated into moderate estimates of θ that varied between 0.088 and 0.199 (Table 3). Allelic differences among taxa were often based on modal size differences, and consequently Φ_{ST} estimates were approximately twice as high as θ on average, varying between 0.120 and 0.486 (Tables 1 and 2).

Much smaller differences in allelic composition were observed among samples within taxa (Table 2). Significant differences in allele frequencies were found at loci SEB33 and SEB30 for *S. fasciatus*, at loci SEB45 and SEB30 for *S. mentella*, at loci SEB31, SEB33, SEB37, SEB9 and SEB30 for *S. marinus*, and at loci SEB37, SEB9 and SEB30 for *S. viviparus*. These differences translated into very small levels of divergence between samples. θ estimates significantly different from zero were found between *S. fasciatus* and *S. viviparus* samples only and a significant Φ_{ST} value was only observed for *S. viviparus* (Table 3). As we could not reject the null hypothesis of a unique gene pool for *S. marinus* and *S. mentella*, assignment tests at the population level were not valid and consequently not performed for these two taxa. The hierarchical gene diversity revealed that the amount of genetic variance imputable to taxa differences was much higher than among samples within a taxon (Table 4). This ratio was approximately 10:1 based on variance in allelic frequencies, and nearly 100:1 based on allelic size variance. This indicated that the accumulation of mutations contributed more substantially to differentiation among taxa relative to that observed among populations within taxon.

Individual relationships

The NJ tree built from the matrix of pairwise D_{AS} distances among all 260 specimens illustrated the potential of multilocus microsatellite genotypes to discriminate redfish taxa. The complete tree was composed of four clusters completely corresponding to taxa, except for six misassigned individual specimens (two *S. marinus* and four *S. mentella*). A smaller subset of 86 specimens (with three misassigned individuals) was used here as a simplified representation of the complete tree

Table 2 Allele frequencies for each population. The codes, location and sample size of each population are described in Table 1

	S. fasciatus		*S. mentella*		*S. marinus*		*S. viviparus*	
Locus-allele (bp)	Famai	Fane	Mene	Metn	Mano1	Mano2	Vino1	Vino2
SEB31								
150	0	0	0	0	0	0	0.017	0
152	0.021	0	0.368	0.441	0	0	0.034	0
154	0.042	0	0.018	0.011	0	0	0.448	0.5
156	0.5	0.5	0.053	0.043	0	0	0.431	0.397
157	0	0	0	0	0	0	0.017	0.052
158	0.417	0.5	0.053	0.032	0	0	0.052	0.034
164	0	0	0	0	0.017	0.036	0	0.017
166	0	0	0	0	0.083	0	0	0
168	0.021	0	0.053	0.011	0.05	0	0	0
170	0	0	0	0	0.067	0.286	0	0
172	0	0	0.035	0.086	0.25	0.304	0	0
174	0	0	0.07	0.108	0.25	0.161	0	0
176	0	0	0.035	0.108	0.017	0	0	0
178	0	0	0.053	0.043	0.05	0.107	0	0
180	0	0	0.053	0.043	0.117	0.054	0	0
182	0	0	0.105	0.022	0.067	0.018	0	0
184	0	0	0	0.022	0.017	0	0	0
186	0	0	0.07	0	0	0	0	0
188	0	0	0	0.011	0.017	0.036	0	0
190	0	0	0	0.011	0	0	0	0
SEB25								
193	0	0	0	0	0	0	0	0.017
195	0	0	0	0.042	0.017	0.036	0.446	0.517
197	0.1	0.086	0.207	0.146	0.05	0.054	0.446	0.35
199	0	0	0.19	0.208	0.3	0.268	0.054	0
201	0	0.029	0.103	0.094	0.05	0	0.054	0.067
203	0	0	0	0.042	0.033	0.125	0	0.05
205	0	0.014	0.052	0.021	0.017	0.018	0	0
207	0.017	0.014	0.017	0.031	0.017	0	0	0
209	0	0.014	0.034	0	0.033	0.036	0	0
211	0	0	0.103	0.031	0.083	0.071	0	0
213	0.05	0.014	0.017	0.01	0.05	0.018	0	0
215	0.067	0.043	0.034	0.021	0.067	0.089	0	0
217	0.083	0.057	0.017	0.073	0.05	0.071	0	0
219	0.133	0.029	0	0.042	0.083	0.036	0	0
221	0.067	0	0	0.031	0.033	0	0	0
223	0.05	0.043	0.017	0.021	0	0	0	0
225	0.117	0.071	0.069	0.052	0.033	0.054	0	0
227	0.217	0.443	0.103	0.094	0	0	0	0
229	0.017	0.057	0.017	0.01	0	0.036	0	0
231	0.017	0.043	0.017	0.031	0.017	0	0	0
233	0.05	0.029	0	0	0.033	0.071	0	0
235	0.017	0.014	0	0	0.017	0.018	0	0
237	0	0	0	0	0.017	0	0	0
SEB33								
220	0	0	0	0	0	0	0	0.169
222	0	0	0.018	0	0	0	0.018	0
224	0	0	0.035	0.01	0	0	0	0
226	0	0	0.018	0	0.018	0	0.036	0.017
228	0	0	0	0	0	0	0.018	0
230	0	0.043	0	0	0	0	0.071	0
232	0.017	0	0	0.031	0	0	0	0
234	0	0	0	0.021	0	0	0	0.017
236	0	0	0.018	0	0	0	0	0
238	0	0.243	0.018	0.021	0	0.036	0.071	0.051

Table 2 *Continued*

	S. fasciatus		*S. mentella*		*S. marinus*		*S. viviparus*	
Locus-allele (bp)	Famai	Fane	Mene	Metn	Mano1	Mano2	Vino1	Vino2
240	0.138	0.043	0.035	0	0	0.018	0.036	0
242	0.034	0.014	0.018	0.021	0	0	0.018	0.017
244	0	0	0	0.031	0	0	0.089	0.136
246	0	0.029	0.035	0.021	0	0	0.036	0.017
248	0.052	0.014	0.018	0.063	0	0	0.054	0.068
250	0.017	0	0.088	0.063	0.105	0.036	0.018	0.051
252	0.034	0	0.018	0.031	0	0.054	0.071	0.068
254	0.034	0.029	0.053	0.073	0	0.018	0	0.051
256	0.086	0	0.07	0.042	0.035	0	0.054	0.051
258	0.052	0.057	0.053	0.052	0.105	0.071	0.036	0.051
260	0.017	0.043	0.07	0.021	0	0.054	0.125	0.051
262	0.034	0.014	0.088	0.021	0	0.018	0.036	0.034
264	0	0.014	0.123	0.094	0.053	0.018	0.054	0.051
266	0.052	0.029	0.053	0.073	0	0.036	0	0.034
268	0.052	0.014	0	0.063	0.035	0.054	0.071	0
270	0	0.214	0.018	0.052	0	0.036	0.018	0.017
272	0.103	0.114	0.018	0.031	0	0.036	0	0
274	0.121	0.014	0.035	0.042	0.018	0.018	0.018	0.034
276	0.034	0.014	0	0.021	0	0.036	0.018	0
278	0.017	0.014	0.035	0	0.018	0.018	0.018	0.017
280	0.086	0	0	0.01	0.035	0.107	0.018	0
282	0	0	0	0.052	0.07	0.054	0	0
284	0	0.029	0	0.01	0.018	0.018	0	0
286	0	0.014	0	0	0.07	0.036	0	0
288	0	0	0.053	0.01	0.088	0.036	0	0
290	0	0	0	0.01	0.105	0	0	0
292	0.017	0	0.018	0	0.035	0.018	0	0
294	0	0	0	0.01	0	0.071	0	0
296	0	0	0	0	0.053	0.089	0	0
298	0	0	0	0	0.053	0.018	0	0
302	0	0	0	0	0.035	0	0	0
308	0	0	0	0	0.018	0	0	0
310	0	0	0	0	0.018	0	0	0
314	0	0	0	0	0.018	0	0	0
SEB30								
163	0	0.014	0	0	0	0	0	0
165	0	0.029	0	0	0	0	0	0
171	0	0	0	0	0	0.036	0	0
173	0.086	0.014	0	0.01	0	0	0	0
175	0	0.014	0	0	0	0	0	0
177	0	0	0	0	0.55	0.446	0	0
179	0	0	0	0.031	0	0.036	0	0.119
181	0.017	0	0	0	0	0	0	0.017
183	0	0	0	0	0	0	0.017	0
185	0	0.014	0	0	0.017	0.018	0	0
187	0.017	0.029	0	0	0.05	0	0	0
189	0.138	0.057	0	0	0	0.018	0.017	0
191	0.017	0.043	0	0	0	0	0.069	0
192	0	0	0	0	0	0	0.017	0
193	0	0	0	0.01	0	0	0	0
195	0	0.029	0	0.01	0.017	0	0	0
197	0.017	0	0.017	0	0.067	0.018	0	0
199	0.069	0.186	0	0.031	0	0	0.034	0
201	0.034	0.014	0	0	0	0.018	0.034	0
205	0	0.057	0.017	0	0	0	0	0
207	0.069	0.014	0	0.01	0	0	0	0.017

Table 2 *Continued*

	S. fasciatus		*S. mentella*		*S. marinus*		*S. viviparus*	
Locus-allele (bp)	Famai	Fane	Mene	Metn	Mano1	Mano2	Vino1	Vino2
209	0.293	0.186	0.069	0	0.017	0.018	0	0.068
211	0.052	0.057	0	0.01	0.017	0.054	0	0
213	0	0.043	0.069	0.042	0.033	0.018	0	0.051
215	0	0.014	0.034	0.01	0.033	0	0.034	0.034
217	0.017	0	0.017	0	0	0.071	0	0.034
219	0.034	0.071	0	0.031	0	0	0	0.017
221	0	0	0.017	0	0.017	0.036	0.017	0.102
223	0.034	0	0.017	0.01	0.033	0.089	0.103	0.034
225	0	0	0.034	0.01	0.017	0.018	0.017	0.068
227	0.034	0	0.086	0.021	0	0.018	0.034	0
229	0	0	0.034	0.01	0.017	0	0.103	0.136
231	0	0.014	0.017	0.042	0	0.018	0.052	0.051
233	0.034	0.014	0.034	0.031	0	0	0.086	0.017
235	0	0.014	0.017	0.042	0	0.018	0.069	0.017
237	0	0.014	0.052	0.208	0	0	0.052	0.034
239	0	0	0.103	0.208	0	0	0.017	0.051
241	0	0	0.207	0.115	0	0	0	0
243	0	0	0.052	0.063	0.033	0	0.034	0.034
245	0	0	0.052	0.031	0	0	0.034	0.034
247	0	0	0.017	0	0	0.018	0.017	0.034
249	0	0	0	0.01	0.033	0	0.034	0
251	0	0	0	0	0	0	0	0.017
253	0	0	0.017	0	0	0	0	0
255	0	0	0.017	0	0	0	0	0
257	0	0.029	0	0	0	0	0	0
265	0	0	0	0	0	0	0.017	0
279	0	0	0	0	0	0	0.017	0
285	0	0	0	0	0.033	0.018	0	0
287	0	0	0	0	0.017	0.018	0	0
289	0	0	0	0	0	0	0.034	0
291	0	0.014	0	0	0	0	0	0
293	0.034	0.014	0	0	0	0	0	0
299	0	0	0	0	0	0	0	0.017
317	0	0	0	0	0	0	0.017	0
353	0	0	0	0	0	0	0.017	0
SEB46								
112	0	0	0	0	0	0.018	0	0
116	0	0	0.362	0.356	0.1	0.107	0.017	0.017
118	0	0.029	0	0	0.05	0.036	0	0
120	0.02	0	0	0	0.05	0.196	0.017	0.05
122	0	0	0	0	0.067	0.071	0.017	0
124	0.4	0.412	0.213	0.1	0.133	0.071	0.05	0.05
126	0	0	0.043	0.022	0	0	0	0
128	0.04	0.029	0	0.022	0.05	0.071	0.133	0.017
130	0.08	0.074	0.043	0.122	0	0.018	0.083	0.117
132	0.02	0.015	0.064	0.067	0.017	0	0.217	0.25
134	0	0.015	0.043	0.044	0.15	0.196	0.067	0.067
136	0.1	0.088	0.085	0.044	0.083	0.036	0.033	0.033
138	0.12	0.147	0.085	0.056	0.05	0	0.017	0.017
140	0.18	0.162	0.064	0.156	0.167	0.071	0.017	0
142	0	0.015	0	0	0.05	0.071	0	0.033
144	0.02	0.015	0	0	0.033	0.018	0	0
146	0	0	0	0	0	0	0	0.017
148	0.02	0	0	0	0	0	0.05	0.033
150	0	0	0	0	0	0	0.067	0
152	0	0	0	0	0	0.018	0.05	0.033

Table 2 *Continued*

	S. fasciatus		*S. mentella*		*S. marinus*		*S. viviparus*	
Locus-allele (bp)	Famai	Fane	Mene	Metn	Mano1	Mano2	Vino1	Vino2
154	0	0	0	0	0	0	0.067	0.067
156	0	0	0	0	0	0	0	0.033
158	0	0	0	0	0	0	0	0.033
160	0	0	0	0	0	0	0.033	0
162	0	0	0	0	0	0	0.017	0.033
166	0	0	0	0.011	0	0	0.017	0
168	0	0	0	0	0	0	0	0.017
174	0	0	0	0	0	0	0	0.033
176	0	0	0	0	0	0	0	0.017
180	0	0	0	0	0	0	0.017	0
186	0	0	0	0	0	0	0.017	0
188	0	0	0	0	0	0	0	0.017
208	0	0	0	0	0	0	0	0.017
SEB37								
211	0	0	0	0	0	0	0	0.017
213	0	0	0	0	0	0	0	0.017
219	0	0	0	0	0	0	0.029	0.033
221	0	0	0	0	0	0	0.029	0
223	0	0	0	0	0	0.018	0	0
229	0	0	0	0	0.362	0.411	0	0
231	0	0	0	0	0.069	0.036	0	0.033
233	0	0	0	0.011	0	0.018	0	0.133
235	0	0	0.286	0.169	0.017	0.071	0.029	0
237	0	0	0.02	0.022	0	0	0.086	0
239	0	0	0	0.034	0	0	0.171	0.083
241	0	0.029	0	0	0	0.018	0.029	0.067
243	0.017	0.029	0.02	0	0	0	0.029	0.017
245	0.083	0.157	0.143	0.011	0	0	0	0.033
247	0.25	0.214	0.061	0.135	0	0.036	0	0
249	0.15	0.114	0.02	0.079	0	0.089	0.086	0
251	0.067	0.014	0.143	0.045	0.069	0.125	0.057	0.05
253	0.05	0.057	0.02	0.056	0.19	0.036	0.029	0
255	0.067	0.086	0.061	0.112	0.052	0	0.057	0.033
257	0.133	0.057	0.02	0.045	0.069	0.054	0.057	0
259	0.033	0.029	0	0.09	0.052	0.071	0	0.05
261	0.067	0.1	0.102	0.011	0.017	0.018	0	0.067
263	0.033	0.014	0.02	0.022	0.052	0	0.057	0.033
265	0	0	0	0.011	0	0	0.029	0.067
267	0.017	0.043	0	0	0	0	0.143	0
269	0.017	0	0.041	0.034	0.017	0	0.029	0.067
271	0	0.014	0	0.022	0	0	0	0.017
273	0	0.014	0	0.022	0.017	0	0	0
275	0.017	0.014	0	0	0	0	0	0.017
277	0	0.014	0	0.022	0.017	0	0.029	0.033
279	0	0	0.02	0.022	0	0	0.029	0
281	0	0	0.02	0.011	0	0	0	0.033
285	0	0	0	0	0	0	0	0.033
287	0	0	0	0	0	0	0	0.05
293	0	0	0	0.011	0	0	0	0
295	0	0	0	0	0	0	0	0.017
SEB09								
95	0	0	0	0	0	0.054	0	0
99	0	0.015	0.036	0.054	0.19	0.232	0	0
101	0.052	0.088	0.071	0.054	0	0	0.017	0.017
103	0.241	0.191	0.018	0.054	0	0	0.017	0.033
105	0.034	0.044	0.018	0.022	0.224	0.054	0	0.167

Table 2 *Continued*

	S. fasciatus		*S. mentella*		*S. marinus*		*S. viviparus*	
Locus-allele (bp)	Famai	Fane	Mene	Metn	Mano1	Mano2	Vino1	Vino2
107	0.345	0.515	0.161	0.109	0.034	0.018	0.259	0.417
109	0.086	0.059	0.179	0.239	0.017	0.018	0.534	0.3
111	0.086	0.044	0.411	0.283	0.017	0	0.172	0.067
113	0.017	0	0.018	0.022	0	0.036	0	0
115	0	0	0	0.033	0.034	0.125	0	0
117	0.138	0.044	0.089	0.12	0.362	0.393	0	0
119	0	0	0	0	0.069	0.071	0	0
125	0	0	0	0	0.052	0	0	0
127	0	0	0	0.011	0	0	0	0
SEB45								
114	0.018	0	0	0	0	0	0	0
116	0	0.015	0	0	0	0	0	0
118	0	0.061	0.018	0.011	0	0	0	0
120	0	0	0.071	0.021	0.05	0.036	0.02	0
122	0	0.015	0	0	0.033	0.054	0	0.017
124	0	0	0.018	0.043	0.033	0	0	0
128	0	0	0.268	0.16	0.2	0.179	0	0
130	0.036	0.015	0	0.011	0.05	0	0.449	0.586
132	0.661	0.621	0.125	0.33	0.033	0.018	0.082	0.086
134	0.071	0.076	0.125	0.149	0.017	0.054	0.143	0.086
136	0.143	0.152	0.143	0.138	0.25	0.125	0.082	0.017
138	0.036	0.015	0.018	0.043	0.283	0.357	0.041	0
140	0	0	0.054	0	0	0.071	0.061	0.052
142	0	0	0	0.032	0	0.018	0	0
144	0.036	0	0	0.011	0.017	0	0.02	0.017
146	0	0	0.018	0.032	0.017	0	0.02	0.034
148	0	0	0	0.011	0	0	0	0
150	0	0.015	0.018	0	0	0	0	0
152	0	0.015	0.036	0	0	0	0	0
154	0	0	0.054	0	0	0	0	0.034
156	0	0	0.018	0	0	0	0.061	0.034
158	0	0	0.018	0.011	0	0	0.02	0.017
160	0	0	0	0	0	0.036	0	0
164	0	0	0	0	0.017	0	0	0
166	0	0	0	0	0	0.036	0	0
168	0	0	0	0	0	0	0	0.017
170	0	0	0	0	0	0.018	0	0

Table 3 Pairwise sample differentiation estimates, based on allelic (θ, lower diagonal) and molecular (Φ_{ST}, upper diagonal) variance analysis at eight microsarellites loci in eight redfish samples. Values in italics indicate intrataxonomic pairwise comparisons

Samples	1	2	3	4	5	6	7	8
1-Famai		*0**	0.329	0.310	0.247	0.208	0.467	0.375
2-Fane1	*0.022*		0.326	0.315	0.314	0.273	0.495	0.348
3-Mene1	0.099	0.137		*0**	0.430	0.395	0.142	0.207
4-Metne	0.088	0.127	*0.009**		0.433	0.398	0.121	0.223
5-Mano1	0.156	0.199	0.094	0.097		*0**	0.381	0.486
6-Mano2	0.152	0.196	0.097	0.098	*0.009**		0.396	0.455
7-Vino1	0.143	0.173	0.108	0.109	0.182	0.177		*0.038*
8-Vino2	0.153	0.179	0.137	0.139	0.192	0.192	*0.016*	

*Not significant following sequential Bonferroni corrections ($\alpha = 0.05$, $k = 28$).

Table 4 Hierarchical analysis of genetic diversity in redfish with eight microsatellite loci, using allelic (θ) or molecular (Φ_{ST}) variance, with percentages of variation (%), variance components (V_a) and probability of components being equal to zero (P).

	%	V_a	P
Allelic variance			
Among taxa	12.84	0.3442	0.00610
Among populations within taxon	1.17	0.0314	< 0.00001
Within populations	85.99	2.3065	< 0.00001
Size variance			
Among taxa	34.88	134.97	< 0.00001
Among populations within taxon	0.35	1.3574	< 0.00001
Within populations	64.77	250.62	< 0.00001

(Fig. 1). This translated into highly significant ($P < 0.0001$) indices of classification; 83% for *S. mentella*, 89% for *S. marinus*, and 100% for both *S. fasciatus* and *S. viviparus*. Some phylogenetic information could also be obtained from the D_{AS} tree. *S. viviparus* and *S. fasciatus* had a sister-group relationship at the first-order clustering level, while *S. marinus* had the most basal position. No consistent clustering was observed among samples within each taxon.

Assignment sucess

High percentages of assignment success were observed among the four taxa based on both the empirical and simulated methods (Fig. 2). Using all loci, 87–100% of

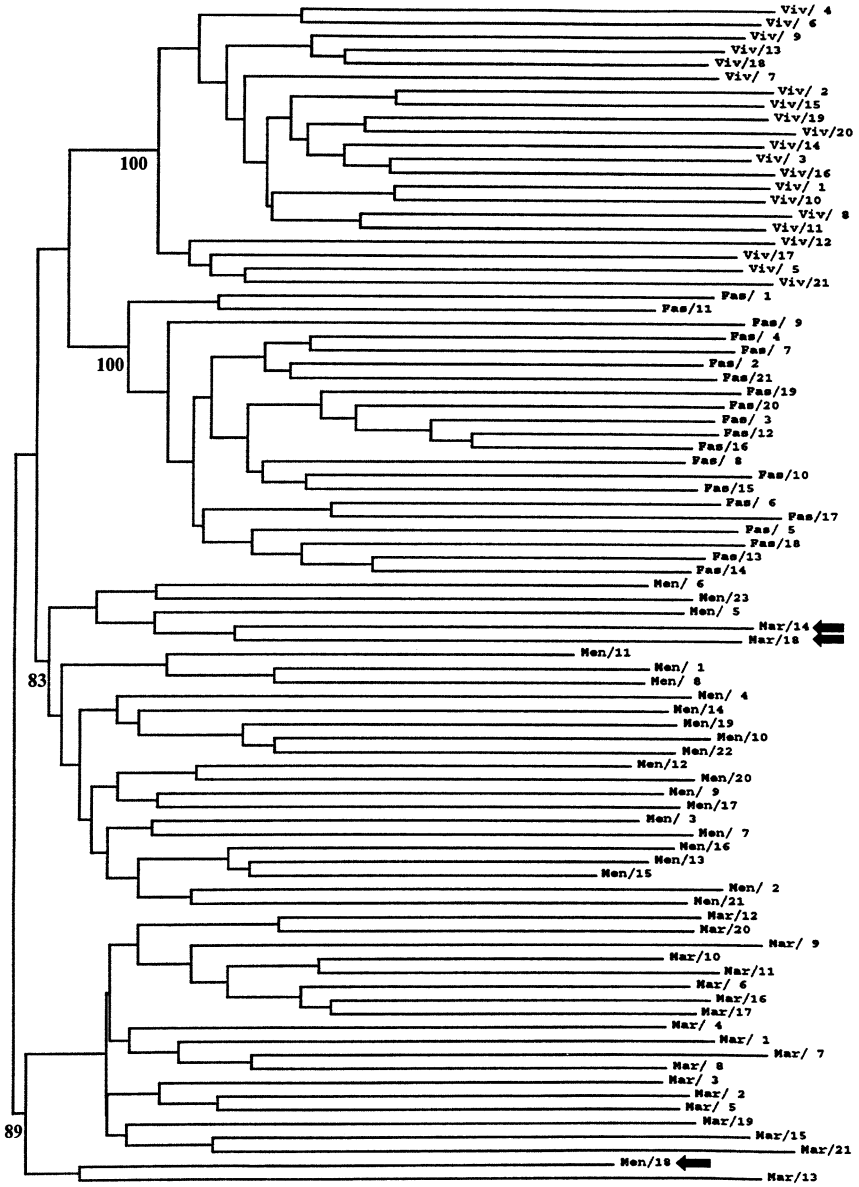

Fig. 1 Neighbour-joining (NJ) tree of 86 redfish specimens based on the allele shared distance (D_{AS}). Arrows indicate misclustered individuals. Values along branches indicate the index of classification for each taxa cluster, based on the analysis of 260 specimens.

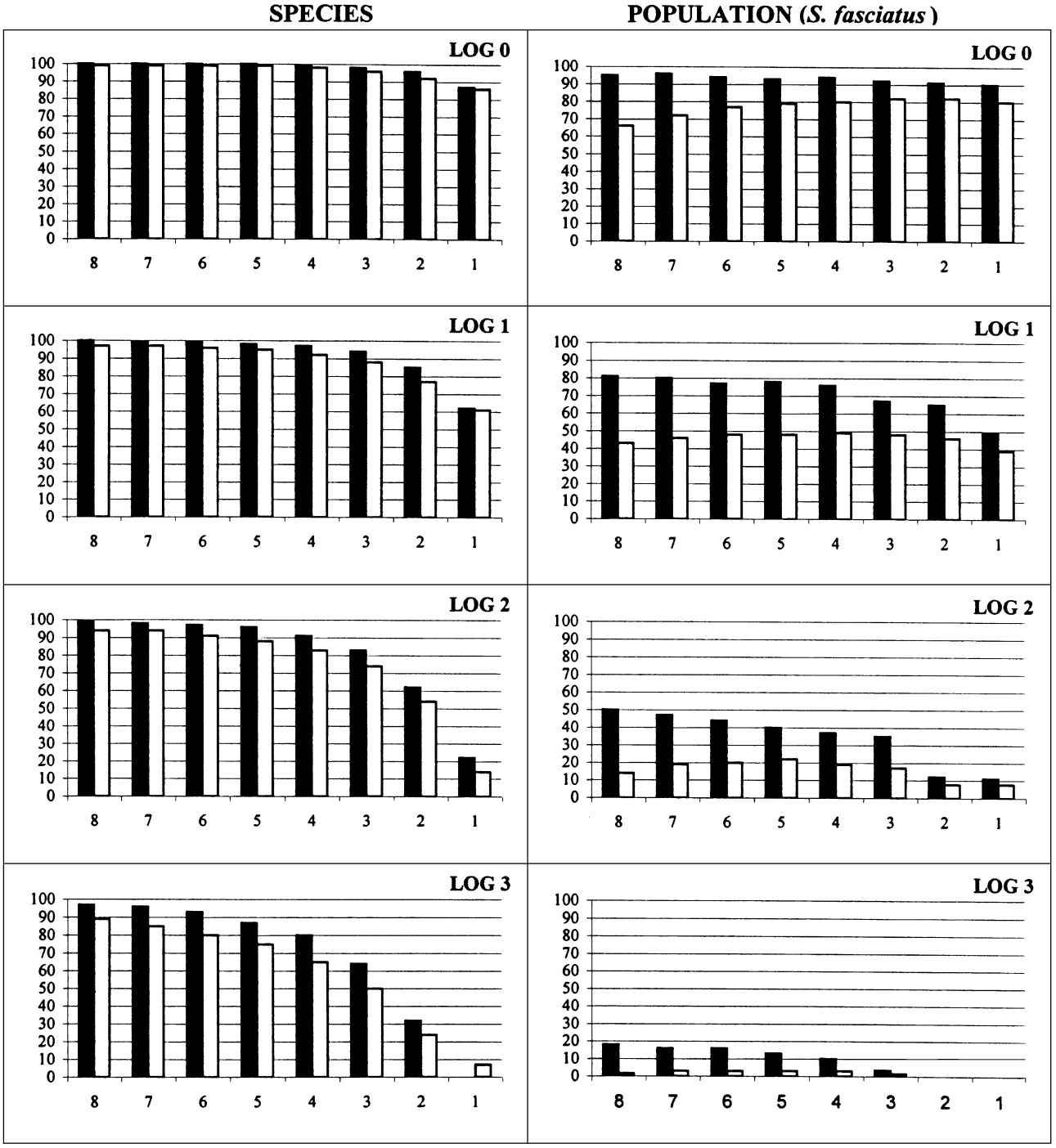

Fig. 2 Results of assignment precision (in percent) among taxa and populations within taxon (*Sebastes fasciatus*) as a function of number of loci (8–1) and threshold level (Log0 to Log3), with the empirical (white bars) and simulated (black bars) methods. The combinations of 1–7 loci with the highest assignment precision are listed below. Numbers refer to reduced locus names (e.g. 31 = SEB31). Taxa: (Log0): 31, 33, 25, 37, 46, 9, 30; 31, 25, 37, 46, 9, 30; 31, 25, 46, 9, 30; 31, 25, 9, 30; 31, 25, 30; 31, 30; 31 (Log1): 31, 33, 25, 37, 46, 9, 30; 31, 25, 37, 46, 9, 30; 31, 25, 46, 9, 30; 31, 45, 46, 30; 31, 25, 30; 31, 30; 31 (Log2): 31, 25, 37, 45, 46, 9, 30; 31, 25, 45, 46, 9, 30; 31, 25, 37, 46, 30; 31, 25, 46, 30; 31, 25, 30; 31, 30; 31 (Log3): 31, 25, 37, 45, 46, 9, 30; 31, 25, 37, 45, 46, 30; 31, 25, 45, 46, 30; 31, 25, 46, 30; 31, 25, 30; 31, 30; 30. *S. fasciatus*: (Log0): 31, 33, 25, 45, 46, 9, 30; 31, 33, 25, 45, 46, 9; 31, 33, 25, 45, 9; 33, 25, 45, 9; 31, 33, 9; 33, 9; 33 (Log1): 31, 33, 25, 37, 45, 9, 30; 31, 33, 25, 45, 46, 30; 31, 33, 45, 9, 30; 33, 25, 45, 30; 31, 33, 30; 33, 30; 33 (Log2): 31, 33, 25, 45, 46, 9, 30; 31, 33, 25, 45, 9, 30; 31, 33, 25, 9, 30; 31, 33, 25, 30; 33, 25, 30; 31, 33; 33 (Log3): 31, 33, 25, 37, 46, 9, 30; 31, 33, 25, 37, 9, 30; 33, 25, 45, 9, 30; 33, 25, 9, 30; 31, 33, 30; 31, 33; 33.

individuals were correctly assigned from thresholds log3 to log0 (Fig. 2). The success remained above 95% at log0 with only four loci, while the number of loci necessary to reach a similar level approximately increased by one for each threshold level. Results of both empirical and simulated methods were comparable at log0, but discrepancy between them increased with the stringency applied. Assignment tests at the population level are only presented for *S. fasciatus*, but very similar results were obtained for *S. viviparus* (Fig. 2). In contrast with the results obtained at the taxa level, results obtained by both methods varied considerably at all threshold levels. Relatively high assignment success was only obtained with the simulated method at log0. Values dropped drastically with increasing threshold levels for both methods. An interesting observation was that assignment success did not clearly improve with an increasing number of loci with the empirical method. In fact, the best assignment success at log0 was obtained with the use of two and three loci.

Discussion

Taxa and population discrimination

This study revealed the usefulness of microsatellite multilocus genotype analysis to discriminate individuals of redfish taxa in the North Atlantic. Specific alleles and/or distinct allelic size modes were observed for all taxa depending on loci. The potential to discriminate individuals from different taxa was illustrated by the D_{AS} tree in which four clusters corresponding to each taxon were defined, with only 2.3% of specimens clustering at the wrong place, which could potentially be due to incorrect identification from other characters. Microsatellites are thus much more informative than previously used genetic markers to discriminate redfishes in the North Atlantic (Nedreaas & Naevdal 1991; Rubec *et al*. 1991; Bentzen *et al*. 1998; M. Black, personal communication). Moreover, these markers can easily be used at any life-history stages (e.g. Bentzen *et al*. 1996), which can be of interest in studies of recruitment and larval ecology (Ruzzante *et al*. 1996).

The analysis of multilocus genotype relationships based on D_{AS} distance also illustrated the potential usefulness of microsatellites to infer shallow phylogenies where other genetic markers have been limited. Barsukov (1972) proposed that although they have a disjunct distribution on both sides of the Atlantic ocean, *Sebastes fasciatus* and *S. viviparus* were more closely related to each other than to other taxa, and that *S. marinus* had basal relationships relative to other redfishes from the North Atlantic. The D_{AS} tree resolved here provides a first empirical support to this hypothesis.

Our results were also indicative that the extent of genetic variance among populations within redfish taxa is much smaller than among taxa, in the order of 10–100 times depending on the parameter used. The extent of intrapopulation allelic diversity and gene diversity estimates observed in this study were very comparable to those reported to date for other marine fishes (e.g. de García León *et al*. 1995; Bentzen *et al*. 1996; Rico *et al*. 1997; Ruzzante *et al*. 1998). Similarly, the very low but significant genetic differentiation quantified between samples within both *S. fasciatus* and *S. viviparus* was in the same range of amplitude reported in those studies.

Assignment precision

Concomitant with the strong discrimination revealed by the D_{AS} tree, microsatellites proved to be powerful in reclassifying specimens to taxon of origin in the assignment procedures. Thus, a nearly perfect assignment success was obtained when using all eight loci at minimal stringency (log0), and nearly 90% could still be assigned correctly with the criterion that a genotype could be assigned if it was 1000-times more likely to be in a given taxon than in any other (log3). Furthermore, the use of the optimal combinations for assignment from 1 to 8 loci allowed us to determine that high success may still be obtained when reducing the number of loci analysed. An obvious advantage of such a procedure is to reduce both cost and time required to realize such studies. When applying the most commonly used criterion of log0, more than 95% assignment success could still be obtained with only four loci. However, given the possible uncertainties as to whether the assumptions required to reliably apply assignment tests are always met, it may sometimes be a risk to rely on the most relaxed criterion to perform such analysis. For instance, the assignment procedure does not take into account the possibility of differential population size among groups. The probability or likelihood that a sampled genotype originates from a given group vs. other groups is not only a function of its probability of occurrence within each group, but also of the relative population sizes of the different groups. Not considering differences in relative population size may lead to misclassifying genotypes to the less abundant groups at the expense of more abundant ones. For instance, a given multilocus genotype may be 10 times more likely in a given group based on its allelic composition, but if that group is 100-times less abundant than a second one, it would be overall 10-times more likely in nature that this genotype belongs to the second group. Although it may often be impossible to precisely know the discrepancy in population size among groups studied, the stringency of threshold procedures can be adjusted to approximate its effect, based on the best knowledge available, thus

reducing potential assignment biases. High threshold levels may also be required in situations where correct assignment is critical, such as in forensics applications (e.g. Shriver *et al*. 1997).

Another observation of this study was that the precision of assignment obtained by the empirical method became lower relative to the simulated one as the stringency level increased (Fig. 2). The contrast between both methods was more stricking when comparing populations within taxa, which was probably related to the lack of power of our set of loci for population assignment within *Sebastes* taxa. Thus, assignment success of both empirical and simulated methods dropped rapidly at all levels of stringency above log0. Also, increased assignment success was obtained with a reduced number of loci with the empirical method. This observation corrobates previous theoretical investigations which showed that adding less segregating loci, generally those with large numbers of low frequency alleles, may actually increase noise rather than power (discussed in Smouse *et al*. 1982; Smouse & Chevillon 1998).

Advantages and limitations of the empirical and simulated methods

The main advantage of the simulation approach lies in the potential of generating an almost unlimited number of individuals (multilocus genotypes). By increasing the number of simulated individuals, one can thus better cover the set of all possible multilocus genotypes in a given group, thus potentially improving the precision of the probability of correct assignment. On the other hand, this method uses the allelic composition of source populations as if sampling errors simply did not exist. One can foresee two consequences of this. First, the simulated genotypes are not exactly distributed according to the real allelic distributions. The second and potentially worse consequence is that the success is very likely to be overestimated by assuming complete knowledge of allelic distributions. Sampling errors can thus have a dramatic effect on the real power of the classification procedure, especially in situations where populations are characterized by many, low-frequency alleles. Suppose that an allele has a sampled frequency (x) of 0.02 but has a true frequency (P) of 0.01. Every time this allele appears within a genotype, the above sampling error has the same impact on the calculation of its likelihood, as if $x = 0.8$ and $P = 0.4$, because the ratio $x/P = 2$ in both cases. However, the latter sampling error is obviously much less likely than the former.

Contrary to the simulated approach, the empirical method generally explores a very restricted portion of the genotypic space (e.g. Paetkau *et al*. 1995). On the other hand, this procedure removes the individual to be assigned from the gene pool of the source population, and consequently eliminates the bias that favours correct allocation. It is thus a more conservative procedure than the simulated method. Considering both the advantages and limitations, the simulation method will probably be the most useful in situations where the groups studied are well differentiated by high allelic frequency differences. When this is not the case, the use of the empirical method will probably be safer. This, however, remains to be explored in further empirical and theoretical investigations.

Acknowledgements

We are grateful to Jean-Marie Sévigny, E. Parent and G. Naevdal for providing tissue samples, Sylvain Martin for technical assistance, and Jean-Marie Cornuet (INRA, France) for kindly providing the program to calculate D_{AS} distance and construct NJ trees on individuals. The manuscript has also been improved by the constructive comments of Per Palsböll and two anonymous reviewers. This study was financially supported by the Department of Fisheries and Oceans (Canada) and a NSERC (Canada) grant to L.B. Contribution to the research programs of GIROQ (Groupe Interuniversitaire de Recherche Océanographique du Québec).

References

Barsukov VV (1968) The systematics relationship of redfishes of the genus Sebastes of the Northwest Atlantic ocean. *Doklady Akad. Nauk. SSSR*, **183**, 479–482.

Barsukov VV (1972) Systematics of the Atlantic redfishes. *Trudy PINRO*, **28**, 128–142.

Bentzen P, Taggart CT, Ruzzante DE, Cook D (1996) Microsatellite polymorphism and the population structure of Atlantic cod (*Gadus morhua*) in the northwest Atlantic. *Canadian Journal of Fisheries and Aquatic Science*, **53**, 2706–2721.

Bentzen P, Wright JM, Bryden LT *et al*. (1998) Tandem repeat polymorphism and heteroplasmy in the mitochondrial control region of red fishes (*Sebastes*: Scorpaenidae). *Journal of Heredity*, **89**, 1–7.

Bossart JL, Pashley Prowell D (1998) Genetic estimates of population structure and gene flow: limitations, lessons and new directions. *Trends in Ecology and Evolution*, **13**, 202–206.

Bowcock AM, Ruiz-Linares A, Tomfohrd J *et al.* (1994) High resolution of human evolutionary trees with polymorphic microsatellites. *Nature*, **368**, 455–457.

Chakraborty R, Jin L (1993) A unified approach to study hypervariable polymorphisms: Statistical considerations of determining relatedness and population distances. In: *DNA Fingerprinting: State and the Science* (eds Pena SDJ, Chakraborty R, Epplen JT, Jeffreys AJ), pp. 153–175. Birkhäuser Verlag, Basel, Switzerland.

Cornuet JM, Aulagnier S, Lek S, Franck P, Solignac M (1996) Classifying individuals among infra-specific taxa using microsatellite data and neural networks. *Conseil de Recherche*

Académique Des Sciences de Paris, Sciences de la Vie, **319**, 1167–1177.

Dempster AP, Laird NM, Rubin DB (1977) Maximum likelihood from incomplete data via the EM algorithm. *Journal of the Royal Statistics Society B*, **39**, 1–38.

Ellegren H, Savolainen P, Rosen B (1996) The genetical history of an isolated population of the endangered grey wolf *Canis lupus*: a study of nuclear and mitochondrial polymorphisms. *Philosophical Transactions of the Royal Society of London B*, **351**, 1661–1669.

Estoup A, Angers B (1998) Microsatellite and minisatellites for molecular ecology: theoretical and experimental considerations. In: *Advances in Molecular Ecology* (ed. Carvhalo GR), pp. 55–86. IOS Press, The Netherlands.

Estoup A, Garnery L, Solignac M, Cornuet JM (1995) Microsatellite variation in honey bee (*Apis mellifera* L.) populations: hierarchical genetic structure and test of the infinite allele and stepwise mutation models. *Genetics*, **140**, 679–695.

de García León FJ, Dallas JF, Chatain B, Canonne M, Versini JJ, Bonhomme F (1995) Development and use of microsatellite markers in sea bass, *Dicentrarchus labrax* (Linnaeus, 1758) (Perciformes: Serrandidae). *Molecular Marine Biology and Biotechnology*, **4**, 62–68.

Guo SW, Thompson EA (1992) Performing the exact test of Hardy–Weinberg proportion for multiple alleles. *Biometrics*, **48**, 361–372.

Jarne PJ, Lagoda JL (1996) Microsatellites, from molecules to populations and back. *Trends in Ecology and Evolution*, **11**, 424–429.

Jones AG, Östlund-Nilsson S, Avise JC (1998) A microsatellite assessment of sneaked fertilizations and eggs thievery in the fifteenspines stickelback. *Evolution*, **52**, 848–858.

Johansen T, Nedreaas K, Naevdal G (1993) Electrophoretic discrimination of blue mouth *Helicolenus* dactylopterus (De La Roche, 1809), from *Sebastes* spp. in the Northeast Atlantic. *Sarsia*, **78**, 25–29.

Kellogg KA, Markert JA, Stauffer JRJ, Kocher TD (1995) Microsatellite variation demonstrates multiple paternity in lekking cichlid fishes from Lake Malawi, Africa. *Proceedings of the Royal Society of London B*, **260**, 79–84.

Michalakis Y, Excoffier L (1996) A generic estimation of population subdivision using distances between alleles with special interest to microsatellite loci. *Genetics*, **142**, 1061–1064.

Nedreaas K, Naevdal G (1991) Identification of 0- and 1-group redfish (genus *Sebastes*) using electrophoresis. *ICES Journal of Marine Sciences*, **48**, 91–99.

Nedreaas K, Johansen T, Naevdal G (1994) Genetic studies of redfish (*Sebastes* spp.) from Icelandic and Greenland waters. *ICES Journal of Marine Sciences*, **51**, 461–467.

Nefyodov GN (1971) Serum haptoglobins in the *Marinus* and mentella types of North Atlantic redfish. *Rapport P.-V. Reunion du Conseil International du l'Exploration de la Mer*, **161**, 126–129.

Ni IH (1981) Separation of the sharp-beaked redfish, *S. fasciatus* and *S. mentella*, from Northeastern Grand Bank by morphology of extrinsic gasbladder musculature. *Journal of the Northwest Atlantic Fisheries Sciences*, **2**, 7–12.

Ni IH (1982) Meristic variation in beaked redfishes, *Sebastes mentella* and *S. fasciatus*, in the Northwest Atlantic. *Canadian Journal of Fisheries and Aquatic Sciences*, **39**, 1664–1685.

Paetkau D (1998) Gene flow between insular, coastal and interior populations of brown bears in Alaska. *Molecular Ecology*, **7**, 1283–1292.

Paetkau D, Calvert W, Stirling I, Strobeck C (1995) Microsatellite analysis of population structure in Canadian polar bears. *Molecular Ecology*, **4**, 347–354.

Payne RH, Ni IH (1982) Biochemical population genetics of redfishes (*Sebastes*) off Newfounland. *Journal of the Northwest Atlantic Fisheries Sciences*, **3**, 169–172.

Power DJ, Ni IH (1985) Morphometric differences between Golden redfish (*Sebastes marinus*) and beaked redfishes (*S. mentella* and *S. fasciatus*). *Journal of the Northwest Atlantic Fisheries Sciences*, **6**, 1–7.

Queller DC, Strassmann JE, Hugues CR (1993) Microsatellites and kinship. *Trends in Ecology and Evolution*, **8**, 285–288.

Raymond M, Rousset F (1995) GENEPOP (Version 1.2): Population genetics software for exact test and ecumenism. *Journal of Heredity*, **86**, 248–249.

Rice WR (1989) Analysing tables of statistical tests. *Evolution*, **43**, 223–225.

Rico C, Ibrahim KM, Rico I, Hewitt GM (1997) Stock composition in North Atlantic populations of whiting, *Merlangius merlangus*, L., using microsatellite markers. *Journal of Fish Biology*, **51**, 462–475.

Roques S, Pallotta D, Sévigny JM, Bernatchez L (1999) Isolation and characterisation of polymorphic microsatellite markers in the North Atlantic redfish (Teleostei: Scorpaenidae, genus *Sebastes*). *Molecular Ecology*, **8**, 685–686.

Rubec PJ, McGlade JM, Trottier BL *et al.* (1991) Evaluation for methods for separation of Gulf of Saint-Lawrence beaked redfishes, *Sebastes fasciatus* and *S. mentella*: malate dehydrogenase mobility patterns compared with extrinsic gasbladder muscle passages and anal fin ray counts. *Canadian Journal of Fisheries and Aquatic Sciences*, **48**, 640–660.

Ruzzante DE, Taggart CT, Cook D, Goddard S (1996) Genetic differentiation between inshore and offshore Atlantic cod (*Gadus morhua*) of Newfoundland: microsatellite DNA variation and antifreeze level. *Canadian Journal of Fisheries and Aquatic Sciences*, **53**, 634–645.

Ruzzante DE, Taggart CT, Cook D (1998) Genetic differentiation between inshore and offshore Atlantic cod (*Gadus morhua*) of Newfoundland: a test of evidence of temporal stability. *Canadian Journal of Fisheries and Aquatic Sciences*, **54**, 2700–2708.

San Cristobal M, Chevalet C (1995) Efficiency of highly polymorphic codominant markers in parent identification. Laboratoire de Génétique Cellulaire. Institut National de la Recherche Agronomique, France, 1–29.

Smouse PE, Chevillon C (1998) Analytical aspects of population-specific DNA fingerprint for individuals. *Journal of Heredity*, **89**, 143–150.

Smouse PE, Spielman RS, Park MH (1982) Multiple-locus allocation of individuals to groups as a function of the genetic variation within and differences among human populations. *The American Naturalist*, **119**, 445–463.

Schneider S, Kueffer JM, Roessli D, Excoffier L (1997) ARLEQUIN, Version 1: an exploratory population genetics software environment. Genetics and Biometry Laboratory, University of Geneva, Geneva, Switzerland.

Shriver MO, Smith MW, Jin L, *et al.* (1997) Ethnic affiliation

estimation by use of population-specific DNA. *American Journal of Human Genetics*, **60**, 957–964.

Walsh PS, Metzger DA, Higuchi R (1991) Chelex 100 as a medium for simple extraction of DNA for PCR-based typing for forensic material. *Biotechniques*, **10**, 506–513.

Waser P, Strobeck C (1998) Genetic signatures of interpopulation dispersal. *Trends in Ecology and Evolution*, **13**, 43–44.

Weir BS, Cockerham CC (1984) Estimating *F*-statistics for the analysis of population structure. *Evolution*, **38**, 1358–1370.

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.B. are in the understanding of the patterns and processes of molecular and organismal evolution, as well as their significance to conservation. P.D. works in L.B.'s laboratory and is responsible for developing analytical tools for population genetics studies.