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Allozyme variation and genetic divergence in the sand goby, *Pomatoschistus minutus* (Teleostei: Gobiidae)

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Samples of the widely distributed sand goby *Pomatoschistus minutus* have been investigated genetically from ten localities in the north-eastern Atlantic, North Sea, western Mediterranean and Adriatic Sea. Levels of genetic diversity and differentiation were assessed with starch (SGE) and cellulose acetate (CAGE) gel electrophoresis for 13 enzyme systems. Genetic differentiation between spatial samples points to a reduction or even absence of gene flow between the Adriatic and the other samples, including the western Mediterranean Sea (pair-wise F_{ST}=0.37 and 0.32 for SGE and CAGE respectively). The sample from the Adriatic Sea was clearly differentiated from the other samples at the lactate dehydrogenase loci *LDH-A** (SGE and CAGE) and *LDH-C** (CAGE). Values for genetic differentiation between Venetian and other sand gobies were of the same order of magnitude as between *P. minutus* and its closest relative *P. lozanoi*, suggesting allopatric speciation in the lagoon of Venice. At locations outside the Adriatic Sea, the sand goby has the typical features of a marine fish with a high level of gene flow and a low degree of genetic differentiation.

INTRODUCTION

The sand goby *Pomatoschistus minutus* (Pallas, 1770) is one of the most abundant and widespread *Pomatoschistus* species in the north-eastern Atlantic. Its distributional range extends from the north of Norway (Tromsø) and the Faroe Islands, along the coasts of western Europe and the British Isles, the Baltic Sea, the Mediterranean Sea and the Black Sea (Miller, 1986). Although not commercially exploited, its ecological importance can hardly be overestimated as it plays a major role in coastal ecosystems.

An anonymous researcher (as reported by Miller, 1986) proposed geographically distinct subspecies, *Pomatoschistus minutus elongatus* (Canestrini, 1861) for the Mediterranean and Black Sea and *Pomatoschistus minutus minutus* for the Atlantic Ocean, morphologically distinguished by a dark chin spot in the females and breast pigmentation in both sexes. Whether this morphological differentiation is the result of phenotypic plasticity, or the result of a genuine reproductive isolation is unknown. Wallis & Beardmore (1984) carried out an extensive allozyme survey of the genus *Pomatoschistus* but only to confirm the systematic relationships between the species. They reported, however, clear-cut allozymatic differences between *P. minutus* from the lagoon of Venice and the Atlantic Ocean (Wallis & Beardmore, 1983), which were confirmed by Stefanni et al. (1996).

It has been argued that cellulose acetate gel electrophoresis (CAGE) has a lower resolution than starch gel electrophoresis (SGE), but other studies have proven a similar resolving power (Richardson et al., 1986). Therefore we took the opportunity to combine the results from two independent allozyme surveys to assess the power of the respective techniques. Although different individuals were screened per sampling site, and thus no strict comparison can be made *in se*, congruence in overall results for both techniques would reinforce any conclusions on the population genetic structure in the sand goby.

In the present work we have compared 13 enzyme systems of *Pomatoschistus minutus* from the north-eastern Atlantic, the North Sea, the western Mediterranean Sea and the Adriatic Sea, to address the following questions: (1) to which extent are the observed differences between geographical populations the result of restricted gene flow rather than phenotypic plasticity? (2) Is there any genetic basis for assigning subspecies status to the Mediterranean *P. minutus (elongatus)*? (3) What is the position of the Venetian *P. minutus*?

MATERIALS AND METHODS

Samples were collected by beam trawl, hand net or bought fresh from local fish markets, frozen for transport and stored at either −50°C or −80°C. Individuals were identified morphologically by the papilla pattern on the head (Miller, 1986) and biochemically according to Wallis & Beardmore (1984). Six hundred and one specimens of *Pomatoschistus minutus* from ten European localities were subjected to allozyme electrophoresis (Figure 1, Appendix I and II). At each locus allele mobility was calculated with reference to the most common allele, which was assigned a mobility of 100.

Allozyme electrophoresis

In total 601 specimens of *Pomatoschistus minutus* from ten localities were subjected to either horizontal SGE or CAGE.

Figure 1. Sampling sites of *Pomatoschistus minutus*.

The SGE samples were obtained from the following sites: Bergen (Ber: Atlantic Ocean, Norway), Ameland (Ame: Wadden Sea, the Netherlands), Oban (Oba: Atlantic Ocean, UK), Plymouth (Ply: English Channel, UK), Mauguio (Mau: western Mediterranean Sea, France) and Venice (Ven: Adriatic Sea, Italy) (Figure 1). Continuous tris-citrate electrophoresis at pH 8.0 was performed accordingly at 150–160 V and 45 mA for 3.5–4 h at 4°C. Eleven enzyme systems were screened (LDH, PGM, GPI, MDH, ME, AK, CK, G6PDH, SOD, EST, IDHP), corresponding to 22 loci.

The CAGE samples from the following localities were screened: Bergen, Oban, Texel (Tex: Wadden Sea, The Netherlands), Frisian Front (FrF: North Sea), Ostend (Ost: North Sea, Belgium), Pérols (Per: western Mediterranean Sea, France) and Venice (Figure 1). Cellulose acetate gel electrophoresis was carried out according to Richardson et al. (1986). Nine enzymes were assayed (AK, CK, LDH, MDH, IDHP, GPI, PGM, AAT, FH), corresponding to 15 loci. Run time varied between 25 and 40 min and was carried out at 220 V and 400 mA at room temperature. Two buffer systems, tris-maleate at pH 7.8 and tris-glycine at pH 8.8, were used. A sample of *P. lozanoi* from the Belgian coast was also subjected to CAGE and used as an outgroup in the analysis.

Statistical analyses

Genetic diversity, the mean number of alleles per locus, the proportion of polymorphic loci (P), Nei's observed (H_o) and unbiased expected (H_e) heterozygosity, were calculated with the program GENETIX version 4.02 (Belkhir et al., 1996–2001). A locus was considered polymorphic when the frequency of its most common allele did not exceed 0.99. Hardy–Weinberg equilibrium (HWE) and linkage disequilibrium were calculated in the program GENEPOP version 3.1b (Raymond & Rousset, 1995). A sequential Bonferroni test was applied to correct significance levels for multiple testing for avoiding type I errors. Estimators of F-statistics were calculated according to Weir & Cockerham (1984) using GENETIX and tested for significance with permutation tests (1000 replicates). Isolation by distance was assessed with a Mantel test in GENETIX. Geographical distances were measured as the shortest coastal distances between sites.

RESULTS

Allele frequencies, HWE and linkage disequilibrium

Values for genetic diversity are presented in Appendix I (SGE) and II (CAGE).

SGE: fifteen out of 22 loci were monomorphic. The lowest level of observed heterozygosity was recorded in the sample from Oban (0.025), the highest value in Mauguio (0.162). Mean number of alleles was 1.43; the highest value (2.00) occurred in the sample from Venice. Tests for HWE revealed significant deviations for locus *PGM-1** in the sample from Venice only. Global tests for linkage disequilibrium between pairs of loci across all sample localities were significant for the combination *PGM-1** and *PGM-2** ($P < 0.01$) in the samples from Mauguio and Bergen.

CAGE: twelve loci out of 15 were polymorphic. Observed heterozygosity was highest in the samples from Bergen and Pérols, while the lowest value was recorded in Oban. Mean number of alleles varied from 1.53 (Oban) to 1.8 (Venice). A significant heterozygote deficit was recorded at *LDH-C** in all samples except the Venetian *Pomatoschistus minutus*. Two significant cases ($P < 0.05$) of linkage disequilibrium were observed: *LDH-C*–PGM-2** (Pérols) and *LDH-C*–PGM-1** (Texel).

Genetic structure

The SGE and CAGE reveal the highest degree of genetic differentiation between the Venetian sample and all the others, including the western Mediterranean. The CAGE results show that this differentiation is of the same order of magnitude as between *P. minutus* and its closest relative *P. lozanoi* (Table 1). Similarly, both CAGE and SGE show little differentiation between the western Mediterranean and the southern North Sea.

Differences in results between both methods relate to the samples from Bergen and Oban. The sample from Bergen is differentiated from all the others using SGE, contrary to CAGE results. However, CAGE shows that the sample from Bergen possesses a unique allele at locus *PGM-1**, albeit in a low frequency (0.02%), and differs also at locus *LDH-C**, with the fast-moving allele *LDH-C*115* being underrepresented compared to all the other samples. The sample from Oban is not differentiated from the others using CAGE, except for the Mediterranean *P. minutus*. The SGE shows that Oban is significantly differentiated from all the others, but due to allele frequency differences at only one locus, *PGM-2** (Appendix I). Allele frequencies at *PGM-2** were also different for CAGE, but due to the low amount of individuals stained (10 fish), *PGM-2** was excluded from further analysis of CAGE data.

Table 1. *Pair-wise estimates of F_{ST} (Weir & Cockerham, 1984), calculated over all loci for all populations. Significance: *, P<0.05; **, P<0.01 and ***, P<0.001 are based on 1000 permutations. Values above diagonal are SGE data, below diagonal are CAGE data.* Pl *refers to the sample of* Pomatoschistus lozanoi.

	Ber	Oba	Ply	Ame	Tex	Ost	FrF	Per	Mau	Ven	*Pl*
Ber	0.000	0.010	0.276***	0.154***	–	–	–	–	0.193***	0.533***	–
Oba	−0.003	0.000	0.268***	0.141***	–	–	–	–	0.187***	0.534***	–
Ply	–	–	0.000	0.014	–	–	–	–	0.007	0.574***	–
Ame	–	–	–	0.000	–	–	–	–	−0.005	0.524***	–
Tex	−0.004	−0.005	–	–	0.000	–	–	–	–	–	–
Ost	0.023	0.007	–	–	−0.002	0.000	–	–			–
FrF	0.035	0.017	–	–	0.004	−0.036	0.000	–	–	–	–
Per	0.077**	0.057***	–	–	0.037**	0.005	0.000	0.000	–	0.550***	–
Mau	–	–	–	–	–	–	–	–	0.000	–	–
Ven	0.697***	0.672***		–	0.651***	0.608***	0.597***	0.582***	–	0.000	–
Pl	0.799***		–	–	0.784***	0.770***	0.768***	0.781***	–	0.870***	0.000

Ber, Bergen; Oba, Oban; Ply, Plymouth; Ame, Ameland; Tex, Texel; Ost, Ostend; FrF, Frisian Front; Per, Pérols; Mau, Mauguio; Ven, Venice—see text for full description of sites.

Isozyme patterns of lactate dehydrogenase

Allele frequencies at the *LDH** loci distinguish the Venetian *Pomatoschistus minutus* from all the others. *LDH** patterns from SGE for the Venetian sample are characterized by unique alleles at two loci: *LDH-A*110* and *LDH-B*105* (Appendix I). The CAGE reveals that allele *LDH-A*135*, occurring only rarely in the other sand goby samples (frequencies from 0 to 0.026), dominates in the Venetian sample (frequency of 0.898) (Appendix II). Allele *LDH-C*100* was not recorded in the Venetian sample but is the most common allele in all other samples (CAGE). Referring to Wallis & Beardmore (1983), we found no indications of a fourth locus, the so-called *'LDH-0*'* in the Venetian sample.

DISCUSSION

Comparison between CAGE and SGE

The results of two different techniques lead to very similar conclusions about the genetic structure of *Pomatoschistus minutus*. Both techniques clearly detected the distinctness of the Venetian population of *P. minutus* while no major differences between the samples from the Atlantic Ocean, the North Sea and the western Mediterranean Sea were recorded.

Genetic diversity

Overall heterozygosity values fit the typical values for marine species, reflecting larger effective population sizes compared to freshwater species. Compared to SGE, CAGE detects more (rare) alleles per locus in all samples. At *LDH-C** little polymorphism was reported using SGE (Wallis & Beardmore, 1984; Stefanni et al., 1996) but with CAGE three alleles were detected. A possible explanation for this discrepancy could be the weak response to staining of this locus on SGE. The significant heterozygote deficit at *LDH-C** in the CAGE results may be due to various reasons such as the Wahlund effect, but then we should notice the same deficit at the other polymorphic loci. Another possibility could be the occurrence of null alleles (Richardson et al., 1986). Selective mortality of heterozygotes has also been recorded, but detailed biochemical studies would be required to support this. Nevertheless, global results on degree of population differentiation remain similar with or without including *LDH-C**.

Genetic structure and gene flow among sand gobies

Both CAGE and SGE show congruence in the following observations: (1) the distinctness of the Venetian sample; (2) the similarity between Atlantic and western Mediterranean *P. minutus*; and (3) a high level of gene flow within the Atlantic basin.

The status of the Venetian population: allopatric speciation or natural selection?

The distinctness of the Venetian *Pomatoschistus minutus* at *LDH-A** has been reported by Wallis & Beardmore (1983). They noticed that the distinct allele for *P. minutus* in the Venice lagoon at *LDH-A** showed the same electrophoretic mobility as in the related estuarine species *P. microps* and suggested this distinct allele being an adaptive response to environmental pressures in a variable, enclosed environment. Another argument in favour of the hypothesis of natural selection is that natural selection acts on specific loci, while in the case of a genuine reproductive isolation, genetic drift should affect all loci similarly. However, comparing pair-wise F_{ST} values between the Venetian sample and all the others, we notice that these are of the same order of magnitude as between *P. minutus* and its closest relative, *P. lozanoi*, suggesting limited or probably absent gene flow with the western Mediterranean (Table 1). Moreover, results of sequence analysis of mtDNA also indicate a significant differentiation on the D-loop (Stefanni & Thorley, 2003) between the Venetian and all the other samples. While we do not argue the fact that natural selection may be operating on *LDH-A**, we are inclined to support the existence of reproductive isolation between the Venetian sample and the others. The large degree of differentiation between the Venetian *P. minutus* and the others raises questions about the taxonomic status of the Venetian (Adriatic) population. Consequently, we

suggest that the taxonomic status of the Venetian *P. minutus* should be reconsidered. However, more detailed studies are needed for final conclusions on the status of this population.

Whether the Venetian sand goby is reproductively isolated from the rest of the Adriatic *Pomatoschistus minutus* is unsure. *Pomatoschistus minutus* is not permanently present in the Venetian lagoon; it migrates towards the sea in winter, and probably carries out a spawning migration towards the sea in the spring as well, as described for all other *P. minutus*. Therefore, gene flow between the Venetian lagoon and the Adriatic Sea is not unlikely. A similar isolation of Adriatic populations has been reported in other fish species. This has been attributed to the presence of a large cyclonic gyre in the South Adriatic, (partially) isolating the northern Adriatic from the rest of the Mediterranean Sea, thus enhancing genetic differentiation in the northern Adriatic Sea (Magoulas et al., 1996). Alternatively, the species might be isolated due to the higher temperatures in the southern part of the Adriatic Sea. *Pomatoschistus minutus* seems to occur mainly along the northern Mediterranean coasts (Miller, 1986); being a boreal species, its occurrence might be constrained by higher water temperatures.

Gene flow of Pomatoschistus minutus *throughout its distributional range*

The low level of genetic differentiation between *P. minutus* from the Atlantic, North Sea and western Mediterranean suggests that gene flow, drift and selection are balancing each other across the natural range. Adults of *P. minutus* probably have a small range of movement, migrations being limited to spawning and (in northern areas) thermal migrations. Thus, any large-scale gene flow probably depends mainly on the planktonic larval stage. The small pair-wise F_{ST} values between the samples from southern France, the English Channel, and the Wadden Sea, as observed with CAGE and SGE, point to a high degree of gene flow between these basins. Thus, our data do not support the proposed differentiation in the two subspecies *P. minutus minutus* in the Atlantic Ocean and *P. minutus elongatus* in the Mediterranean Sea.

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Appendix I. *Number of individuals screened, allele frequencies, observed (H_o) and non-biased expected heterozygosity (H_e) and Hardy–Weinberg equilibrium (HWE) for* Pomatoschistus minutus *based on SGE results.*

		SGE samples					
Locus	Allele	Ply	Ven	Mau	Ame	Ber	Oba
*GPI-2**	**105*	0.000	0.038	0.000	0.000	0.000	0.000
	**100*	1.000	0.962	1.000	1.000	1.000	1.000
N		40	40	30	40	40	40
H_e		0.000	0.073	0.000	0.000	0.000	0.000
H_o		0.000	0.075	0.000	0.000	0.000	0.000
HWE		–	n.s.	–	–	–	–
*IDHP-2**	*105	0.013	0.000	0.000	0.000	0.000	0.000
	**100*	0.988	1.000	1.000	1.000	1.000	1.000
N		40	40	30	40	40	40
H_e		0.025	0.000	0.000	0.000	0.000	0.000
H_o		0.025	0.000	0.000	0.000	0.000	0.000
HWE		n.s.	–	–	–	–	–
*LDH-A**	**110*	0.000	0.813	0.000	0.000	0.000	0.000
	**100*	1.000	0.188	1.000	1.000	1.000	1.000
N		40	40	30	40	40	40
H_e		0.000	0.309	0.000	0.000	0.000	0.000
H_o		0.000	0.375	0.000	0.000	0.000	0.000
HWE		–	n.s.	–	–	–	–
*LDH-B**	**105*	0.000	0.013	0.000	0.000	0.000	0.000
	**100*	1.000	0.988	1.000	1.000	1.000	1.000
N		40	40	30	40	40	40
H_e		0.000	0.000	0.000	0.000	0.000	0.000
H_o		0.000	0.000	0.000	0.000	0.000	0.000
HWE		–	n.s.	–	–	–	–
*MDH-1**	**107*	0.000	0.000	0.017	0.000	0.000	0.000
	**100*	1.000	1.000	0.983	1.000	1.000	1.000
N		40	40	30	40	40	40
H_e		0.000	0.000	0.033	0.000	0.000	0.000
H_o		0.000	0.000	0.033	0.000	0.000	0.000
HWE		–	–	n.s.	–	–	–
*PGM-1**	**100*	0.875	0.850	0.900	0.838	0.900	0.925
	**96*	0.125	0.038	0.100	0.163	0.000	0.075
	**93*	0.000	0.113	0.000	0.000	0.100	0.000
N		40	40	30	40	40	40
H_e		0.222	0.267	0.183	0.276	0.182	0.141
H_o		0.200	0.200	0.133	0.275	0.200	0.150
HWE		n.s.	$P<0.05$	n.s.	n.s.	n.s.	n.s.
*PGM-2**	**102*	0.588	0.013	0.483	0.425	0.100	0.063
	**100*	0.388	0.975	0.517	0.525	0.850	0.813
	**98*	0.025	0.013	0.000	0.050	0.050	0.125
N		40	40	30	40	40	40
H_e		0.510	0.048	0.508	0.548	0.268	0.324
H_o		0.300	0.050	0.967	0.300	0.250	0.025
HWE		$P<0.05$	n.s.	$P<0.001$	$P<0.01$	n.s.	$P<0.001$
$P_{0.99}$		0.429	0.714	0.429	0.286	0.286	0.286
MNA		1.57	2.00	1.43	1.43	1.43	1.43
Mean H_e		0.108	0.103	0.104	0.118	0.064	0.066
Mean H_o		0.075	0.104	0.162	0.082	0.064	0.025
Overall HWE		$P<0.05$	n.s.	$P<0.001$	$P<0.05$	n.s.	$P<0.001$

Following loci are fixed for the same allele: *AK**, *CK-A**, *G6PDH**, *LDH-C**, *MDH-2**, *GPI-1**. MNA, to mean number of alleles; N, number of fish screened; n.s, not significant; $P_{0.99}$, proportion of polymorphic loci. Ply, Plymouth; Ven, Venice; Mau, Mauguio; Ame, Ameland; Ber, Bergen; Oba, Oban—see text for full description of sites.

Appendix II. *Number of individuals screened, allele frequencies, observed (H_o) and non-biased expected heterozygosity (H_e) with standard deviation between parentheses and Hardy–Weinberg equilibrium (HWE), based on CAGE results.*

		CAGE samples						
Locus	Allele	Ost	Ber	Frf	Tex	Oba	Ven	Per
*AAT**	*100	1.000	1.000	1.000	1.000	1.000	0.938	0.993
	*150	0.000	0.000	0.000	0.000	0.000	0.062	0.007
N		47	47	23	35	55	24	68
H_e		0.000	0.000	0.000	0.000	0.000	0.120	0.015
H_o		0.000	0.000	0.000	0.000	0.000	0.125	0.015
HWE		–	–	–	–	–	n.s.	–
*GPI-2**	*125	0.000	0.009	0.000	0.000	0.000	0.000	0.000
	*116	0.010	0.051	0.000	0.014	0.000	0.021	0.000
	*100	0.990	0.941	1.000	0.986	1.000	0.979	0.993
	*90	0.000	0.000	0.000	0.000	0.000	0.000	0.007
N		51	59	35	36	64	47	68
H_e		0.020	0.113	0.000	0.028	0.000	0.042	0.015
H_o		0.020	0.119	0.000	0.028	0.000	0.043	0.015
HWE		–	n.s.	–	–	–	–	n.s.
*IDPH-2**	*115	0.000	0.000	0.000	0.000	0.036	0.000	0.000
	*100	1.000	1.000	0.917	1.000	0.964	1.000	1.000
	*86	0.000	0.000	0.083	0.000	0.000	0.000	0.000
N		3	49	12	29	14	30	19
H_e		0.000	0.000	0.159	0.000	0.071	0.000	0.000
H_o		0.000	0.000	0.000	0.000	0.071	0.000	0.000
*LDH-A**	*135	0.000	0.000	0.014	0.014	0.008	0.898	0.026
	*100	0.990	1.000	0.972	0.986	0.992	0.092	0.974
	*60	0.010	0.000	0.014	0.000	0.000	0.010	0.000
N		51	53	36	36	61	49	58
H_e		0.020	0.000	0.055	0.028	0.016	0.187	0.051
H_o		0.020	0.000	0.056	0.028	0.016	0.163	0.052
HWE		–	–	n.s.	–	–	n.s.	n.s.
*LDH-B**	*112	0.020	0.000	0.000	0.014	0.008	0.000	0.009
	*100	0.980	1.000	1.000	0.986	0.992	1.000	0.991
N		51	45	27	36	61	49	58
H_e		0.039	0.000	0.000	0.028	0.016	0.000	0.017
H_o		0.039	0.000	0.000	0.028	0.016	0.000	0.017
HWE		n.s.	–	–	–	–	–	–
*LDH-C**	*120	0.000	0.000	0.000	0.000	0.016	0.000	0.000
	*115	0.256	0.091	0.271	0.139	0.139	0.978	0.366
	*107	0.167	0.136	0.188	0.194	0.115	0.022	0.152
	*100	0.578	0.773	0.542	0.667	0.721	0.000	0.482
	*90	0.000	0.000	0.000	0.000	0.008	0.000	0.000
N		51	22	24	36	61	46	56
H_e		0.578	0.385	0.611	0.506	0.451	0.043	0.616
H_o		0.373	0.227	0.292	0.333	0.344	0.044	0.446
HWE		$P<0.05$	$P<0.05$	$P<0.05$	$P<0.05$	$P<0.05$	n.s.	$P<0.05$
*MDH-1**	*140	0.000	0.000	0.014	0.014	0.000	0.000	0.000
	*100	0.990	1.000	0.986	0.986	1.000	1.000	1.000
	*60	0.010	0.000	0.000	0.000	0.000	0.000	0.000
N		52	56	36	36	64	42	68
H_e		0.019	0.000	0.028	0.028	0.000	0.000	0.000
H_o		0.019	0.000	0.028	0.028	0.000	0.000	0.000
HWE		–	–	–	–	–	–	–
*MDH-2**	*100	1.000	1.000	1.000	1.000	1.000	1.000	0.985
	*60	0.000	0.000	0.000	0.000	0.000	0.000	0.015
N		52	51	36	36	64	42	68
H_e		0.000	0.000	0.000	0.000	0.000	0.000	0.029
H_o		0.000	0.000	0.000	0.000	0.000	0.000	0.029
HWE		–	–	–	–	–	–	n.s

(*continued overleaf*)

Appendix II. *(Continued)*.

		CAGE samples						
Locus	Allele	Ost	Ber	Frf	Tex	Oba	Ven	Per
*PGM-1**	**114*	0.020	0.034	0.027	0.015	0.000	0.022	0.008
	**100*	0.857	0.839	0.838	0.882	0.892	0.848	0.918
	**86*	0.122	0.102	0.135	0.103	0.108	0.130	0.075
	**73*	0.000	0.025	0.000	0.000	0.000	0.000	0.000
N		49	59	37	34	60	46	67
H_e		0.253	0.286	0.283	0.214	0.195	0.266	0.153
H_o		0.184	0.322	0.270	0.177	0.150	0.304	0.164
HWE		n.s.	n.s.	$P<0.05$	n.s.	n.s.	n.s.	n.s.
*PGM-2**	**110*	0.000	0.019	0.000	0.000	0.000	0.000	0.000
	**106*	0.379	0.472	0.625	0.523	0.000	0.034	0.268
	**103*	0.017	0.009	0.000	0.000	0.000	0.000	0.049
	**100*	0.466	0.444	0.292	0.409	1.000	0.852	0.561
	**90*	0.138	0.056	0.083	0.068	0.000	0.114	0.122
N		29	54	12	22	10	44	41
H_e		0.631	0.581	0.540	0.568	0.000	0.263	0.603
H_o		0.552	0.593	0.250	0.409	0.000	0.227	0.488
HWE		n.s.	n.s.	$P<0.05$	n.s.	–	n.s.	n.s.
$P_{0.99}$		0.267	0.267	0.400	0.467	0.200	0.400	0.333
Mean H_e		0.104 (0.213)	0.091 (0.181)	0.112 (0.205)	0.093 (0.188)	0.050 (0.122)	0.061 (0.099)	0.010 (0.211)
Mean H_o		0.080 (0.165)	0.084 (0.172)	0.060 (0.111)	0.069 (0.132)	0.040 (0.094)	0.060 (0.098)	0.082 (0.162)
MNA		1.73	1.73	1.67	1.67	1.53	1.60	1.80
Overall HWE		$P<0.05$	$P<0.05$	$P<0.05$	$P<0.05$	$P<0.05$	n.s.	n.s.

Following loci are fixed for the same allele: *AK**, *CK**, *FH**, *GPI-1**, *IDHP-1**. MNA, mean number of alleles; N, number of fish screened; n.s., not significant; $P_{0.99}$, proportion of polymorphic loci. Ost, Ostend; Ber, Bergen; FrF, Frisian Front; Tex, Texel; Oba, Oban; Ven, Venice; Per, Pérols—see text for full description of sites.