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Genetic divergence in natural populations of the Mediterranean brackish-water killifish *Aphanius fasciatus*

Ferruccio Maltagliati*

Dipartimento di Scienze dell'Uomo e dell'Ambiente, Università di Pisa, Via Volta 6, I-56126 Pisa, Italy

ABSTRACT: Samples of the brackish-water cyprinodontid fish *Aphanius fasciatus* from 11 Mediterranean coastal brackish-water habitats were examined for variation at 43 allozyme loci. Sixteen loci were polymorphic in at least 1 population. Estimates of genetic variability revealed low levels of polymorphism, with mean effective number of alleles per locus ranging from 1.02 (SE 0.01) to 1.10 (SE 0.04) and average expected heterozygosity values from 0.013 (SE 0.007) to 0.062 (SE 0.022). The mean Weir & Cockerham f = 0.060 (SE 0.019) confirmed the general concordance with Hardy-Weinberg equilibrium within populations assessed by exact tests. The presence of various private alleles and the mean value of the coancestry coefficient, θ = 0.507 (SE 0.078), indicated a marked genetic divergence among populations. Nei's genetic distance values were characteristic of populations within species, ranging from 0.001 (SE 0.000) to 0.098 (SE 0.044). Genetic affinities obtained by UPGMA cluster analysis were consistent with the geographical distribution of populations. The high degree of genetic divergence among *A. fasciatus* populations corresponds to the naturally fragmented distribution of the species and to restricted gene flow between populations, due to the limited dispersal potential of the species. Furthermore, genetic and geographical distances between populations are consistent with the prediction that the species is genetically structured by isolation by distance.

KEY WORDS: Isolation by distance · Genetic structure · Heterozygosity · Life history traits · Cyprinodontidae · Allozymes · Mediterranean

INTRODUCTION

The study of the population genetic structure of a species and the mechanisms that could have generated that pattern requires knowledge of the geographical distribution of genetic variation. The study of the genetic structure of a species, which can be caused by both life history traits and habitat constraints, may allow us to infer the extent to which these aspects affect the distribution of genetic variation (Soulé & Wilcox 1986, Lande 1988). In particular, the dispersal potential related to life history characteristics appears to play an important role in determining the genetic variability and population structure of fish species (Gyllensten 1985, Waples 1987, Ward et al. 1994). The isolation of natural populations is particularly considerable where strong habitat constraints exist, such as in fresh- or brackish-water habitats. In fact, the evolutionary and ecological importance of these habitats depends on the ways in which species use them and on the extent of the portion of the life cycle spent in such habitats.

Aphanius fasciatus Nardo, 1827, is a cyprinodontid euryhaline fish naturally located in many brackish-water habitats along Mediterranean coasts. It is an endemic species found in the whole Mediterranean region, with the exception of the easternmost and westernmost coasts, where it is replaced by *A. dispar* (Rüppel, 1828) and *A. iberus* (Valenciennes, 1846), respectively (Villwock 1982, Bianco 1995). *A. fasciatus* may spend its whole life cycle in brackish waters, showing fidelity to its habitats. The species has a rela-

*E-mail: maltagli@discat.unipi.it

tively sedentary life history, with large demersal eggs and without larval dispersal stages. Despite adults showing high mobility, it has been hypothesised that their migration from one population to another occurs only as a consequence of occasional and stochastic events, such as exceptional rainfalls or floods, which force migration along coastal seawater and/or through transitory inland water pathways (Maltagliati 1998a,b). Therefore, all the above-mentioned characteristics, together with the natural fragmentation of brackish-water habitats, favour isolation of populations.

Since *Aphanius fasciatus* is important neither as a source of food nor for aquariology purposes, the translocation of individuals, which could result in the establishment of less fit genotypes reducing population fitness through the disruption of coadapted genomes (Shaklee et al. 1993), can be excluded. Thus, this fish is particularly appropriate for the study of the genetic variation resulting from natural events, given that populations have not been manipulated and can be considered to reflect the natural distribution of genotypes. Previous preliminary allozyme investigations have shown considerable levels of population genetic divergence in *A. fasciatus*, allowing the hypothesis of a species genetic structure following the isolation by distance (Maltagliati 1998a,b).

The aim of this work was to extend the study of the genetic variability within and among populations of *Aphanius fasciatus* by means of allozyme markers, in order to gain information on the genetic structure of the species and on the relationships among natural populations of this teleost. Furthermore, the present study highlights the importance of interactions between life history characteristics, species biology, and environmental constraints in determining the genetic structure and the dispersal potential of *A. fasciatus*.

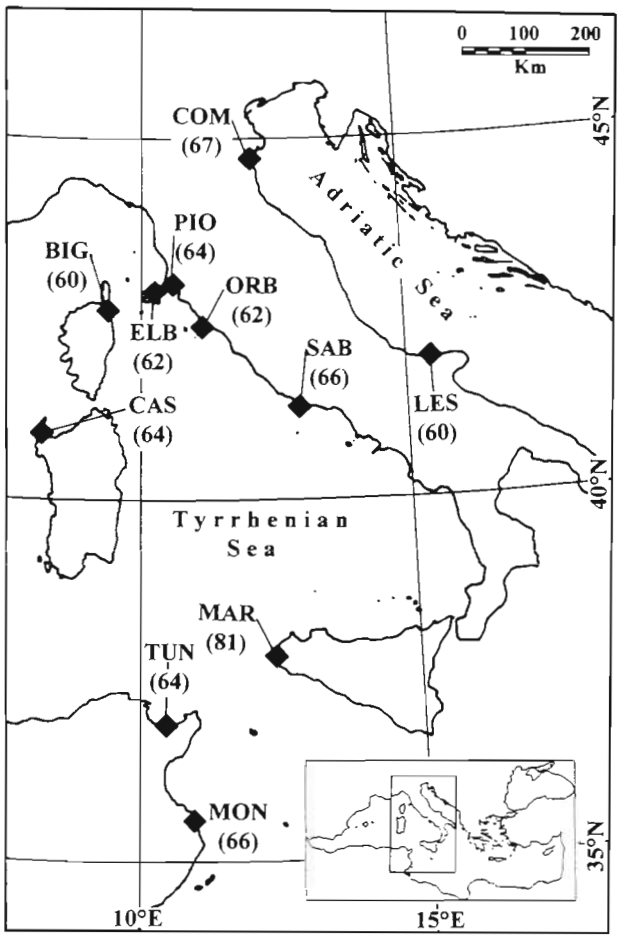

Fig. 1. Localities where *Aphanius fasciatus* was sampled. The numbers of individuals analysed for each locality are in parentheses. See 'Materials and methods' for explanation of population abbreviations

MATERIALS AND METHODS

Sample collection. Samples of *Aphanius fasciatus* were collected from natural populations in 11 brackish-water habitats of the central Mediterranean (Fig. 1) in the period between October 1994 and June 1996. The sites were Biguglia coastal pond (BIG, 42° 38' N, 09° 28' E); a man-made canal near the city of Piombino (PIO, 42° 57' N, 10° 29' E); a brackish microhabitat at Elba Island (ELB, 42° 48' N, 10° 19' E); the eastern basin of Orbetello lagoon (ORB, 42° 26' N, 11° 12' E); Sabaudia lake (SAB, 41° 18' N, 13° 02' E); Casaraccio coastal pond (CAS, 40° 54' N, 08° 15' E); Marsala lagoon (MAR, 37° 50' N, 12° 29' E); the lake of Tunis (TUN, 36° 49' N, 10° 16' E); Monastir lagoon (MON, 35° 46' N, 10° 47' E); Lesina lake (LES, 41° 53' N, 15° 22' E); and Comacchio lagoon (COM, 44° 41' N, 12° 11' E). Thirty individuals collected in the CAS, ELB, ORB, PIO and SAB populations have been analysed in previous research, representing pilot studies for the present investigation (Maltagliati 1998a,b). Samples were obtained using either a hand-net or small fish-traps, baited with anchovy fillets and placed in shallow water with abundant benthic vegetation. Live specimens of some samples were brought to the laboratory and killed in distilled water and ice, others were shipped to the laboratory in dry ice. All individuals were placed in separate plastic bags and stored at –80°C until processed for electrophoresis.

Electrophoretic methods. Cellulose acetate electrophoresis was conducted following the procedures outlined in Maltagliati (1998a). Twenty-two enzymatic systems were analysed: alcohol dehydrogenase (ADH, E.C. 1.1.1.1); adenylate kinase (AK, E.C. 2.7.4.3); aldolase (ALDO, E.C. 4.1.2.13); aldehyde oxidase (AO,

E.C. 1.2.3.1); creatine kinase (CK, E.C. 2.7.3.2); fructose biphosphatase (FBP, E.C. 3.1.3.11); fumarate hydratase (FH, E.C. 4.2.1.2); glyceraldehyde-3-phosphate dehydrogenase (GAPDH, E.C. 1.2.1.12); glucose dehydrogenase (GDH, E.C. 1.1.1.47); glycerate dehydrogenase (GLYDH, E.C. 1.1.1.29); glycerol-3-phosphate dehydrogenase (GPD, E.C. 1.1.1.8); glucose-6-phosphate dehydrogenase (G6PDH, E.C. 1.1.1.49); glucose-6-phosphate isomerase (GPI, E.C. 5.3.1.9); isocitrate dehydrogenase ($NADP^+$) (IDH, E.C. 1.1.1.42); L-lactate dehydrogenase (LDH, E.C. 1.1.1.27); malate dehydrogenase (MDH, E.C. 1.1.1.37); malic enzyme (ME, E.C. 1.1.1.40); mannose-6-phosphate isomerase (MPI, E.C. 5.3.1.8); phosphogluconate dehydrogenase (PGDH, E.C. 1.1.1.44); phosphoglucomutase (PGM, E.C. 5.4.2.2); pyrroline dehydrogenase (PYDH, E.C. 1.5.1.12); sorbitole dehydrogenase (SORD, E.C. 1.1.1.14).

For each locus the most common allele of the reference population (Orbetello lagoon population) was designated as **100*, the other alleles were designated as greater or lower values according to their electrophoretic mobility. Calibration of alleles was accomplished by running specimens from different populations on the same cellulose acetate membrane. Nomenclature of enzymes, loci and alleles followed the recommendations of Shaklee et al. (1990).

Statistical analyses. Allozyme data for all populations were analysed using the computer packages DISPAN (Ota 1993: computer program distributed by the author), FSTAT 1.2 (Goudet 1994), GENEPOP 3.1 (Raymond & Rousset 1995), GENETIX 3.3 (Belkhir et al. 1996) and GDA 1.0 (Lewis & Zaykin 1996 computer program distributed by the authors). Given that *Aphanius fasciatus* possess a number of chromosomes $2n$ = 48 (Vitturi et al. 1995), statistics for diploid organisms was employed. Genetic variability was determined by using the mean number of alleles per locus, the mean effective number of alleles per locus, the percentage of polymorphic loci at the 95% level, and the average observed and expected unbiased heterozygosity per locus, calculated according to Nei (1978). Genotype frequencies within populations were tested for deviation from Hardy-Weinberg equilibrium using an exact significance probability test (Louis & Dempster 1987). Multiple tests were adjusted by the sequential Bonferroni correction to control for Type I error (Hochberg 1988).

Levels of population structuring and genetic divergence were studied by Wright's (1978) *F*-statistics, using Weir & Cockerham (1984) jackknifed unbiased estimators. The variances of these parameters were calculated by jackknifing across loci (Weir & Cockerham 1984, Weir 1996). Estimates of *F*-statistics were tested for difference from zero using permutation procedures with 1000 replicates (Goudet 1994). To determine genetic affinities among populations, Nei's (1978) genetic distance was calculated and standard errors were obtained by jackknifing over loci. On the basis of the matrix of Nei's genetic distances a UPGMA (unweighted pair-group method using arithmetic average) dendrogram was constructed and subjected to a bootstrapping analysis with 1000 replicates. Finally, to verify the correlation between genetic and geographical distance matrices, Mantel's test of the association between 2 parameters in data matrices with internal correlation (Manly 1991) was carried out. Probabilities were read directly from the distribution of 5000 randomised matrices computed by permutations.

RESULTS

Samples were surveyed for 43 presumptive gene loci of which 16 were polymorphic in at least 1 population (Table 1). Heterozygotes were 2-banded (implying a monomeric quaternary structure) for *FBP**, *MPI** and *PGM**; 3-banded (dimeric structure) for *G6PDH-1**, *G6PDH-2**, *GPI-1**, *GPI-2**, *ME-2**, *ME-3**; 5-banded (tetrameric structure) for *LDH-1** and *LDH-3**; and broad but undefined (implying a multimeric but uncertain structure) for *AO-1**, *AO-2**, *GDH-2**, *GPD-2** and *GPD-3**. The genetic basis of the banding pattern in *Aphanius fasciatus* was assessed and discussed in a previous work (Maltagliati 1998a). With respect to that pilot study, the increase of the sample size to 62 specimens in the Orbetello population determined the detection of the low frequency allele *GPD-2* 102*, which had previously gone undetected. Furthermore, due to the extension of the analysis to 11 populations in the present research, polymorphism was observed in 4 additional loci (*AO-1**, *FBP**, *LDH-1** and *ME-3**), which were monoallelic in the preliminary study.

Allele frequencies for the polymorphic loci vary among populations (Table 1). Eight private alleles (exclusive to 1 population) were observed: *MPI* 96* in COM; *GPDH-2* 98*, *GPI-1* 96*, *ME-3* 98*, *PGM* 96* in LES; *ME-2* 102* in MON; *GPD-2* 102* in ORB; and *GPI-2* 96* in SAB. Nevertheless, with the exception of *GPI-2* 96* in SAB, their frequencies of occurrence were extremely low. Other alleles were distributed in populations according to the existence of geographical entities: *AO-2* 102* in COM and LES (Adriatic populations); *FBP* 102* in MAR, MON and TUN (southernmost populations); *GPI-1* 102* and *LDH-1* 98* in MON and TUN (Tunisian populations) (Table 1). The percentage of polymorphic loci ranged from 2.33 to 20.93; the mean number of alleles per locus and the mean effective number of alleles per locus ranged from 1.12 (SE 0.05) to 1.35 (SE 0.09) and from 1.02 (SE 0.01) to 1.10 (SE 0.04), respectively (Table 2). The latter esti-

Table 1. *Aphanius fasciatus*. Allele frequencies at 16 loci in 11 natural populations. N: sample size. See 'Materials and methods' for locality abbreviations. Loci *ADH-1**, *ADH-2**, *ALDO-1**, *ALDO-2**, *ALDO-3**, *AK**, *CK-1**, *CK-2**, *FH**, *GAPD-1**, *GAPD-2**, *GDH-1**, *GLYDH**, *GPD-1**, *IDH-1**, *IDH-2**, *LDH-2**, *MDH-1**, *MDH-2**, *MDH-3**, *MDH-4**, *ME-1**, *PGDH**, *PYDH-1**, *PYDH-2**, *SORD-1**, and *SORD-2** showed no allelic variation in all samples analysed

Locus	Allele	BIG	CAS	COM	ELB	LES	MAR	MON	ORB	PIO	SAB	TUN
*AO-1**	(N)	(59)	(54)	(46)	(57)	(43)	(50)	(52)	(48)	(56)	(59)	(47)
	*100	0.958	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	0.926
	*98	0.042	–	–	–	–	–	–	–	–	–	0.074
*AO-2**	(N)	(58)	(56)	(55)	(52)	(52)	(57)	(47)	(46)	(59)	(55)	(39)
	*102	–	–	0.155	–	0.038	–	–	–	–	–	–
	*100	0.966	1.000	0.845	1.000	0.962	–	–	1.000	1.000	0.900	–
	*98	0.034	–	–	–	–	0.518	0.904	–	–	0.100	0.949
	*96	–	–	–	–	–	0.482	0.096	–	–	–	0.051
*FBP**	(N)	(60)	(63)	(61)	(62)	(47)	(59)	(62)	(59)	(60)	(66)	(64)
	*102	–	–	–	–	–	0.017	0.008	–	–	–	0.008
	*100	1.000	1.000	1.000	1.000	1.000	0.983	0.992	1.000	1.000	1.000	0.992
*GDH-2**	(N)	(58)	(21)	(47)	(21)	(33)	(19)	(33)	(25)	(34)	(34)	(33)
	*102	0.086	0.078	–	–	–	0.007	–	–	–	–	–
	*100	0.914	0.922	1.000	1.000	1.000	0.558	1.000	1.000	1.000	0.992	0.960
	*98	–	–	–	–	–	0.435	–	–	–	0.008	0.040
*GPD-2**	(N)	(53)	(55)	(54)	(44)	(51)	(35)	(61)	(44)	(52)	(63)	(63)
	*102	–	–	–	–	–	–	–	0.011	–	–	–
	*100	0.783	1.000	0.917	0.614	0.971	0.957	0.795	0.682	0.606	0.865	0.460
	*98	0.217	–	0.083	0.386	0.029	0.043	0.205	0.307	0.394	0.135	0.540
*GPD-3**	(N)	(59)	(61)	(65)	(62)	(60)	(80)	(65)	(61)	(64)	(66)	(64)
	*102	0.051	–	–	0.048	–	0.013	–	0.033	0.055	0.008	–
	*100	0.949	1.000	1.000	0.952	1.000	0.987	1.000	0.967	0.945	0.992	1.000
*G6PDH-1**	(N)	(52)	(59)	(63)	(60)	(60)	(49)	(56)	(60)	(62)	(63)	(46)
	*102	0.106	0.178	–	–	–	0.122	–	–	–	–	–
	*100	0.894	0.822	1.000	1.000	1.000	0.878	1.000	1.000	1.000	1.000	1.000
*G6PDH-2**	(N)	(60)	(60)	(63)	(62)	(50)	(56)	(55)	(62)	(63)	(61)	(45)
	*102	–	–	–	–	–	–	0.100	–	–	0.082	0.178
	*100	1.000	1.000	1.000	1.000	0.970	1.000	0.900	1.000	1.000	0.918	0.822
	*98	–	–	–	–	0.030	–	–	–	–	–	–
*GPI-1**	(N)	(60)	(59)	(66)	(62)	(55)	(72)	(61)	(44)	(64)	(66)	(51)
	*102	–	–	–	–	–	–	0.008	–	–	–	0.284
	*100	1.000	0.932	–	1.000	–	0.764	0.238	1.000	1.000	0.326	0.696
	*98	–	0.068	1.000	–	0.991	0.236	0.754	–	–	0.674	0.020
	*96	–	–	–	–	0.009	–	–	–	–	–	–
*GPI-2**	(N)	(53)	(58)	(66)	(59)	(57)	(61)	(64)	(47)	(64)	(64)	(64)
	*102	–	–	0.015	–	–	0.057	0.047	–	–	–	0.008
	*100	0.792	1.000	0.985	0.483	0.772	0.926	0.953	0.787	0.336	0.734	0.820
	*98	0.208	–	–	0.517	0.228	0.016	–	0.213	0.664	–	0.172
	*96	–	–	–	–	–	–	–	–	–	0.266	–
*LDH-1**	(N)	(60)	(64)	(67)	(62)	(60)	(80)	(66)	(62)	(64)	(66)	(62)
	*100	1.000	1.000	1.000	1.000	1.000	1.000	0.689	1.000	1.000	1.000	0.992
	*98	–	–	–	–	–	–	0.311	–	–	–	0.008
*LDH-3**	(N)	(53)	(64)	(57)	(62)	(60)	(72)	(42)	(62)	(62)	(64)	(41)
	*102	–	–	–	–	–	0.007	0.012	–	–	–	–
	*100	0.377	1.000	0.956	0.387	1.000	0.993	0.452	0.387	0.113	0.539	0.963
	*98	0.623	–	0.044	0.613	–	–	0.536	0.613	0.887	0.461	0.037
*ME-2**	(N)	(59)	(63)	(67)	(59)	(60)	(80)	(66)	(62)	(64)	(66)	(64)
	*102	–	–	–	–	–	–	0.008	–	–	–	–
	*100	0.941	0.960	1.000	1.000	1.000	0.981	0.992	1.000	1.000	1.000	1.000
	*98	0.059	0.040	–	–	–	0.019	–	–	–	–	–
*ME-3**	(N)	(59)	(64)	(67)	(59)	(60)	(80)	(66)	(62)	(64)	(66)	(64)
	*100	1.000	1.000	1.000	1.000	0.975	1.000	1.000	1.000	1.000	1.000	1.000
	*98	–	–	–	–	0.025	–	–	–	–	–	–
*MPI**	(N)	(60)	(64)	(67)	(62)	(60)	(81)	(66)	(61)	(64)	(66)	(64)
	*102	0.467	–	–	0.306	–	0.043	–	0.344	0.250	0.553	–
	*100	0.533	1.000	0.008	0.694	1.000	0.926	0.561	0.656	0.750	0.439	0.297
	*98	–	–	0.985	–	–	0.031	0.439	–	–	0.008	0.703
	*96	–	–	0.007	–	–	–	–	–	–	–	–
*PGM**	(N)	(59)	(64)	(67)	(62)	(54)	(74)	(65)	(62)	(64)	(66)	(64)
	*100	0.949	0.773	–	0.968	–	0.108	0.015	1.000	0.922	1.000	0.820
	*98	0.051	0.227	1.000	0.032	0.991	0.892	0.985	–	0.078	–	0.180
	*96	–	–	–	–	0.009	–	–	–	–	–	–

Table 2. *Aphanius fasciatus*. Measures of genetic variability calculated for each population. Standard errors are in parentheses. p_{95} is the 0.95 level of polymorphism. See 'Materials and methods' for locality abbreviations

Population	BIG	CAS	COM	ELB	LES	MAR	MON	ORB	PIO	SAB	TUN
Mean sample size per locus	57.6 (0.7)	60.4 (0.9)	62.0 (1.5)	58.9 (1.2)	54.9 (1.4)	65.9 (3.5)	60.0 (1.9)	56.4 (1.9)	61.9 (0.9)	63.9 (0.8)	56.5 (2.4)
Percentage of loci polymorphic (p_{95})	20.93	9.30	4.65	9.30	2.33	16.28	16.28	9.30	13.95	16.28	18.60
Mean no. of alleles per locus	1.26 (0.07)	1.12 (0.05)	1.14 (0.06)	1.14 (0.05)	1.16 (0.06)	1.35 (0.09)	1.30 (0.09)	1.14 (0.06)	1.14 (0.05)	1.23 (0.07)	1.33 (0.09)
Mean effective no. of alleles per locus	1.09 (0.03)	1.03 (0.02)	1.02 (0.01)	1.06 (0.03)	1.02 (0.01)	1.08 (0.04)	1.10 (0.04)	1.07 (0.04)	1.07 (0.03)	1.10 (0.04)	1.10 (0.04)
Mean observed heterozygosity	0.049 (0.016)	0.020 (0.009)	0.012 (0.006)	0.048 (0.022)	0.016 (0.009)	0.048 (0.017)	0.060 (0.022)	0.041 (0.019)	0.037 (0.016)	0.057 (0.022)	0.056 (0.018)
Mean expected heterozygosity	0.057 (0.019)	0.023 (0.011)	0.013 (0.007)	0.048 (0.021)	0.015 (0.009)	0.052 (0.019)	0.062 (0.022)	0.041 (0.019)	0.041 (0.018)	0.057 (0.021)	0.062 (0.021)

mate measures the evenness of allele frequencies, i.e. evenness of the most common alleles, considering that rare alleles contribute little to the total value (Berg & Hamrick 1997). The mean observed and expected heterozygosity values were very close, indicating a general accordance to Hardy-Weinberg equilibrium. The values of the mean observed and expected heterozygosity ranged from 0.012 (SE 0.006) to 0.060 (SE 0.022) and from 0.013 (SE 0.007) to 0.062 (SE 0.022), respectively (Table 2).

Within the 87 probability tests, only 1 significant departure from Hardy-Weinberg equilibrium was detected at *LDH-3** in BIG ($p = 0.0027$); however, this was not significant after the Bonferroni correction. All single-locus estimates of F (indicating the deviance from Hardy-Weinberg equilibrium within the total population) were significant at $p < 0.001$, with the exception of *GPD-3** ($p < 0.05$) and *FBP**, *ME-2** and *ME-3** (not significant) (Table 3). All single-locus significant values were positive, evidence of a general deficit of heterozygotes. In contrast, the mean f and its single-locus values were not significant, with the exception of *AO-1**, *GPD-2**, *G6PDH-1** and *GPI-1** (significant at $p < 0.05$ for deficit of heterozygotes), confirming the general within-population accordance to Hardy-Weinberg expectations (Table 3). The single-locus estimates of the coancestry coefficient θ, which indicates the genetic divergence among populations, were highly significant ($p < 0.001$) (the *FBP** value being the only exception), giving a highly significant mean value ($\theta = 0.507$, SE 0.078) (Table 3).

The minimum Nei's (1978) genetic distance was 0.001 (SE 0.000) (between BIG and ORB) and the maximum was 0.098 (SE 0.044) (between COM and PIO). The genetic affinities among populations can be observed in the UPGMA dendrogram of Nei's genetic distances (Fig. 2). UPGMA analysis showed a general consistency between genetic affinity and geographical distance. Three main clusters are evident and supported by maximum bootstrap values (= 1000); however, within each cluster some nodes were associated with low bootstrap values (<500), indicating that there is essentially no meaningful resolution at a smaller spatial scale (Fig. 2). Mantel's test confirmed that genetic distances between populations are positively correlated with geographical distances ($g = 4.133$; $p < 0.001$).

Table 3. *Aphanius fasciatus*. Weir & Cockerham (1984) estimates of F-statistics for individual loci in 11 populations. Standard errors are in parentheses

Locus	F	θ	f
*AO-1**	0.309 (0.078)^c	0.044 (0.024)^c	0.279 (0.098)^a
*AO-2**	0.755 (0.071)^c	0.732 (0.084)^c	0.096 (0.088)
*FBP**	−0.003 (0.002)	0.003 (0.005)	−0.006 (0.005)
*GDH-2**	0.542 (0.230)^c	0.463 (0.242)^c	0.135 (0.024)
*GPD-2**	0.265 (0.068)^c	0.184 (0.066)^c	0.099 (0.032)^a
*GPD-3**	0.066 (0.081)^a	0.019 (0.006)^c	0.048 (0.081)
*G6PDH-1**	0.273 (0.103)^c	0.113 (0.032)^c	0.177 (0.089)^a
*G6PDH-2**	0.186 (0.033)^c	0.095 (0.039)^c	0.100 (0.017)
*GPI-1**	0.739 (0.110)^c	0.697 (0.127)^c	0.142 (0.063)^a
*GPI-2**	0.286 (0.125)^c	0.297 (0.099)^c	−0.019 (0.043)
*LDH-1**	0.627 (0.303)^c	0.559 (0.270)^c	0.096 (0.047)
*LDH-3**	0.483 (0.136)^c	0.474 (0.118)^c	0.012 (0.099)
*ME-2**	−0.013 (0.006)	0.039 (0.013)^c	−0.054 (0.015)
*ME-3**	0.000 (0.000)	0.034 (0.016)^c	−0.034 (0.016)
*MPI**	0.507 (0.145)^c	0.487 (0.148)^c	0.039 (0.027)
*PGM**	0.823 (0.076)^c	0.804 (0.084)^c	0.100 (0.044)
Mean	0.537 (0.077)^c	0.507 (0.078)^c	0.060 (0.019)

$^a p < 0.05$; $^b p < 0.01$; $^c p < 0.001$

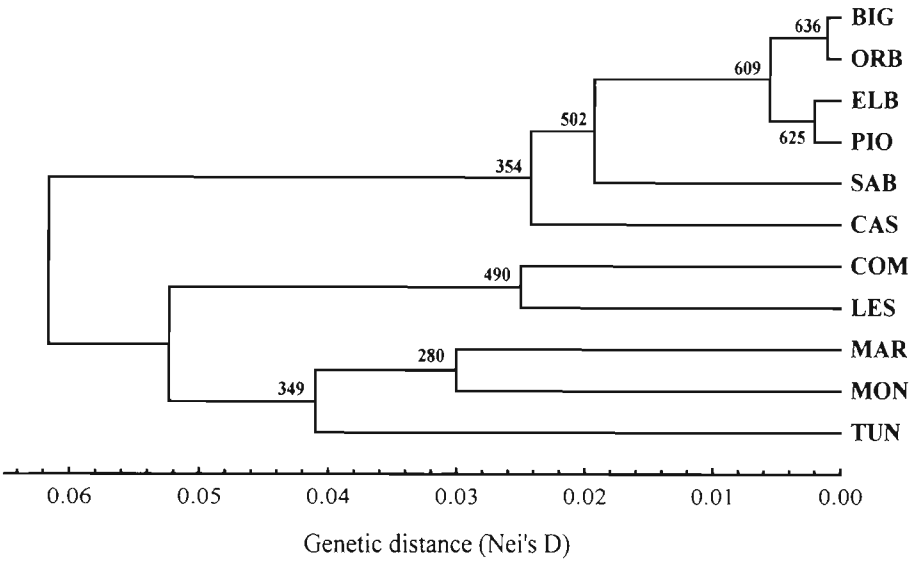

Fig. 2. *Aphanius fasciatus*. UPGMA dendrogram of Nei's (1978) genetic distances between samples. Node values are indicated on branching points and represent the number of times a particular cluster group was formed in 1000 bootstrap iterations. See 'Materials and methods' for explanation of population abbreviations

DISCUSSION

The results of the present study highlight low levels of genetic variability and high genetic divergence between natural populations of *Aphanius fasciatus*. The levels of electrophoretic mobility and variation of individual alleles found in the present study are consistent with results obtained in previous preliminary investigations on this species (Maltagliati 1998a,b). In all populations analysed, the percentage of polymorphic loci was low, because 27 loci showed no allelic variation in all samples analysed and several alleles had frequencies lower than 0.05. The mean number of alleles was low (≤1.35), with the mean effective number of alleles substantially lower (≤1.10). This is due to the presence of many alleles at low frequencies, indicating that populations of *A. fasciatus* are demographically stable, namely they have apparently not been subjected to recent founder events or recurrent bottlenecks.

In the 9 populations of *Aphanius fasciatus* the mean expected heterozygosities over the 43 examined loci was lower than 0.065 and differences among populations were not interpretable either by geographical distribution or by ecological characteristics of single habitats. Moreover, the results of the present investigation are broadly comparable with those obtained by other researchers for closely related species such as *A. dispar* (Kornfield & Nevo 1976) and *A. iberus* (Doadrio et al. 1996). In fact, heterozygosity values ranged from 0.024 to 0.063 in 5 populations of *A. dispar* and from 0.023 to 0.109 in 12 populations of *A. iberus*. The consistency of genetic variability estimates in these closely related species with the values obtained in *A. fasciatus* could be explained by the presence of common characteristics of brackish-water cyprinodontids. Life history traits, constraints given by the natural fragmentation of their habitats and environmental conditions of biotopes finally result in similar levels of genetic variability.

The mean value of f, not significantly different from zero, confirms the general accordance with Hardy-Weinberg expectations at the intrapopulational level, as demonstrated by the exact significance probability tests. This suggests the absence of population substructuring and excludes the occurrence of inbreeding. The within-population genetic homogeneity found in *Aphanius fasciatus* could be explained by the high mobility of adult individuals within isolated brackish-water habitats, preventing population genetic substructuring. Nevertheless, the loci *AO-1**, *GPD-2**, *G6PDH-1** and *GPI-1** showed significant values of f (Table 3), i.e. a general within-population deficit of heterozygotes at those loci. Generally, explanations for this take into account the Walhund effect, inbreeding, presence of null alleles and/or selection. Nevertheless, the first and second effects are to be discarded, because it would be expected that they occur across all variable loci; however, the action of selection or the presence of null alleles at those loci cannot be excluded.

The marked differences in allele frequencies, the presence of various private alleles and the values of the coancestry coefficients found in *Aphanius fasciatus* populations suggest that the distribution of genetic variability is highly structured within the species and that gene flow between populations is reduced. The degree of genetic divergence among populations is

very high, as estimated by the mean value of the coancestry coefficient (θ = 0.507, SE 0.078), and comparable to that detected by Doadrio et al. (1996) in the closely related species *A. iberus* and that obtained in freshwater teleosts living in completely separated habitats (Gyllensten 1985, Ward et al. 1994). Restricted gene flow implies that populations are substantially self-replenishing on a local scale, favouring genetic divergence over time. The correspondence between gene flow and dispersal capabilities has been studied for a number of marine, anadromous and freshwater fishes (Gyllensten 1985, Waples 1987, Ward et al. 1994). Generally, species with pelagic eggs and long-lived planktonic larvae possess high dispersal capabilities and are less likely to show high levels of genetic divergence between populations. Conversely, species with low dispersal capabilities are more likely to be structured in isolated populations, with reduced genetic exchange and increased genetic divergence. *A. fasciatus* is no exception to this trend. In fact, its specialised life history, with large demersal eggs, without larval dispersal stages and habitat fidelity markedly limits dispersal and gene flow. Superimposed on these effects is the naturally fragmented distribution of brackish habitats also favouring genetic divergence among *A. fasciatus* populations. The present study did not focus on the adaptive significance of the genetic differences among populations, but it is clear that isolated brackish habitats strongly contribute to genetic divergence.

The values of Nei's (1978) index detected in the present study are characteristic of conspecific populations. They do not indicate extremely high genetic distances, due to the presence of the same allele fixed in all populations for 27 of the loci analysed. Striking genetic heterogeneity was observed on a wide regional scale and to a lesser extent among populations within a region. A strong correspondence between the pattern of genetic variability and the geographical distribution of populations was found. In fact, alleles characteristic of populations from particular regions, namely *AO-2*102* in Adriatic Sea populations, *FBP*102* in southernmost populations and *GPI-1*102* in Tunisian populations, can be taken as representative of a substantial geographical trend of genetic variation.

Models of geographical population divergence are typically constructed under the assumptions of selective neutrality and focus on the analysis of divergence that results from mutation, genetic drift and gene flow (Kimura 1983). Therefore, assuming neutrality of the genetic markers used in the present study, the positive significant correlation between geographical and genetic distances observed in *Aphanius fasciatus* populations supports Wright's (1943) isolation by distance model of species genetic structure. As already hypothesised in previous preliminary allozyme studies (Maltagliati 1998a,b), the observed pattern of population structure is most likely maintained by a very limited gene flow between populations, within which genetic variability is primarily influenced by genetic drift. However, gene flow is sufficient to maintain the isolation by distance genetic structure. In addition, the disjunct coastal distribution of *A. fasciatus* suggests that its populations may form a 1-dimensional metapopulation structure, the coastline being the dimension, with restricted gene flow occurring between adjacent populations.

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