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A preliminary investigation of allozyme genetic variation and population geographical structure in *Aphanius fasciatus* from Italian brackish-water habitats

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A total of 150 individuals from *Aphanius fasciatus* from coastal brackish-water habitats was analysed by allozyme electrophoresis to collect data on its genetic variation. From 22 enzymes, 43 putative enzyme-coding loci were resolved, 12 of which were polymorphic at $P_{0.99}$ level. Only one of the 31 probability tests showed a significant departure from the Hardy–Weinberg equilibrium. *Aphanius fasciatus* showed low levels of genetic polymorphism, with expected heterozygosity values ranging from 0·027 (S.E.=0·013) to 0·064 (S.E.=0·023). Nei's genetic distances between populations ranged from 0·002 to 0·042. Weir & Cockerham *F*-statistics showed high levels of genetic heterogeneity among populations (jackknifed θ=0·302, S.E.=0·045) and estimates of N_m were <1, indicating restricted gene flow. Significant positive correlation between genetic distance and geographical distance matrices, detected by Mantel's test (g=1·941; P<0·001), is consistent with the prediction that the species is genetically structured by isolation-by-distance.

Key words: *Aphanius fasciatus*; brackish-water habitats; allozymes; genetic variation; genetic distance; gene flow; isolation-by-distance.

INTRODUCTION

Subdivision between populations within a species increases the potential for genetic differentiation due either to selection or drift. Gyllensten (1985) and subsequently Ward *et al.* (1994) discussed the differences of the levels of genetic substructuring in marine, anadromous and freshwater teleosts. Generally marine species have the potential for long-distance dispersal of eggs, larvae, juveniles and adults, resulting in genetic connection between populations over large distances. Conversely, some characteristics of species living in disjunct habitats, such as fresh- or brackish-water sites and the nature of the habitats occupied produce a higher likelihood of evolutionary divergence among populations (Carvalho, 1993): benthic eggs, absence of larvae, low mobility of adults, presence of geographical and ecological barriers, fragmentation of habitats, respectively.

The main objectives of the present study were to quantify the levels of allozyme genetic variation within populations of *Aphanius fasciatus* Nardo, 1827 from five different localities, to gain information about the genetic structure of the species; and to relate the observed genetic heterogeneity and postulated levels of gene flow to certain biological and ecological characteristics of the species. *Aphanius fasciatus* is a small cyprinodontid fish (7–8 cm max L_T).

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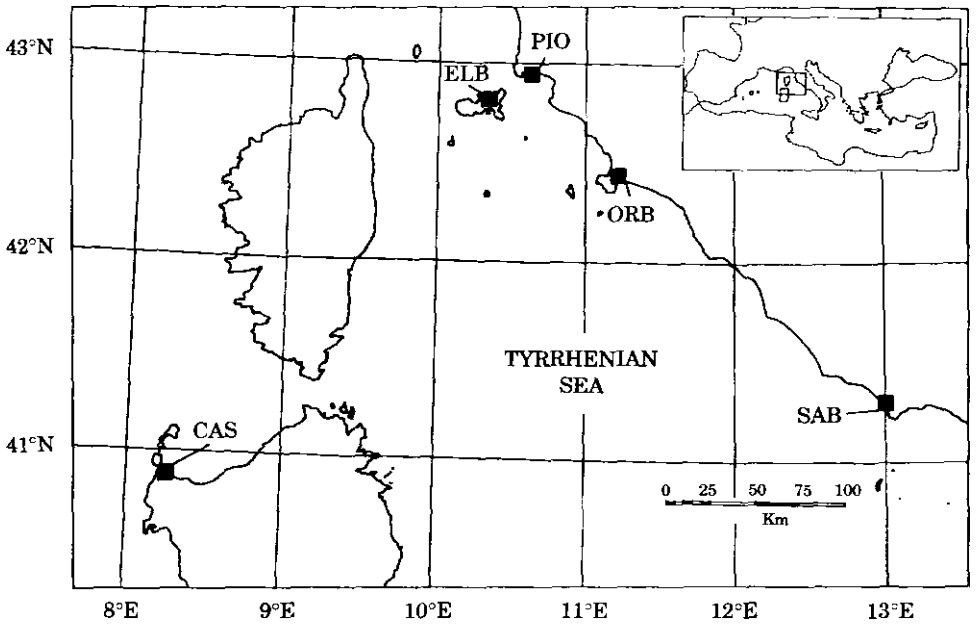

FIG. 1. Location of sampling sites of *A. fasciatus*. CAS, Casaraccio Pond; ELB, Elba Island microhabitat; ORB, Orbetello Lagoon; PIO, a canal near Piombino; SAB, Sabaudia coastal lake.

discontinuously distributed in brackishwater habitats along the coasts of the Mediterranean Sea: it occurs from Egypt westward to Algeria; along the coasts from southern Turkey to France; and along coastal habitats of many islands (Villwock, 1970). It aso occurs in some rivers (Tigano & Parenti, 1988; Parenti & Tigano, 1993) or oases (Kraiem, 1983). *Aphanius fasciatus* is relatively sedentary, with large benthic eggs, no larval dispersal stages and limited migrating capabilities of the adults. It is an ideal organism for an investigation focusing on the importance of drift, selection and gene flow in determining population divergence, because the potential for restricted gene flow, the ecological variation and the discontinuity of distribution along the coasts are extensive. Furthermore, since *A. fasciatus* is not a species of commercial interest and is of scarce relevance for aquariologists, the distribution of genotypes among its populations should not be directly affected by human activities. Morphological differentiation among *A. fasciatus* populations has been detected particularly from Sicily, Sardinia and the Adriatic Sea (Tigano & Parenti, 1988; Parenti & Tigano, 1993). Moreover, variability of the number of active nucleolus organizer regions (NORs) was found both at the intra- and interpopulation level (Vitturi *et al.*, 1995).

MATERIALS AND METHODS

COLLECTION OF SAMPLES

Samples of 30 fishes were collected between October 1994 and July 1995 from each of the following five coastal localities (Fig. 1): Casaraccio coastal pond (CAS) (40°54′ N, 08°15′ E); a brackish-water microhabitat at Elba Island (ELB) (42°48′ N, 10°19′ E); Orbetello Lagoon (ORB) (42°26′ N, 11°12′ E); a man-made canal near Piombino (PIO)

(42°57′ N, 10°29′ E); and Sabaudia coastal lake (SAB) (41°02′ N, 10°19′ E). These locations were separated by 30–400 km. Baited fish-traps were laced in shallow water with abundant benthic vegetation, and the catches were transferred alive to the laboratory, killed in iced water and placed immediately in single plastic bags for storage at −80° C until the analyses.

ELECTROPHORESIS

Samples of epaxial muscle, eye and liver were analysed by electrophoresis. Tissue samples were ground in three volumes of the extracting buffer [Tris (0·05 M), EDTA (0·01 M), PMSF (0·1 M), Mercaptoethanol (0·2%), Triton X100 (0·1%), corrected to pH 8 with HCl] then centrifuged for 10 min at 4000 *g*. Supernatant fractions were applied immediately to cellulose acetate membranes (Sartophor® system, Sartorius). Samples were kept at <5° C at all stages of preparation. All electrophoesis runs were performed at 300 V for 25 min. A preliminary screening was conducted on some specimens from Orbetello Lagoon to optimize analytical conditions for the activity and resolution of enzymes using cellulose acetate electrophoresis. A total of 39 enzyme systems (Table I) was assayed using the TEM buffer [Tris (0·1 M), EDTA (0·01 M), $MgCl_2$ (0·001 M), corrected to pH 7·8 with maleic acid]. Enzymes were analysed following the staining procedures of Richardson *et al.* (1986) and Pasteur *et al.* (1987), slightly modified. Nomenclature for protein-coding loci followed the recommendations of Shaklee *et al.* (1990). The most common allele was assigned arbitrarily the value 100, slower and faster bands on the zymograms, representing other alleles, were given lower or higher numbers corresponding to their relative mobilities, respectively. Calibration of alleles was accomplished by running individuals from different populations on the same electrophoretic membrane.

DATA ANALYSIS

Diploidy of *A. fasciatus* ($2n=48$) (Villwock, 1970: Vitturi *et al.*, 1995) allowed the application of classical statistics for electrophoretic data from diploid organisms. Statistical analyses were performed using the computer packages FSTAT (Goudet, 1994) and GENEPOP 3.1 (Raymond & Rousset, 1995). The allele frequencies, the proportion of polymorphic loci ($P_{0.99}$ criterion), the mean number of alleles per locus and the observed and expected heterozygosity per locus were calculated for each population. Deviations from the expected Hardy–Weinberg genotypic proportions within populations were assessed using an exact significance probability test (Louis & Dempster, 1987). Levels of population structuring were quantified for all loci with Weir & Cockerham (1984) estimators of *F*-statistics. The following parameters were calculated: f, the correlation of genes within individuals within populations and θ, the correlation of genes of different individuals in the same population. Estimates of the variance of these parameters were obtained by jackknifing across loci (Weir & Cockerham, 1984; Weir, 1996). *F*-statistics were tested for difference from zero permuting (1000 replicates) alleles within samples (f) and alleles between samples (F and θ) over all loci. To determine multilocus genetic relationships among populations, Nei's (1978) and modified Rogers' (Wright, 1978) genetic distances were computed and denominated here D_N and D_R, respectively. On the basis of Nei's (1978) genetic distance matrix, a dendrogram was generated with the unweighted pair group method (UPGMA) cluster analysis (Sneath & Sokal, 1973) and subjected to bootstrapping with 5000 replicates. Populations were ordinated in a bi-dimensional space with multidimensional scaling (MDS) to examine the relationship depicted in the original distance matrix (Lessa, 1990). The modified Rogers' genetic distance was used because metricity of the distance measure is required in MDS analysis (Lessa, 1990). To verify the correlation among geographical and genetic distance matrices, a Mantel's test of the association between two parameters in data matrices with internal correlation (Manly, 1985) was used with 120 permutations ($n!$ permutations, where n is the number of populations). Indirect estimates of gene flow (N_m, the effective number of migrants per generation) were calculated using both Wright's (1943) island model, where $\theta=1/(4N_m+1)$; and Slatkin's (1985) private alleles method,

TABLE I. *A. fasciatus*, enzyme systems assayed

Abbreviation	Enzyme	E.C. No.	Act*	Res†	Tis‡	No. loci
AAT	Aspartate aminotransferase	2.6.1.1	—	—	—	—
ACO	Aconitase	4.2.1.3	+	—	—	—
ACP	Acid phosphatase	3.1.3.2	—	—	—	—
ADA	Adenosine deaminase	3.5.4.4	+	—	—	—
ADH	Alcohol dehydrogenase	1.1.1.1	+	+	L	2
AK	Adenylate kinase	2.7.4.3	+	+	L	1
ALDO	Aldolase	4.1.2.13	+	+	M, L	3
ALP	Alkaline phosphatase	3.1.3.1	—	—	—	—
AO	Aldehyde oxidase	1.2.3.1	+	+	L	2
CK	Creatine kinase	2.7.3.2	+	+	L	2
DR5P	Desoxyribose-5-P aldolase	4.1.2.4	+	—	—	—
FBP	Fructose biphosphatase	3.1.3.11	+	+	L	1
FDH	Formaldehyde dehydrogenase	1.2.1.1	—	—	—	—
FH	Fumarate hydratase	4.2.1.2	+	+	L	1
FK	Fructose kinase	2.7.1.4	+	—	—	—
GAPDH	Glyceraldehyde-3-P dehydrogenase	1.2.1.12	+	+	M	2
GDA	Guanine deaminase	3.5.4.3	+	—	—	—
GDH	Glucose dehydrogenase	1.1.1.47	+	+	L	2
GLUDH	Glutamate dehydrogenase	1.4.1.3	+	—	—	—
GLYDH	Glycerate dehydrogenase	1.1.1.29	+	+	L	1
GPD	Glycerol-3-P dehydrogenase	1.1.1.8	+	+	M, L	3
G6PDH	Glucose-6-P dehydrogenase	1.1.1.49	+	+	M, L	2
GPI	Glucose-6-P isomerase	5.3.1.9	+	+	M, E	2
HAOX	Hydroxyacid oxidase	1.1.3.15	+	—	—	—
HBDH	3-Hydroxybutyrate dehydrogenase	1.1.1.30	+	—	—	—
HK	Hexokinase	2.7.1.1	+	—	—	—
IDH	Isocitrate dehydrogenase ($NADP^+$)	1.1.1.42	+	+	M, L	2
LDH	L-Lactate dehydrogenase	1.1.1.27	+	+	E	3
MDH	Malate dehydrogenase	1.1.1.37	+	+	M	4
ME	Malic enzyme	1.1.1.40	+	+	M	3
MPI	Mannose-6-P isomerase	5.3.1.8	+	+	M	1
NP	Purine-nucleoside phosphorylase	2.4.2.1	—	—	—	—
PGDH	Phosphogluconate dehydrogenase	1.1.1.44	+	+	L	1
PGM	Phosphoglucomutase	5.4.2.2	+	+	M	1
PYDH	Pyrroline dehydrogenase	1.5.1.12	+	+	L	2
SKDH	Shikimate dehydrogenase	1.1.1.25	—	—	—	—
SOD	Superoxide dismutase	1.15.1.1	+	—	—	—
SORDH	Sorbitole dehydrogenase	1.1.1.14	+	+	L	2
XDH	Xanthine dehydrogenase	1.2.1.37	+	—	—	—

Act, Activity; Res, resolution; Tis, tissue. *+, Good; —, poor or absent. †+, Good; —, not resolvable. ‡M, Epaxial muscle; L, liver; E, eye.

$\ln[p(1)] = a\ln(N_m) + b$ [where $p(1)$ is the average frequency of alleles found in only one population sampled; a and b are constants that depend on the number of individuals sampled per population]. The former method assumes an island model of population structure; however, it has been demonstrated that it will yield a relatively sound estimate for other models of population structure, such as a stepping-stone array (Slatkin, 1994). These methods estimate gene flow rates assuming that, on average, allozyme variation across loci is neutral.

RESULTS

PILOT STUDY

Seventeen of the 39 enzymes assayed were not scorable either because of inadequate staining or because the resulting patterns were uninterpretable (Table I). ALDO, GPD, G6PDH and IDH gave different electrophoretic patterns for apaxial muscle and liver tissues, and GPI had two different patterns for epaxial muscle and eye tissues, thus each of these enzymes was analysed for the two tissues (Table I).

The genetic basis of the banding patterns should be determined before commencing any statistical analysis of electrophoretic data (Weir, 1996); in most cases, however, it is impractical or impossible to carry out such direct tests. In these cases it is necessary to rely on indirect evidence to verify the genetic basis of the observed variation. Lacking direct genetic evidence for *A. fasciatus*, the following indirect criteria were adopted to establish the genetic nature of the observed variation in enzyme banding patterns: (1) the isozyme banding patterns of presumed heterozygotes agreed with those predicted from the known subunit structure of these enzymes in other fishes for GDH, GPD, GPI, LDH, ME, MPI, PGDH, PGM (Markert *et al.*, 1975; Bolch *et al.*, 1993; Lee *et al.*, 1995) and in other vertebrates (Richardson *et al.*, 1986); (2) with the exception of only the *GPD-2** locus, all probability tests for agreement with Hardy–Weinberg expectations failed to reveal significant deviations. The only sigificant departure from Hardy–Weinberg equilibrium occurred at *GPD-2** locus in the Piombino population (exact $P=0{\cdot}021$) and was probably due to a sampling error. For this locus the deviation from equilibrium was due to an excess of heterozygote individuals.

The requirement of a pilot study were widely satisfied, since more than a sufficient number (12) of suitable genetic markers were identified and detailed information on the genetic structure of the species was found.

GENETIC VARIATION WITHIN POPULATIONS

Twelve of the 43 loci examined in *A. fasciatus* were polymorphic (Table II). For each population, no more than two alleles per locus were observed, with the exception of three at *MPI** in the sample from Sabaudia Lake. The mean number of alleles per locus was 1·2 in Sabaudia population and 1·1 in all the other populations (Table III). The lowest percentage of polymorphic loci was observed in the population from Casaraccio Pond and Orbetello Lagoon ($P_{0{\cdot}99}=12$ each), whereas the highest value occurred in the population from Sabaudia Lake ($P_{0{\cdot}99}=21$) (Table III). Mean values of observed and expected heterozygosity ranged from 0·024 (S.E.=0·011) to 0·060 (S.E.=0·023) and from 0·027 (S.E.=0·013) to 0·064 (S.E.=0·023), respectively. The average jackknifed f, indicating the level of genetic heterogeneity within populations, was 0·023 (S.E.=0·035), not significantly different from zero ($P=0{\cdot}261$).

GENETIC VARIATION AMONG POPULATIONS

A high degree of genetic variability among populations is shown by the presence of various private alleles: *GDH-2**102, *G6PDH-1**102, *ME-2**98 in Casaraccio Pond population and *AO-2**98, *GDH-2**98, *G6PDH-2**102, *GPI-2**96, *MPI**98 in the Sabaudia Lake population (Table II). For all samples the

TABLE II. *A. fasciatus*, allele frequencies at 12 polymorphic loci†

Locus	Allele	Population				
		CAS	ELB	ORB	PIO	SAB
*AO-2**	100	1·000	1·000	1·000	1·000	0·893
	98	0·000	0·000	0·000	0·000	0·107
(*n*)		(26)	(20)	(24)	(28)	(28)
*GDH-2**	102	0·074	0·000	0·000	0·000	0·000
	100	0·926	1·000	1·000	1·000	0·983
	98	0·000	0·000	0·000	0·000	0·017
(*n*)		(27)	(28)	(29)	(30)	(30)
*GPD-2**	100	1·000	0·412	0·646	0·340	0·817
	98	0·000	0·588	0·354	0·660	0·183
(*n*)		(21)	(17)	(24)	(25)	(30)
*GPD-3**	102	0·000	0·050	0·067	0·100	0·017
	100	1·000	0·950	0·933	0·900	0·983
(*n*)		(27)	(30)	(30)	(30)	(30)
*G6PDH-1**	102	0·204	0·000	0·000	0·000	0·000
	100	0·796	1·000	1·000	1·000	1·000
(*n*)		(27)	(30)	(28)	(28)	(30)
*G6PDH-2**	102	0·000	0·000	0·000	0·000	0·133
	100	1·000	1·000	1·000	1·000	0·867
(*n*)		(26)	(30)	(30)	(29)	(30)
*GPI-1**	100	0·900	1·000	1·000	1·000	0·367
	98	0·100	0·000	0·000	0·000	0·633
(*n*)		(25)	(30)	(12)	(30)	(30)
*GPI-2**	100	1·000	0·483	0·900	0·433	0·672
	98	0·000	0·517	0·100	0·567	0·000
	96	0·000	0·000	0·000	0·000	0·328
(*n*)		(25)	(29)	(15)	(30)	(29)
*LDH-3**	100	0·000	0·600	0·550	0·917	0·483
	98	1·000	0·400	0·450	0·083	0·517
(*n*)		(30)	(30)	(30)	(30)	(30)
*ME-2**	100	0·950	1·000	1·000	1·000	1·000
	98	0·050	0·000	0·000	0·000	0·000
(*n*)		(30)	(27)	(30)	(30)	(30)
*MPI**	102	0·00	0·317	0·259	0·233	0·567
	100	1·000	0·683	0·741	0·767	0·417
	98	0·000	0·000	0·000	0·000	0·017
(*n*)		(30)	(30)	(29)	(30)	(30)
*PGM**	100	0·733	0·983	1·000	0·917	1·000
	98	0·267	0·017	0·000	0·083	0·000
(*n*)		(30)	(30)	(30)	(30)	(30)

n, Number of individuals sampled for each locus.
Population abbreviations are as in Fig. 1.
†Loci *ADH-1**, *ADH-2**, *ALDO-1**, *ALDO-2**, *ALDO-3**, *AK**, *AO-1**, *CK-1**, *CK-2**, *FBP**, *FH**, *GAPD-1**, *GAPD-2**, *GDH-1**, *GLYDH**, *GPD-1**, *IDH-1**, *IDH-2**, *LDH-1**, *LDH-2**, *MDH-1**, *MDH-2**, *MDH-3**, *MDH-4**, *ME-1**, *ME-3**, *PGDH**, *PYDH-1**, *PYDH-2**, *SORDH-1**, *SORDH-2** showed no allelic variation in all samples analysed.

magnitude of genetic subdivision, measured as the average jackknifed θ was 0·302 (S.E.=0·045). This value was significantly greater than zero ($P<0·001$). Values of N_m calculated by Wright's (1943) method and by Slatkin's (1985)

TABLE III. *A. fasciatus*, measures of genetic variability calculated for each population

Population	CAS	ELB	ORB	PIO	SAB
Mean sample size per locus	26·0 (0·8)	28·3 (0·6)	25·4 (1·0)	28·1 (0·5)	29·4 (0·4)
Mean no. of alleles per locus	1·1 (0·0)	1·1 (0·1)	1·1 (0·0)	1·1 (0·1)	1·2 (0·1)
Percentage of loci polymorphic	12	14	12	14	21
Mean observed heterozygosity	0·024 (0·011)	0·048 (0·022)	0·037 (0·017)	0·045 (0·021)	0·060 (0·023)
Mean expected heterozygosity	0·027 (0·013)	0·048 (0·022)	0·039 (0·018)	0·042 (0·018)	0·064 (0·023)

Standard errors are in parentheses. Population abbreviations are as in Fig. 1.

TABLE IV. *A. fasciatus*, values of Nei's (1978) genetic distance (above diagonal) and modified Rogers' (Wright, 1978) genetic distance (below diagonal)

	CAS	ELB	ORB	PIO	SAB
CAS	—	0·029	0·015	0·042	0·027
ELB	0·167	—	0·005	0·002	0·021
ORB	0·122	0·074	—	0·010	0·015
PIO	0·202	0·053	0·103	—	0·029
SAB	0·163	0·143	0·123	0·168	—

Population abbreviations are as in Fig. 1.

private alleles method were 0·578 and 0·445 effective migrants per generation, respectively, both indicating low levels of gene flow among populations.

Analyses of population genetic differentiation carried out using Nei's and modified Rogers' genetic distances gave values ranging from D_N=0·002 to 0·042 and from D_R=0·053 to 0·202, respectively (Table IV). The presence of 27 loci fixed for the same allele in all populations lowered these values greatly. The relatively high value of cophenetic correlation (r=0·870) suggests that the dendrogram of Nei's genetic distances (Fig. 2) represents the original genetic distance matrix accurately. The value of stress (s=0·000) indicates that distances among populations on the MDS plot represent the genetic distances accurately (Fig. 3). MDS showed population subdivision consistent with both the genetic relationship depicted in the UPGMA dendrogram and the geographical distribution of sites. Mantel's test confirmed that genetic differentiation among populations is correlated with geographical distance (g=1·941; P<0·001).

DISCUSSION

GENETIC VARIATION WITHIN POPULATIONS

The five populations of *A. fasciatus* analysed are characterized by low levels of allozymic polymorphism (Table III), with expected heterozygosity comparable

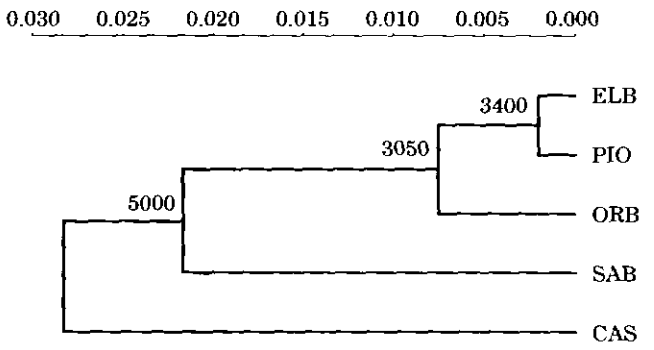

FIG. 2. UPGMA dendrogram of Nei's (1978) genetic distance among populations sampled. The dendrogram is a consensus tree based on bootstrapping with 5000 replicates; node values indicated on branching points represent the number of times a particular cluster group of *A. fasciatus* was formed out of 5000 iterations. Population abbreviations are as in Fig. 1.

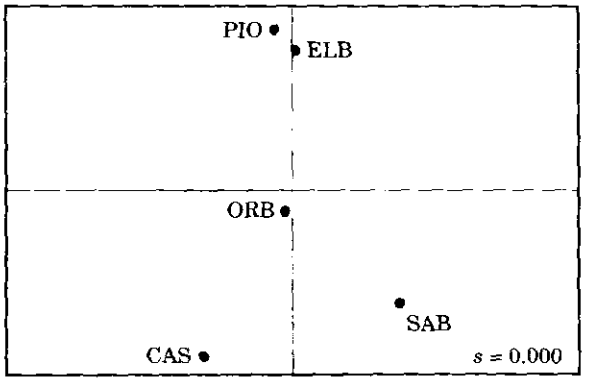

FIG. 3. Multidimensional scaling of modified Rogers' (Wright, 1978) genetic distances. Population abbreviations are as in Fig. 1.

to the mean values for anadromous and freshwater fish species reported by Gyllensten (1985), but lower than those reported by Ward *et al.* (1994). The results reflect the characteristically low levels of polymorphism in many typical brackishwater invertebrate species (Battaglia *et al.*, 1978; Abbiati & Maltagliati, 1996). Furthermore, the closely related species, *A. iberus* (Valenciennes), having ecological and biological characteristics similar to *A. fasciatus*, also showed low levels of genetic polymorphism, with slightly higher values of heterozygosity (H_{exp} range=0·023–0·109) (Doadrio *et al.*, 1996). The different values obtained for *A. iberus* are probably due to either the lower number of loci analysed, or the different set of enzymes assayed. The low levels of genetic variability in *A. fasciatus* can be explained by random loss of alleles in isolated local populations through the effects of genetic drift. Alternatively, one among the most extended theories reported for explaining low polymorphism in brackishwater species is related to adaptation to brackishwater habitats, which requires tolerance of considerable fluctuation of physicochemical parameters, such as salinity, temperature and oxygen concentration (Cognetti, 1994 and references therein).

GENETIC VARIATION AND GENE FLOW AMONG POPULATIONS

A high degree of isolation among populations, with F_{ST} values usually >0·450, has been detected in populations of various species of brackish- and freshwater cyprinodontids (Ashbaugh *et al.*, 1994; Doadrio *et al.*, 1996). *Aphanius fasciatus* is no exception to this trend, and population structuring was as strong as in other cyprinodontid fishes. Weir & Cockerham's (1984) coancestry coefficient showed that *A. fasciatus* populations are differentiated genetically with a mean value of jackknifed θ of the same magnitude of the average value found by Gyllensten (1985) (reported as G_{ST} by the author) in 10 freshwater fish species. Despite the low mean number of alleles per locus in all populations (Table III), the presence of various allelic forms (Table II) is evidence of great genetic heterogeneity among populations. The small values of the mean number of alleles per locus are due to the presence of various private alleles, which can be found in only one or few samples. This genetic characteristic produces moderate Nei's (1978) genetic distances, ranging from 0·002 to 0·042, which are typical of conspecific populations of cyprinodontid species. In fact, they are of the same magnitude of those observed among populations of *Cyprinodon macularius* Baird & Girard (D_N=0·002–0·042) (Turner, 1983) and the Mediterranean populations of *A. iberus* (D_N=0·002–0·096) (Doadrio *et al.*, 1996).

Generally life-history adaptations have evolved in response to selection for restricted dispersal, thus the likelihood of reproductive isolation of populations is increased and their genetic differentiation is likely (Waples, 1987). Life-history traits of *A. fasciatus*, such as benthic eggs and absence of larval stages and habitat preferences, determine a low potential for dispersal, which is consistent with the observed high degree of genetic differentiation among its populations. In addition, the genetic structure of *A. fasciatus* results from the interaction of life-history traits and natural fragmentation of habitats, which determine the observed isolation of local populations.

A clear geographical trend of genetic differentiation among populations emerged from the analyses of genetic distances (Figs 2 and 3) and it was confirmed by a significant positive correlation between the genetic and geographical distance matrices. These analyses support Wright's (1943) isolation-by-distance model of genetic structuring in *A. fasciatus*. Although *A. fasciatus* is considered a non-migratory species, exchange of individuals from one habitat to another is possible, in fact some specimens have been recorded in the open sea mixed with juveniles of *Sardinia pilchardus* Risso, 20–70 m from the coast (Torchio, 1967). The presence of this species in the marine environment is considered occasional and probably is connected with unpredictable events, such as exceptional rainfalls or floods that might flush individuals of *A. fasciatus* away from brackishwater habitats to the open sea.

Analyses of gene flow carried out using either Wright's (1943) or Slatkin's (1985) methods were consistent and gave very low values of N_m. From an ecological perspective, these estimates of gene flow confirm the occurrence of restricted migration between populations and imply that genetic differentiation over time may have occurred due to genetic drift. The natural fragmentation of the brackish-water habitats, contributing to the disjunct coastal distribution of *A. fasciatus* and the observed estimates of gene flow among populations suggest that the one-dimensional stepping-stone model (Kimura & Weiss, 1964; Slatkin,

1994) may be a more apppropriate model to describe the mechanisms of genetic substructuring of the species. However, the present study provides only a rough insight into the pattern of gene flow among populations of *A. fasciatus*, given that only five localities were analysed. Further investigations on a greater number of individuals and populations will allow the application of powerful methods, such as the regression approach, recently introduced by Slatkin (1993, 1994), to demonstrate the modality of gene flow clearly in this cyprinodontid species.

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