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Genetic structure of the common sole *Solea vulgaris* at different geographic scales

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Abstract The genetic structure of the flatfish *Solea vulgaris* was investigated on several spatial scales and at the temporal level through analysis of electrophoretic variation at 8 to 12 polymorphic enzyme loci. No differentiation was apparent at the temporal scale. Some differentiation was detected at and above the regional scale. Isolation by distance was evidenced by the significant correlation between genetic and geographic distances, and by the consistency of the results of multiple-locus correspondence analysis with geographic sampling patterns. The analysis suggested that the geographic unit of population structure (i.e. a geographical area corresponding to a panmictic or nearly panmictic population) lies within a radius of the order of 100 km. The isolation-by-distance pattern in *S. vulgaris* contrasted with the known genetic structures of other flatfish species of the northeastern Atlantic and Mediterranean in a way that may be related to the range of their respective temperature tolerances for eggs and larvae.

Introduction

Marine fish species usually show little geographic divergence (Gyllensten 1985), and examples of homogeneity among populations of marine fish separated by thousands of kilometres are numerous (Grant et al. 1984; Shaklee and Samollow 1984; Grant et al. 1987 among others). Counterexamples, with significant genetic heterogeneity among samples from localities separated by distances short enough to lie within the animals' potential migration range, are also known (Lacson et al. 1989; Planes et al. 1994). Waples (1987) studied the structures and gene flow in several coastal species differing in the duration of their larval stages, and found that population differentiation was negatively correlated with the dispersal potential of the pelagic stages, implying that ecological factors did not exert differential pressures and that populations had already reached equilibrium with respect to migration and genetic drift. The comparison over their entire geographic range (northeastern Atlantic and Mediterranean), of the genetic structures of turbot (*Scophthalmus maximus*), brill (*S. rhombus*) and flounder (*Platichthys flesus*), three flatfishes with similar habitat and life-history features including larval stage durations, lead to different conclusions. There was a high degree of population differentiation in the flounder (Galleguillos and Ward 1982; Borsa et al. 1987), no differentiation apparent in the brill, and an intermediate pattern in the turbot (Blanquer et al. 1992), suggesting that factors other than the duration of the pelagic stage are important, or have been so in the past.

The studies referred to above did not address the question of the geographical scale of population differentiation. Assessing the degree of differentiation between geographical units as a function of spatial scale makes it possible to define the limit beneath which populations can be considered panmictic or nearly so, and above which they can be considered genetically structured. In addition, investigation of population differentiation in the temporal dimension is expected to yield information on natural selection and on variability in the parental origin of successive cohorts. Because evolutionary forces may act on populations at specific and differing scales, multiple-scale investigation of their genetic structure is necessary.

The present work reports on a multiple spatial-scale study of the genetic structure of the common sole *Solea vulgaris* (Quensel) over most of its distributional range (northeastern Atlantic and Mediterranean). The choice of this species, which is also of economic importance, meets several of the requirements expressed above. Extensive information on its biology, ecology and behaviour has been gathered recently, with emphasis on the pelagic and juve-

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nile stages (e.g. Coggan and Dando 1988; Koutsikopoulos et al. 1991; Rijnsdorp et al. 1992; Rogers 1992). The biogeography of fish communities in the northeastern Atlantic–Mediterranean region has been subject to some research (Hureau and Monod 1973; Klausewitz 1973; Quignard 1978), and the within-species level has been addressed in a few flatfish species (Galleguillos and Ward 1982; Borsa et al. 1987; Blanquer et al. 1992).

Materials and methods

Sampling

Solea vulgaris was collected from various locations along the coasts of the French Atlantic Ocean and the western and eastern Mediterranean Sea (Fig. 1) between 1981 and 1986. Samples were obtained by trawling, except in the Mediterranean lagoons Arnel, Mauguio, Pierre-Blanche, Prévost, where an artisanal network of nets and traps ("capetchade") was used.

The sampling design was hierarchical, examining successive spatial scales: geographic regions (thousands of kilometres), zones within each region (hundreds of kilometres), and localities within zones (tens of kilometres). The coastal circulation [according to Ovchinnikov (1966) for the Mediterranean and Harden Jones (1968) for the northeastern Atlantic] exhibits major discontinuities in Galicia, in the Gibraltar strait–Alboran Sea region, and between the eastern and the western Mediterranean basins. The three sampling regions of *Solea vulgaris* – the northeastern Atlantic and the western and eastern Mediterranean, thus corresponded to objective geographical (oceanographical) regions.

Five localities (Vilaine estuary, Ebro delta, Agde, Grau du Roi, and Marseille) were re-sampled up to four times on different sampling dates, thereby introducing a temporal scale into the investigation of genetic structure. The sample collected at Vilaine in July 1986 had a non-overlapping, bimodal length-distribution. This sample was separated into two subsamples (V2 and V4 in Table 1) under the assumption that it consisted of two distinct cohorts (0+ and 1+).

Allozyme electrophoresis

The sole were transported on ice to the laboratory immediately after collection, or later frozen in dry ice (whole fish) or in liquid nitrogen (tissues). A piece of muscle and a piece of liver of each individual were dissected and homogenised at 0 to 4°C in an equal volume of 0.1 *M* Tris/HCl/EDTA/NADP buffer (pH 6.8). The supernatant obtained after centrifugation at 22 000 $\times g$ for 30 min at 4°C was used as the soluble enzyme extract. It was stored at –80°C until it was run on horizontal starch gel for allozyme electrophoresis.

Genetic variation was investigated at 12 presumed allozyme loci (Table 2) found to be polymorphic from the total set of 23 presumed allozyme loci scored by Pasteur et al. (1985). Electrophoresis and staining were carried out as described by Pasteur et al. (1985, 1987).

Electrophoretic variation observed for all enzymes in the present study except LDH-2 conformed to a simple Mendelian model of codominance according to the known quaternary structure of each enzyme (Quignard et al. 1984; Pasteur et al. 1985). Patterns observed for LDH-2 were atypical, with two (instead of five) bands observed in presumed heterozygotes (Pasteur et al. 1985).

Parameters of population structure

Single-locus *F*-statistics (Wright 1969) were estimated using the parameters f (for correlation between alleles in an individual relative to its subpopulation, F_{IS}) and θ (for correlation between alleles in a subpopulation relative to the total population, F_{ST}) of Weir and Cockerham (1984). Monolocus f values were compared to zero, assuming that $f\sqrt{N}$ (N=sample size) has a normal distribution under equilibrium (Brown 1970). Multiallelic, single-locus f and θ, and multilocus f and θ values were estimated as weighted averages over alleles and over loci [Eq. (10) of Weir and Cockerham], respectively, and their standard deviations over loci were estimated using jack-

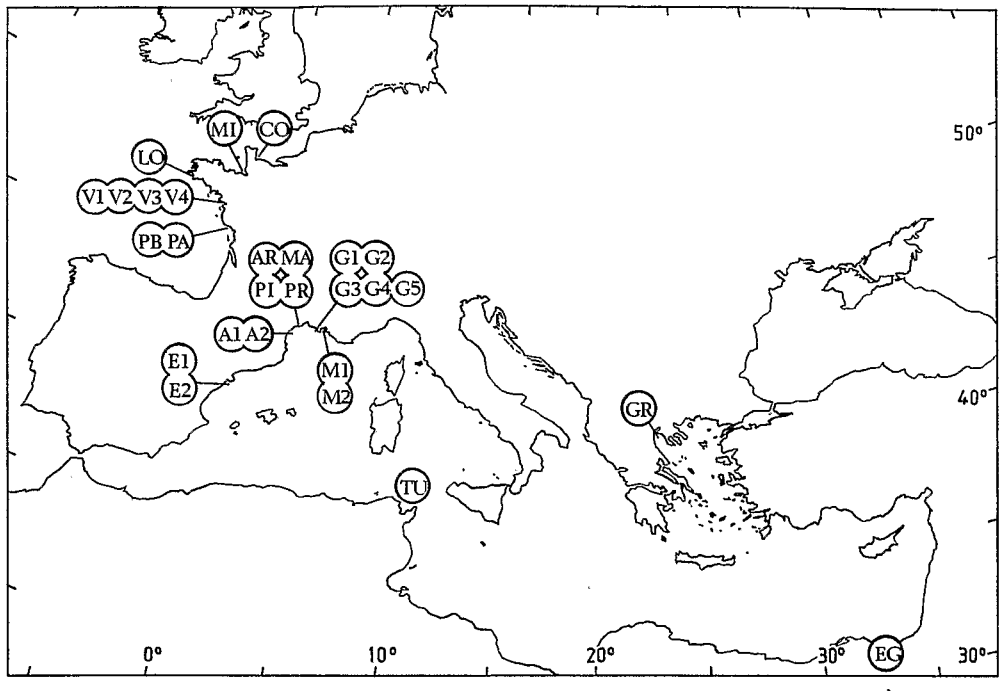

Fig. 1 Sampling locations for *Solea vulgaris* in northeastern Atlantic and Mediterranean Sea. Locations detailed in Table 1

Table 1 *Solea vulgaris*. Samples collected for allozyme analysis (*N* sample size; *Adults* fish of large size; *Genitors* adult fish caught during reproductive period; *Juveniles 0+*, *1+*, *2+* young of the year, 1 yr-old and 2 yr-old individuals, respectively; *Heterogeneous* samples including several of the foregoing categories)

Sample		Date	(*N*)	Observations on age
Northeastern Atlantic				
Manche				
CO	Cotentin	Nov. 1981	(29)	Adults
MI	Mont Saint-Michel	Nov. 1981	(30)	Adults
Bretagne				
LO	Loctudy	Apr. 1982	(12)	Adults
Golfe de Gascogne				
V1	Vilaine estuary	Nov. 1981	(29)	Adults
V2	Vilaine estuary	July 1986	(120)	Juveniles 0+
V3	Vilaine estuary	Sep. 1986	(115)	Juveniles 0+
V4	Vilaine estuary	July 1986	(120)	Juveniles 1+
PB	Pertuis breton	Aug. 1986	(81)	Juveniles 1+
PA	Pertuis d'Antioche	Aug. 1986	(44)	Juveniles 1+
Western Mediterranean				
Ebro delta				
E1[a]	Ebro delta	Mar. 1985	(30)	Adults
E2	Ebro delta	Feb. 1986	(68)	Juveniles 1+ and 2+
Golfe du Lion				
A1	Agde	Mar. 1984	(30)	Genitors
A2	Agde	Sep. 1984	(26)	Adults
AR	Etang de l'Arnel	1981–1982	(15)	Heterogeneous
MA	Etang de Mauguio	1981–1982	(20)	Heterogeneous
PI	Etang de Pierre-Blanche	June 1981	(67)	Juveniles 0+
PR	Etang du Prévost	Oct. 1981	(21)	Adults
G1	Grau du Roi	Jan. 1981	(27)	Adults
G2	Grau du Roi	1981	(8)	Adults
G3	Grau du Roi	Apr. 1981	(80)	Genitors
G4	Grau du Roi	Nov. 1983	(71)	Adults
G5	Grau du Roi	Mar. 1984	(74)	Genitors
M1	Marseille	Mar. 1984	(23)	Genitors
M2	Marseille	Sep. 1984	(29)	Adults
Golfe de Tunis				
TU[a]	Tunis	1980	(8)	Adults
Eastern Mediterranean				
Egean Sea				
GR	Thermaikos gulf	Apr. 1986	(64)	Adults
Egypt				
EG[a]	Suez canal	Apr. 1985	(10)	Adults

[a] Samples analysed prior to present study. E1 by She et al. (1987 a), TU by Pasteur et al. (1985), EG by She et al. (1987 b)

knife resampling. Single-locus f values over samples were estimated as unweighted averages and their standard deviations were estimated using jackknife resampling. F-statistics were computed from the matrix of raw individual genotypic data using the F_{ST} procedure of the software GENETIX (Bonhomme et al. 1993). Genetic distances (D) between pairs of populations were computed as $D=-\ln(1-\theta)$ (Reynolds et al. 1983). Each set of single-locus θ values was tested for normality using the package BIOMECO (Lebreton et al. 1990). In this test, critical values have been corrected according to Lilliefors (1967), with rejection of normality when the observed largest difference had a probability of occurrence of < 0.2. In all, 12 sets of Monolocus θ were each tested for normality. Hence we rejected normality when the probability of the largest difference was less than $0.2/12=0.017$. Averages of f and θ were compared to zero using a Student's t-test (Sokal and Rohlf 1969).

Other calculations included estimations of genetic variability, using the number of electromorphs per locus by region (A), and the single-locus genetic diversity index H, unbiased for sample size (N):

$$H=2N/(2N-1)\times(1-\sum_i x_i^2),$$

where x_i is the frequency of the ith electromorph in the sample. The average genetic diversity per sample by region was calculated at each locus as a weighted average of values per sample. Heterogeneities among H and A distribution sets by region were tested using the Kruskal–Wallis test (Sokal and Rohlf 1969) implemented in BIOMECO.

Test for isolation by distance

Pairwise genetic (D) and geographic (d) distance calculations were made between samples pooled at the zone level: Cotentin (CO), Michel (MI), Loctudy (LO), Vilaine (V1 to V4), Pertuis (PB+PA), Ebro (E1 to E2), Lion (A1–A2+AR+MA+PI+PR+G1–G5+M1 to M2), Tunis (TU), Egean (GR) and Egypt (EG) (abbreviations refer to notation in Table 1). Pooled sample sizes ranged from 8 to 491 individuals. The choice of the regional scale was guided both by the results of the multiple-scale analysis of population structure and by the biological observations designating the zone as the objective unit of habitat for a population. The geographic distances between localities were estimated approximately by measuring the coastline on a 1:10 000 000 map.

Mantel's test for the comparison of distance matrices with internal correlation (Manly 1985) was used for testing the correlation

Table 2 *Solea vulgaris*. Protocols for routine screening of electrophoretic variation at 12 polymorphic allozyme loci (*TC* continuous Tris-citrate buffer; *TCN* TC buffer with NADP in gel; *TCB* discontinuous Tris-citrate-borate buffer)

Enzyme	E.C. No.	Tissue	Buffer	(pH)	Loci scored
Aspartate aminotransferase	2.6.1.1	muscle	TC	(8.0)	*Aat-3*
α-glycerophosphate dehydrogenase	1.1.1.8	muscle	TC	(8.0)	*αGpd-1*
		liver	TCN	(6.7)	*αGpd-2*
Glucose-phosphate isomerase	5.3.1.9	muscle	TCB	(8.7)	*Gpi-1, Gpi-2*
Isocitrate dehydrogenase	1.1.1.42	muscle	TC	(6.7)	*Idh-1*
		liver	TCN	(6.7)	*Idh-2*
Lactate dehydrogenase	1.1.1.27	muscle	TCB	(8.7)	*Ldh-2, Ldh-3*
Malate dehydrogenase	1.1.1.37	muscle	TC	(8.0)	*Mdh-2*
6-phosphogluconate dehydrogenase	1.1.1.43	liver	TCN	(6.7)	*6-Pgd*
Phospho-glucomutase	2.7.5.1	muscle	TCB	(8.7)	*Pgm*

between D and d. This test has been implemented as the MANTEL procedure in GENETIX (Bonhomme et al. 1993).

Multidimensional genetic-structure analysis

Correspondence analysis (CA; Benzécri 1973) was used to assess overall relationships between samples. Samples of individuals from each location were each defined by an n-dimensional vector (n=number of characters), so they were positioned as a cluster in an n-dimensional character hyperspace. Multiple-locus allozyme data input consisted of an unweighted sample × allele matrix, where each sample was defined by its allelic counts at the 8 loci (*Aat-3*, *αGpd-1*, *Gpi-1*, *Gpi-2*, *Ldh-2*, *Ldh-3*, *Mdh-2* and *Pgm*) that were investigated over the widest set of samples. The samples taken into account were all those of Table 1 except V1, G1 and TU, in order to maximize both the number of samples and the information available for characterizing them.

CA was performed using the BIOMECO software package (Lebreton et al. 1990). The significance of the results was tested empirically by the use of internal replicates, and by internal re-sampling.

Results

Genetic diversity

Genetic diversity (H) was estimated for each locus in each sample of *Solea vulgaris* (data not shown) on the basis of the electromorph frequency data of Tables 3 to 5. Since the estimator of genetic diversity at a locus was unbiased for sample size, average values over loci with different sample sizes could be calculated. These averages were calculated only in the samples where all loci were scored, thereby allowing comparisons unbiased by locus sampling. The numbers of alleles and the average H values for each locus in the three regions sampled (northeastern Atlantic, western and eastern Mediterranean) are reported in Table 6.

No significant heterogeneity was detected among regions in their distributions of single-locus H and A values (Kruskal–Wallis test; χ^2=1.20, 2 *df*, p>0.5 and χ^2=3.17, 2 *df*, p>0.20 for H and A, respectively), although the average A in the eastern Mediterranean (A=2.25) was smaller than in both the western Mediterranean and the northern Atlantic (A=2.67 in both regions).

Fixation indices

Wright's fixation index was estimated as f for each locus in each sample as an assessment of deviations from Hardy–Weinberg expectations (Table 7). The bottom row of the table yields locus-averaged information for each sample, whereas the last column presents f values for specific loci, averaged over all samples. Only significant positive f values, indicating heterozygote deficiencies at some loci in some samples, were noted. Some extreme f values (f=1) were observed at loci *Aat-3* and *Ldh-2* in Samples A1 and A2, respectively, and both were caused by the occurrence in the sample of a single homozygote for a rare allele, present only in the heterozygous state in other samples. On the whole, no heterozygote deficiency was detected at Locus *Aat-3* (mean f over all samples=0.068; NS); hence we assumed that f=1 at this locus in Sample A2 was an artifact of sampling. The pattern observed at Locus *Ldh-3* was similar [one significant value in sample V4 (p< 0.001), but mean f values over samples=0.056 (NS)] and deserves the same assumption. A significant heterozygote deficiency was apparent at Locus *Ldh-2* in another sample (PA), and so was the value over all samples (mean f over samples=0.178; Student's t-test; 25 *df*; p< 0.05). A slight but significant heterozygote excess was noted at Locus *Mdh-2* (mean f over samples=–0.018; Student's t-test; 24 *df*; p< 0.05).

Only Sample PI could be considered as deviating from Hardy–Weinberg expectations (mean f over loci=0.117; Student's t-test; 10 *df*; p< 0.05). On the whole, however, there was a significant heterozygote deficiency (mean f over samples and loci=0.045; Student's t-test; 11 *df*; p< 0.01), confirming the general trend for heterozygote deficiency at most loci (mean f over samples was positive at 8 loci out of 11).

Heterozygote deficiency was related to age using the Vilaine data set (Samples V1 to V4: individualized cohorts differed from each other by their age). We observed that f(V1)<f(V4)<f(V3)<f(V2) (Table 7), while conversely Age (V1)>Age(V4)>Age(V3)>Age(V2) (Table 1).

Differentiation as a function of scale

Single-locus θ values were calculated at up to 12 loci for replicated samples within the same localities (temporal dimension), among local samples from each of the following zones: Manche, Vilaine+Pertuis and Golfe du Lion (intra-zone scale), among samples pooled for each zone from the following regions: north-eastern Atlantic, west-

Table 3 *Solea vulgaris*. Electromorph frequencies in nine samples from northeastern Atlantic Ocean. Location and enzyme abbreviations as in Tables 1 and 2 (*N* sample size)

Locus, electromorph	Sample								
	CO	MI	LO	V1	V2	V3	V4	PB	PA
Aat-3									
100	1	1	1	1	0.97	0.97	0.99	0.99	1
80	0	0	0	0	0.03	0.03	0.01	0.01	0
(*N*)	(29)	(30)	(12)	(1)	(120)	(114)	(120)	(81)	(44)
αGpd-1									
120	0	0	0	0	0	0	0	0.01	0
115	0.16	0.13	0.13	0.17	0.13	0.11	0.08	0.10	0.10
100	0.84	0.87	0.88	0.83	0.87	0.89	0.91	0.88	0.89
85	0	0	0	0	0	0	0.02	0.01	0.01
(*N*)	(29)	(30)	(12)	(6)	(120)	(97)	(120)	(81)	(44)
αGpd-2									
115	–	–	–	–	0.06	0.02	0.08	0.06	0.07
110	–	–	–	–	0	0	0	0	0
100	–	–	–	–	0.90	0.96	0.86	0.90	0.87
95	–	–	–	–	0	0	0.01	0	0
80	–	–	–	–	0.04	0.02	0.05	0.04	0.06
(*N*)	–	–	–	–	(100)	(84)	(114)	(78)	(43)
Gpi-1									
140	0	0	0	0	0	0	0	0	0
120	0.05	0	0	0.03	0.00	0	0	0	0
115	0	0	0.04	0	0.04	0.01	0.05	0.04	0
105	0	0	0	0	0	0	0	0	0
100	0.95	0.98	0.96	0.97	0.94	0.98	0.95	0.96	1
90	0	0	0	0	0.01	0.01	0	0	0
80	0	0.02	0	0	0.01	0	0	0	0
(*N*)	(29)	(30)	(12)	(29)	(120)	(102)	(120)	(80)	(42)
Gpi-2									
120	0	0	0	0.04	0	0	0.01	0.01	0
115	0	0.02	0	0	0	0	0	0	0
110	0.19	0.13	0.13	0.14	0.11	0.15	0.17	0.14	0.09
100	0.81	0.85	0.88	0.79	0.86	0.81	0.80	0.84	0.91
95	0	0	0	0	0	0	0	0	0
90	0	0	0	0.04	0	0	0.00	0	0
85	0	0	0	0	0.03	0.03	0.01	0.01	0
70	0	0	0	0	0	0	0	0	0
(*N*)	(29)	(30)	(12)	(28)	(120)	(104)	(120)	(81)	(44)
Idh-1									
120	–	–	–	–	0.01	0.01	0.01	0.01	0.02
100	–	–	–	–	0.98	0.96	0.99	0.99	0.97
85	–	–	–	–	0	0.01	0	0	0
80	–	–	–	–	0.01	0.02	0	0.01	0.01
(*N*)	–	–	–	–	(120)	(110)	(120)	(81)	(44)
Idh-2									
120	–	–	0.04	–	0.04	0.01	0.03	0	0.01
100	–	–	0.63	–	0.65	0.64	0.70	0.71	0.64
80	–	–	0.33	–	0.31	0.35	0.26	0.29	0.35
(*N*)	–	–	(12)	–	(120)	(107)	(120)	(81)	(44)
Ldh-2									
120	0	0	0	0	0	0	0	0	0
110	0.02	0.05	0	0.07	0.04	0.04	0.04	0.06	0.03
100	0.98	0.95	1	0.93	0.95	0.94	0.96	0.92	0.97
90	0	0	0	0	0.00	0.02	0	0.02	0
70	0	0	0	0	0	0	0	0	0
(*N*)	(29)	(30)	(12)	(29)	(120)	(92)	(119)	(80)	(44)
Ldh-3									
100	1	0.98	1	1	0.99	0.99	0.98	0.98	0.98
70	0	0.02	0	0	0.01	0.01	0.02	0.02	0.01
58	0	0	0	0	0	0	0	0	0.01
(*N*)	(29)	(30)	(12)	(8)	(120)	(74)	(120)	(81)	(44)

(continued overleaf)

Table 3 (continued)

Locus, electromorph	Sample								
	CO	MI	LO	V1	V2	V3	V4	PB	PA
Mdh-2									
130	0	0.02	0.04	0	0	0	0	0	0
120	0	0	0	0	0	0	0	0	0
110	0	0	0	0	0	0	0	0	0
105	0	0	0	0	0.01	0	0	0	0
100	0.90	0.95	0.96	0.98	0.94	0.96	0.96	0.98	0.95
85	0	0	0	0	0	0.02	0.01	0.01	0.03
80	0.10	0.03	0	0.02	0.05	0.02	0.03	0.01	0.01
(*N*)	(29)	(30)	(12)	(29)	(120)	(115)	(120)	(81)	(44)
6-PgdN									
110	–	–	–	–	0.01	0	0.02	0	0
100	–	–	–	–	0.56	0.50	0.58	0.53	0.60
80	–	–	–	–	0.44	0.50	0.39	0.47	0.40
60	–	–	–	–	0	0	0	0	0
(*N*)	–	–	–	–	(100)	(25)	(109)	(81)	(44)
Pgm									
135	0	0	0	0	0	0	0	0	0
130	0.03	0.02	0	0	0	0.	0.00	0	0
115	0	0	0	0	0.01	0.01	0.02	0.01	0
100	0.95	0.98	1	0.98	0.98	0.98	0.98	0.99	0.98
80	0.02	0	0	0.02	0.01	0.00	0.00	0	0.02
(*N*)	(29)	(30)	(12)	(29)	(120)	(104)	(120)	(81)	(44)

Table 4 *Solea vulgaris*. Electromorph frequencies in nine samples from Mediterranean (other than Golfe du Lion). Further details in legend to Table 3

Locus, electromorph	Sample								
	E1	E2	A1	A2	M1	M2	TU	GR	EG
Aat-3									
100	1	1	0.98	0.96	0.98	0.98	1	0.99	1
80	0	0	0.02	0.04	0.02	0.02	0	0.01	0
(*N*)	(30)	(68)	(30)	(26)	(23)	(29)	(5)	(64)	(10)
αGpd-1									
120	0	0	0	0	0	0	0	0.01	0
115	0.18	0.21	0.15	0.15	0.15	0.17	0	0.04	0.15
100	0.82	0.79	0.85	0.85	0.83	0.83	1	0.93	0.85
85	0	0	0	0	0.02	0	0	0.02	0
(*N*)	(30)	(68)	(30)	(26)	(23)	(29)	(8)	(64)	(10)
αGpd-2									
115	0	0	0.05	0	0.02	0	0	0	–
110	0.04	0.01	0	0	0	0	0	0	–
100	0.96	0.94	0.92	1	0.93	1	1	1	–
95	0	0	0	0	0	0	0	0	–
80	0	0.04	0.03	0	0.04	0	0	0	–
(*N*)	(28)	(68)	(30)	(26)	(23)	(29)	(8)	(64)	–
Gpi-1									
140	0	0	0	0	0	0	0	0	0
120	0	0	0	0	0	0	0	0	0
115	0.05	0.10	0.05	0.06	0.02	0	0	0.17	0.15
105	0.02	0	0	0	0	0	0	0	0
100	0.92	0.88	0.92	0.94	0.98	0.98	1	0.83	0.85
90	0	0	0	0	0	0	0	0	0
80	0.02	0.02	0.03	0	0	0.02	0	0	0
(*N*)	(30)	(68)	(30)	(26)	(23)	(29)	(8)	(64)	(10)

Table 4 (continued)

Locus, electro-morph	Sample								
	E1	E2	A1	A2	M1	M2	TU	GR	EG
Gpi-2									
120	0	0.01	0	0	0	0	0	0	0
115	0	0	0	0	0	0	0	0	0
110	0.02	0.07	0.05	0.06	0.07	0.07	0.06	0	0.05
100	0.97	0.89	0.93	0.94	0.93	0.93	0.94	0.98	0.95
95	0	0	0	0	0	0	0	0.01	0
90	0	0	0	0	0	0	0	0.01	0
85	0.02	0.02	0.02	0	0	0	0	0	0
70	0	0.01	0	0	0	0	0	0	0
(*N*)	(30)	(68)	(30)	(26)	(23)	(29)	(8)	(64)	(10)
Idh-1									
120	0	0	0	0	0	0	–	0	0
100	1	1	1	1	1	1	–	0.99	1
85	0	0	0	0	0	0	–	0	0
80	0	0	0	0	0	0	–	0.01	0
(*N*)	(30)	(68)	(30)	(1)	(23)	(29)	–	(64)	(10)
Idh-2									
120	–	0.01	–	–	–	–	–	0	–
100	–	0.71	–	–	–	–	–	0.45	–
80	–	0.29	–	–	–	–	–	0.55	–
(*N*)	–	(68)	–	–	–	–	–	(64)	–
Ldh-2									
120	0	0	0	0	0	0	0	0	0
110	0.07	0.07	0.13	0.10	0.15	0.14	0	0.24	0.25
100	0.93	0.92	0.87	0.87	0.85	0.86	0.94	0.76	0.75
90	0	0.01	0	0.04	0	0	0	0	0
70	0	0	0	0	0	0	0.06	0	0
(*N*)	(30)	(68)	(30)	(26)	(23)	(29)	(8)	(64)	(10)
Ldh-3									
100	1	0.99	1	1	1	1	1	1	1
70	0	0	0	0	0	0	0	0	0
58	0	0.08	0	0	0	0	0	0	0
(*N*)	(30)	(68)	(30)	(26)	(23)	(29)	(8)	(64)	(10)
Mdh-2									
130	0	0	0	0	0	0	0	0	0
120	0.02	0	0.03	0	0.04	0	0	0	0
110	0	0	0	0	0	0	0	0	0.05
105	0	0	0	0	0	0	0	0	0
100	0.93	0.97	0.92	0.98	0.93	0.98	0.94	1	0.95
85	0	0.02	0	0	0	0	0	0	0
80	0.05	0.01	0.05	0.02	0.02	0.02	0.06	0	0
(*N*)	(30)	(68)	(30)	(26)	(23)	(29)	(8)	(64)	(10)
6-Pgd									
110	0	0	0	0	0	0	–	0	–
100	0.43	0.48	0.60	0.42	0.48	0.62	–	0.38	–
80	0.57	0.52	0.40	0.58	0.52	0.38	–	0.62	–
60	0	0	0	0	0	0	–	0.01	–
(*N*)	(29)	(68)	(29)	(26)	(23)	(29)	–	(64)	–
Pgm									
135	0	0	0	0	0	0	0	0	0
130	0	0.01	0	0	0	0.02	0	0	0
115	0	0	0.02	0	0	0	0	0	0.15
100	1	0.99	0.97	1	1	0.98	1	1	0.85
80	0	0.01	0.02	0	0	0	0	0	0
(*N*)	(30)	(68)	(30)	(26)	(23)	(29)	(8)	(64)	(10)

Table 5 *Solea vulgaris*. Electromorph frequencies in 9 samples from Golfe du Lion. Further details in legend to Table 3

Locus, electromorph	Sample								
	AR	MA	PI	PR	G1	G2	G3	G4	G5
Aat-3									
100	1	0.96	0.99	0.98	–	0.94	1	1	0.99
80	0	0.04	0.01	0.02	–	0.06	0	0	0.01
(*N*)	(13)	(12)	(67)	(21)	–	(8)	(80)	(71)	(74)
αGpd-1									
120	0	0	0	0	0	0	0	0	0
115	0	0.08	0.14	0.05	0.17	0.13	0.19	0.17	0.07
100	1	0.90	0.86	0.95	0.83	0.88	0.81	0.83	0.92
85	0	0.03	0	0	0	0	0	0	0.01
(*N*)	15)	(20)	(67)	(21)	(27)	(8)	(80)	(69)	(74)
αGpd-2									
115	–	–	0.02	–	–	0	0.01	0.03	0.03
110	–	–	0.02	–	–	0.06	0.02	0.03	0
100	–	–	0.93	–	–	0.94	0.95	0.92	0.96
95	–	–	0	–	–	0	0	0	0
80	–	–	0.02	–	–	0	0.02	0.03	0.01
(*N*)	–	–	(67)	–	–	(8)	(73)	(71)	(71)
Gpi-1									
140	0	0	0	0	0	0	0	0	0.01
120	0.03	0.03	0.01	0.02	0	0	0.03	0.01	0
115	0	0	0.04	0	0	0	0.08	0.04	0.07
105	0	0	0	0	0	0	0	0	0
100	0.97	0.98	0.94	0.93	1	1	0.89	0.94	0.93
90	0	0	0.01	0	0	0	0	0	0
80	0	0	0	0.05	0	0	0	0.01	0
(*N*)	(15)	(20)	(67)	(21)	(27)	(8)	(80)	(70)	(74)
Gpi-2									
120	0	0	0	0	0	0	0	0.01	0.01
115	0	0	0	0	0	0	0	0	0.01
110	0	0.08	0.13	0.05	0.02	0.06	0.06	0.07	0.05
100	1	0.92	0.87	0.95	0.98	0.94	0.94	0.89	0.91
95	0	0	0	0	0	0	0	0	0
90	0	0	0.01	0	0	0	0.01	0.03	0.01
85	0	0	0	0	0	0	0	0	0
70	0	0	0	0	0	0	0	0	0
(*N*)	(8)	(12)	(67)	(21)	(25)	(8)	(80)	(70)	(73)
Idh-1									
120	–	–	0	–	–	0	0	0	0.01
100	–	–	0.99	–	–	1	1	1	0.99
85	–	–	0	–	–	0	0	0	0
80	–	–	0.01	–	–	0	0	0	0.01
(*N*)	–	–	(67)	–	–	(8)	(80)	(71)	(74)
Idh-2									
120	–	–	0.01	–	–	–	–	–	–
100	–	–	0.71	–	–	–	–	–	–
80	–	–	0.28	–	–	–	–	–	–
(*N*)	–	–	(67)	–	–	–	–	–	–
Ldh-2									
120	0	0	0	0	0	0	0	0.01	0
110	0.10	0.10	0.10	0.05	0.13	0.06	0.11	0.08	0.12
100	0.83	0.90	0.90	0.95	0.87	0.88	0.86	0.89	0.86
90	0.07	0	0	0	0	0.06	0.03	0.01	0.02
70	0	0	0	0	0	0	0.01	0	0
(*N*)	(15)	(20)	(67)	(21)	(27)	(8)	(80)	(71)	(74)
Ldh-3									
100	1	1	1	1	1	0.94	0.99	1	1
70	0	0	0	0	0	0	0	0	0
58	0	0	0	0	0	0.06	0.01	0	0
(*N*)	(15)	(17)	(67)	(21)	(27)	(8)	(80)	(71)	(74)

Table 5 (continued)

Locus, electromorph	Sample								
	AR	MA	PI	PR	G1	G2	G3	G4	G5
Mdh-2									
130	0	0	0	0	0	0	0	0	0
120	0	0	0	0	0	0	0.01	0	0.02
110	0	0	0	0	0	0	0	0	0
105	0	0	0	0	0	0	0	0	0
100	1	0.98	0.99	0.98	0.96	0.94	0.98	0.98	0.97
85	0	0	0	0	0	0	0	0	0
80	0	0.03	0.01	0.02	0.04	0.06	0.01	0.02	0.01
(*N*)	(15)	(20)	(67)	(21)	(27)	(8)	(80)	(71)	(74)
6-Pgd									
110	–	–	0.01	–	–	-	0	0	0
100	–	–	0.41	–	–	-	0.5	0.52	0.56
80	–	–	0.58	–	–	-	0.5	0.48	0.44
60	–	–	0	–	–	-	0	0	0
(*N*)	–	–	(67)	–	–	-	(64)	(69)	(72)
Pgm									
135	0	0	0	0	0	0	0	0	0.01
130	0	0	0.01	0	0	0	0.02	0	0
115	0.03	0	0	0.02	0.06	0	0	0.01	0.01
100	0.97	0.97	0.99	0.98	0.94	0.94	0.98	0.99	0.99
80	0	0.03	0	0	0	0.06	0	0	0
(*N*)	(15)	(19)	(67)	(21)	(9)	(8)	(80)	(70)	(74)

Table 6 *Solea vulgaris*. Statistics on electromorph variability by region [*N* total sample size by region; *H* genetic diversity per locus (weighted average of *H* over samples); *A* number of electromorphs in random subsamples of individuals whose size has been chosen for each locus as the *N* for the eastern Mediterranean]

Locus	N. Atlantic			W. Mediterranean			E. Mediterranean		
	A	*H*	(*N*)	A	*H*	(*N*)	A	*H*	(*N*)
Aat-3	0.032	2	(551)	0.020	2	(557)	0.017	2	(74)
αGpd-1	0.208	3	(539)	0.250	3	(595)	0.152	4	(74)
αGpd-2	0.188	3	(419)	0.102	4	(504)	0	1	(64)
Gpi-1	0.075	3	(564)	0.126	3	(596)	0.282	2	(74)
Gpi-2	0.286	3	(568)	0.152	3	(578)	0.036	4	(74)
Idh-1	0.043	3	(475)	0.006	2	(481)	0.002	2	(64)
Idh-2	0.456	3	(472)	0.418	3	(135)	0.499	2	(64)
Ldh-2	0.097	2	(555)	0.208	3	(597)	0.372	2	(74)
Ldh-3	0.028	2	(539)	0.006	1	(594)	0	1	(74)
Mdh-2	0.086	3	(580)	0.059	3	(597)	0.001	2	(74)
6-Pgd	0.501	2	(359)	0.498	2	(477)	0.475	3	(64)
Pgm	0.039	3	(369)	0.029	3	(577)	0.036	2	(74)
Mean	0.170	2.67		0.156	2.67		0.158	2.25	

ern Mediterranean and eastern Mediterranean (regional scale), and among samples pooled for each region (biogeographical scale). These values, and their weighted averages over loci with their respective jackknife standard deviations are reported in Tables 8 to 10.

The distributions of single-locus θ did not significantly depart from normality except in the Golfe du Lion (Table 10). The relatively large θ value observed at locus *Aat-3* in the latter sample was clearly an outlier, and thus contributed to the observed departure from normality. However, the high largest difference in this data set should be disregarded, since the average θ was negative and the monolocus θ was small. Since the distributions of single-locus θ did not otherwise depart from normality, a right-tailed Student's *t*-test with $n-1$ degrees of freedom (n=number of loci) could be used for testing the significance of departure of average θ values from zero.

No differentiation was evident at the temporal scale (Table 8), with weighted average θ values all well within their respective confidence intervals (mean±2 *SD*). Analyses conducted at the intra-zone scale did not reveal any differentiation (Table 9), and those conducted at the regional scale were no more conclusive except possibly for the eastern Mediterranean region (Table 10). Evidence for differentiation appeared at the biogeographical scale (Table 10), where single-locus θ values ranged in a continuous fashion, and their weighted average over all 12 loci was significantly greater than zero ($\theta/SD=4.2$; Student's *t*-test, one-tailed, 11 *df*; $p< 0.001$). Thus, according to a single-locus approach, populations from different regions were strongly differentiated, whereas little or no differentiation could be detected beneath that scale.

Isolation by distance

A more detailed account of the results at the above-regional scale is presented in Table 11, where genetic distances be-

Table 7 *Solea vulgaris*. Fixation-index values in all 27 samples with weighted averages over loci (*wmean*), unweighted averages over samples (*mean*) and their jackknife standard deviations (*SD*) [*ND* no data; – one allele fixed in sample; * $p< 0.05$, ** p 0.01, *** $p< 0.001$ after Bonferroni's corrections for multiple tests (Weir 1990)]

Locus	Sample														
	CO	MI	LO	V1	V2	V3	V4	PB	PA	E1	E2	A1	A2	AR	MA
Aat-3	–	–	–	–	–0.026	–0.023	–0.004	0	–	–	–	0	1***	–	0
αGpd-1	0.097	0.437	–0.100	–0.111	–0.066	–0.009	–0.031	–0.108	0.230	0.015	0.087	–0.160	–0.163	–	0.476
αGpd-2	ND	ND	ND	ND	–0.072	–0.027	–0.037	0.126	0.006	–0.019	0.219	–0.051	–	ND	ND
Gpi-1	–0.037	0	0	–0.018	–0.044	–0.013	0.136	–0.038	–	–0.047	0.243	–0.051	–0.042	0	0
Gpi-2	0.008	–0.140	–0.100	–0.174	0.174	0.333	0.001	0.108	–0.089	–0.012	–0.090	–0.046	–0.042	–	–0.048
Idh-1	ND	ND	ND	ND	–0.012	–0.021	–0.004	–0.003	–0.016	–	–	–	–	ND	ND
Idh-2	ND	ND	0.203	ND	–0.030	–0.169	0.044	–0.103	–0.196	ND	–0.044	ND	ND	ND	ND
Ldh-2	0	–0.036	–	0.477	–0.042	0.146	0.182	0.428	0.661*	–0.055	0.129	1***	–0.101	–0.111	0.465
Ldh-3	–	0	–	–	–0.004	–0.007	0.495***	–0.019	–0.006	–	0	–	–	–	–
Mdh-2	–0.098	–0.024	0	0	–0.048	–0.026	–0.026	–0.008	–0.027	–0.040	–0.017	–0.051	0	–	0
6-Pgd	ND	ND	ND	ND	0.282	0.140	0.030	0.064	–0.175	0.243	0.388	–0.063	0.023	ND	ND
Pgm	–0.024	0	–	0	–0.009	–0.013	–0.018	–0.006	–0.012	–	–0.004	–0.009	–	0	0
WMEAN	0.004	0.077	0.055	–0.039	0.061	0.053	0.030	0.041	–0.055	0.079	0.141	0.082	0.086	–0.077	0.220
(SD)	(0.038)	(0.174)	(0.129)	(0.118)	(0.077)	(0.089)	(0.016)	(0.054)	(0.074)	(0.095)	(0.095)	(0.163)	(0.117)	(0.061)	(0.129)

Table 8 *Solea vulgaris*. Single-locus θ values for replicated samples within same localities (temporal scale), and weighted averages over loci (*wmean*) with jackknife estimates of standard deviation (*SD*). Normality test for each data set according to Lilliefors (1967), with Bonferroni-type correction for assessment of significance

Locus	Vilaine	Ebro	Agde	Grau	Marseille
Aat-3	0.0001	–	–0.0215	0.0350	–0.0193
αGpd-1	–0.0005	–0.0102	–0.0152	0.0110	–0.0215
αGpd-2	0.0136	–0.0003	0.0430	–0.0047	0.0389
Gpi-1	0.0040	–0.0051	–0.0105	0.0094	–0.0093
Gpi-2	0.0007	0.0173	0.0157	–0.0005	–0.0187
Idh-1	0.0028	–	–	0.0007	–
Idh-2	0.0031	–	–	–	–
Ldh-2	–0.0041	–0.0117	–0.0211	–0.0068	–0.0287
Ldh-3	–0.0067	–0.0068	–	0.0313	–
Mdh-2	–0.0003	0.0132	0.0126	–0.0014	0.0090
6-Pgd	–0.0021	–0.0123	0.0443	–0.0034	0.0110
Pgm	–0.0038	–0.0041	0.0057	0.0061	–0.0041
Normality	accepted	accepted	accepted	accepted	accepted
WMEAN	0.0013	–0.0054	0.0096	0.0006	–0.0027
(SD)	(0.0017)	(0.0044)	(0.0181)	(0.0032)	(0.0142)

Table 9 *Solea vulgaris*. θ values among samples from Manche, Vilaine and Pertuis, and Golfe du Lion (intra-zone scale). First column and normality test as in Table 8

Locus	Manche	Vilaine + Pertuis	Golfe du Lion
Aat-3	–	0.0036	0.0027
αGpd-1	–0.0198	–0.0013	–0.0009
αGpd-2	–	–0.0009	–0.0010
Gpi-1	0.0150	0.0001	0.0014
Gpi-2	–0.0072	0.0005	–0.0015
Idh-1	–	–0.0014	–0.0018
Idh-2	–	–0.0008	–
Ldh-2	–0.0003	–0.0010	–0.0014
Ldh-3	–0.0006	0.0001	–0.0016
Mdh-2	0.0124	0.0012	0.0014
6-Pgd	–	–0.0027	–0.0016
Pgm	–0.0028	–0.0009	–0.0014
Normality	accepted	accepted	rejected
WMEAN	–0.0049	–0.0010	–0.0010
(SD)	(0.0064)	(0.0006)	(0.0005)

tween pairs of populations from each zone are given, together with the corresponding geographic distances. The genetic distance was positively correlated with geographic distance (Mantel's test; 1000 permutations; $p< 0.01$).

Multidimensional analysis

The samples appeared ordinated according to geographic gradients on the plane defined by Factorial Axes 1 and 2 of CA, from west to east along Factorial Axis 1, and from south to north along Axis 2 (Fig. 2). Where several samples from the same locality (here considered as replicates) were available, these grouped together on the CA's principal plane, an indication of the robustness of the CA's output. The external stability (Greenacre 1984) of the image formed on the CA's principal plane (Fig. 2) was assessed by both (1) subtracting from the input matrix each set of replicated samples (V3–V4, E2, G3–5 and M2) and (2) jackknifing the set of loci. In all cases, the arrangement of samples in Fig. 2 remained the same (data not shown). The only changes revealed were minor variations in the inertias of the factorial axes and in the relative contributions of samples of individuals to the output, a confirmation of the robustness of the results with respect to both the sampling of populations and the sampling of loci.

Table 7 (continued)

PI	PR	G1	G2	G3	G4	G5	M1	M2	TU	GR	EG	*f* values MEAN	(SD)
0	0	ND	0	–	–	0	0	0	–	0	–	0.068	(0.072)
0.088	–0.026	0.085	–0.077	0.022	0.016	–0.027	0.281	0.051	–	–0.045	–0.125	0.033	(0.033)
0.193	ND	ND	ND	0.486*	0.300	–0.027	–0.031	–	–	–	ND	0.082	(0.048)
–0.042	–0.035	–	–	0.043	0.224	–0.066	0	–	–	–0.090	–0.125	0.000	(0.019)
0.024	–0.026	0	0	–0.054	–0.082	–0.061	–0.048	0	0	–0.004	0	–0.014	(0.019)
–0.008	ND	ND	–	–	–	–0.003	–	–	ND	0	–	–0.008	(0.003)
0.086	ND	ND	0.300	ND	ND	–0.006	ND	ND	ND	–0.001	ND	0.008	(0.045)
0.240	–0.026	–0.130	–0.037	–0.029	0.130	0.245	0.179	0.719	0	–0.056	0.250	0.178*	(0.057)
–	–	–	0	0	–	–	–	–	–	–	–	0.051	(0.056)
–0.008	0	–0.020	0	–0.009	–0.015	–0.016	–0.031	0	0	–	0	–0.018*	(0.005)
0.099	ND	ND	ND	–0.180	–0.155	0.196	0.151	–0.008	ND	0.093	ND	0.071	(0.041)
–0.008	0	0	0	–0.013	–0.007	–0.003	–	0	–	–	–0.125	–0.012	(0.006)
0.117*	–0.021	–0.010	0.089	–0.024	0.005	0.046	0.127	0.140	0	–0.005	0	0.045**	(0.013)
(0.026)	(0.005)	(0.072)	(0.115)	(0.079)	(0.085)	(0.055)	(0.045)	(0.156)	(0)	(0.038)	(0.010)		

Table 10 *Solea vulgaris.* θ values among samples from northeastern Atlantic (*N. Atl.*), western Mediterranean (*W. Med.*) and eastern Mediterranean (*E. Med.*) (regional scale), and among these three regions (whole distribution range, *Whole*). First column and normality test as in Table 8. *** $p < 0.001$, one-tailed Student's *t*-test

Locus	N. Atl.	W. Med.	E. Med.	Whole
Aat-3	0.0004	–0.0001	–0.0252	–0.0001
αGpd-1	–0.0006	0.0063	0.0352	0.0039
αGpd-2	–	–0.0010	–	0.0113
Gpi-1	0.0002	0.0041	–0.0251	0.0182
Gpi-2	–0.0018	–0.0016	0.0185	0.0205
Idh-1	–	–0.0002	–0.0252	0.0034
Idh-2	–	–0.0074	–	0.0224
Ldh-2	–0.0007	0.0021	–0.0292	0.0287
Ldh-3	–0.0016	–0.0013	–	0.0038
Mdh-2	0.0027	0.0001	0.1412	0.0020
6-Pgd	–	0.0003	–	0.0096
Pgm	0.0009	–0.0012	0.3832	–0.0002
Normality	accepted	accepted	accepted	accepted
WMEAN	–0.0005	–0.0004	0.0127	0.0147***
(SD)	(0.0007)	(0.0022)	(0.0365)	(0.0035)

Discussion

Gene flow counteracts differentiation among populations caused by genetic drift or differential selection. When the species has a more or less continuous distribution across a range, the balance between these antagonistic forces may result in clines which may be gradual or sharp. In the latter case isolation by distance ensues, where genetic differentiation at neutral loci increases with geographic distance (Kimura and Weiss 1964). While the life-history features in *Solea vulgaris*, i.e. high fecundity and long-lived planktonic larvae, are expected to facilitate gene flow, other factors may favour geographic isolation. These include physical barriers to dispersion of pelagic stages such as sharp temperature and salinity gradients, the homing behaviour of spawners, hydrodynamic eddies favouring larval retention, and the active vertical swimming of larvae by which they can avoid passive transport off nursery areas (Colton 1959; Harden Jones 1968; Iles and Sinclair 1982; Rijnsdorp et al. 1985). The positive correlation between genetic and geographic distances, and the consistency of the pattern resulting from CA with the geographical pattern of distribution of the samples suggested isolation by distance in *S. vulgaris* (see Slatkin 1993). Because of the strength of the correlation, it is likely that this species is at equilibrium between a high level of gene flow and genetic drift. Hence populations of *S. vulgaris* have probably been present, undisturbed, in their current geographical range for a long time. This is consistent with the conclusions of Mediterranean biogeographers that the species may have entered from the Atlantic into the Mediterranean during the early Pliocene and have settled there since then (e.g. Klausewitz 1973; Quignard 1978).

The sampling design of the present survey made it possible to investigate the genetic structure of *Solea vulgaris* at several spatial scales: between samples within a zone, between zones within a region and between regions over its whole distribution range. The strongest result emerging from CA was an east to west pattern of population differentiation, corresponding to the recognition of separate, regional populations. We also considered the north to south pattern (Axis 2 of the CA results) to be significant: this indicates that a separation between zones within the northeastern Atlantic region is effective. Hence the scale of differentiation between populations was that of the "zone" (that is, a geographical area gathering populations from neighbouring localities within a radius of the order of 100 km). These inferences about the genetic structure of *S. vulgaris* met the expectations, drawn from biological data,

Table 11 *Solea vulgaris*. Matrix of pairwise genetic distances (D, above diagonal) and geographic distances (in km, below diagonal)

	Cotentin	Michel	Loctudy	Vilaine	Pertuis	Ebro	Lion	Tunis	Egean	Egypt
Cotentin		−0.005	−0.014	0.004	0.012	0.023	0.029	0.017	0.126	0.069
Michel	200		−0.024	−0.006	−0.008	0.009	0.005	−0.002	0.094	0.061
Loctudy	600	400		0.016	−0.017	−0.004	0.002	−0.001	0.093	0.061
Vilaine	1000	800	400		0.000	0.022	0.016	−0.002	0.090	0.072
Pertuis	1200	1000	600	200		0.017	0.008	−0.004	0.085	0.069
Ebro	3800	3600	3200	2800	2600		0.007	0.027	0.063	0.034
Lion	4300	4100	3700	3300	3100	500		0.005	0.046	0.030
Tunis	4400	4200	3800	3400	3200	2600	3100		0.075	0.069
Egean	7900	7700	7300	6900	6700	4100	3600	6000		0.013
Egypt	7400	7200	6800	6400	6200	5600	6100	3000	3000	

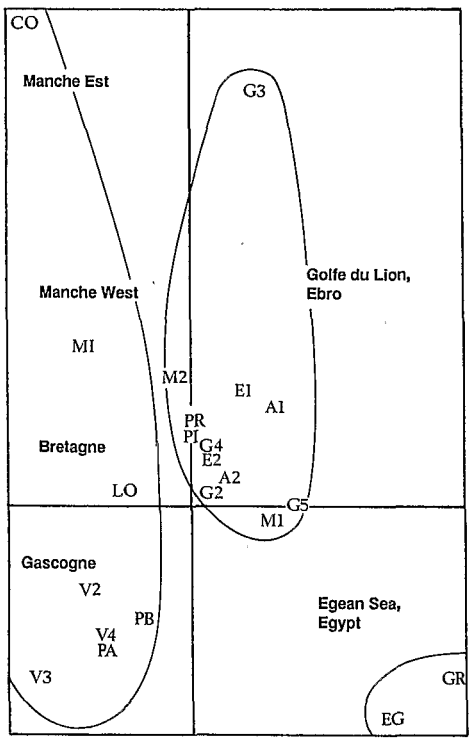

Fig. 2 *Solea vulgaris*. Correspondence analysis. Projection of samples defined by multiple-locus genotype counts. Axis 1, horizontal, = 24.6% total inertia; Axis 2, vertical, = 14.3% total inertia. Sample abbreviations as in Table 1

that panmixia or near-panmixia should occur at the scale of the zone, because of the mixing in spawning areas of mature individuals originating from adjacent nurseries (Dorel et al. 1991), and because of the random, passive diffusion of eggs and larvae back to nursery areas (Koutsikopoulos et al. 1991). Thus, multiple-scale genetic-structure analyses complemented biological observations and vice versa, to the point of agreement that in *S. vulgaris* the basic unit of population structure is likely to be an ensemble of neighbouring populations within a zone.

The overall heterozygote deficiency in the samples of *Solea vulgaris* may be interpreted as a deviation from Hardy–Weinberg genotypic frequencies, either due to some reproductive structure within populations or to selection against heterozygotes at one stage in the life-cycle. Two observations indicated that heterozygote deficiency may be more pronounced in juvenile than in adult *S. vulgaris*: (1) the most significant positive f value was observed in a sample (PI) of early juveniles; (2) in the only data set where such a comparison was possible (V1 to V4), we observed that the older the individuals, the weaker the heterozygote deficiency in a sample, with even a negative f value (indicating an heterozygote excess) in V1, the only sample in the series that consisted of adult individuals. Beardmore and Ward (1977) reported a similar negative trend between heterozygote deficiency and age in plaice. Such a correlation has also been documented in marine bivalves (Zouros and Foltz 1987 and references therein). Flatfishes have some life-history features similar to those of many bivalve species, among which is the release by each female of large numbers of pelagic eggs ($\gtrsim 1\,000\,000$) and their subsequent development into pelagic larvae until metamorphosis. Embryogenesis, larval life and metamorphosis have been generally considered as critical stages for individual survival. Considering that at demographic equilibrium only two of a female's eggs will on the average reach reproductive adulthood, there is wide room for genotype-dependent selection during early stages. Selection against heterozygotes taking place during early development followed by counterselection (Blanc and Bonhomme 1987; Hawkins et al. 1989) may account for more pronounced heterozygote deficiency in post-metamorphosed juveniles than in adults.

The calculations of genetic diversity (H=0.156 to 0.170) were based on allele frequency data at 12 polymorphic allozyme loci, those found to be the most polymorphic in preliminary studies. For a comparison with genetic diversity data from the literature, this bias towards polymorphism was removed by including into the calculations other allozyme loci investigated preliminarily and later discarded for their monomorphism (nine loci: *Ck*, *Est-1*, *Glo*, *Ldh-1*, *Mdh-1*, *Me-1*, *Me-2*, *Sod-1* and *Sod-2*) or because of difficulties in scoring large numbers of individuals (five

loci: *Aat-1*, *Aat-2*, *Acp*, *Est-3* and *Sdh-2*) (Pasteur et al. 1985; Kotoulas 1989). The average *H* value obtained (now over 26 loci) was 0.118 in the western Mediterranean region. It was significantly higher than the average for marine teleosts (H=0.055±0.036; Smith and Fujio 1982) but ranged among the values observed in other flatfishes (*Pleuronectes platessa*, H=0.118; *Platichthys flesus*, H=0.095; *Scophthalmus maximus*, H=0.027 and *S. rhombus*, H=0.121: see Ward and Beardmore 1977; Borsa et al. 1987; and Blanquer et al. 1992; respectively). Several possible explanations have been provided for the variability in the genetic diversity of species. These include (1) the selective effect of habitat, with genetic variability depending on whether the species is a habitat specialist or a habitat generalist (Smith and Fujio 1982), and (2) the neutral effect of effective population size, which affects the equilibrium between mutation and genetic drift towards high or low diversities in large and small populations, respectively (see Soulé 1976). The effective population size can be defined instantaneously as the effective number of individuals contributing to the next generation. It can also be calculated over generations as the harmonic mean of effective population sizes at each generation. It is thus important to note that flatfishes such as the variable *P. platessa* and *Solea vulgaris* are considered to have large population sizes stable over time (Beverton 1962; De Veen 1978), whereas coastal pelagic species (of the genera *Clupea*, *Sardinella*, *Sardinops*), exhibiting levels of genetic variability ranging from low to moderately low (H=0.008 to H=0.052; see Grant 1985; Wilson and Alberdi 1991; other references in Gyllensten 1985) are known to have experienced successions of demographic flushes and crashes in recent geological time (Cury 1988). Population size may be related to the extent of geographic habitat in a species undergoing substantial gene flow between its local populations, but it can also reflect the ability of a species to occupy a broad (or a large array of different) ecological habitats. *Solea vulgaris* conforms to the type of species with either broad geographic and ecological habitats, or large and stable population sizes, or substantial gene flow at the regional scale.

No pattern emerged from the comparison between regions of their estimates of internal genetic variability. Such comparisons could reveal a reduction in genetic variability specific to one area (a signature of local reduction in population size or a population bottleneck). To be observed, such a bottleneck must be severe and the immediate loss in genetic variability (the number of alleles in particular) must have been maintained to the present by geographical isolation of the population (Nei et al. 1975). Moreover, the observed pattern of isolation by distance is hardly compatible with a model of geographic isolation.

The temperature interval for development in common sole eggs ranges from 7 to ~20°C (Fonds 1979 and references therein). Temperatures outside these limits are lethal, and approximately coincide with the actual temperatures above the thermocline off Norway in the summer (Robinson et al. 1979) and in the southeastern Mediterranean in the winter (Robinson 1973). These are respectively the northernmost, coldest and the warmest locations where the species occurs. Within these limits, the planktonic stage duration is known to decrease with increasing temperature. This may explain the somewhat larger θ values in the eastern Mediterranean region, which lies at the edge of the habitat (temperature) tolerances of *Solea vulgaris*. Other flatfishes of the northeastern Atlantic–Mediterranean exhibit stronger geographical structure than *S. vulgaris*. The flounder *Platichthys flesus* consists of four separate populations which are totally geographically isolated from each other (Borsa et al. 1987). The Mediterranean populations, confined to the coastal waters of the North of the Golfe du Lion, the Adriatic Sea, and the Egean Sea, and the Atlantic population are separated from each other by southward peninsulas, the coastal waters of which do not have temperatures lower than 14°C (Robinson 1973). This is beyond the upper limit of the interval of temperature tolerance for eggs and larvae of *P. flesus* (2 to 12°C; Apstein, *in* Russell 1976). The turbot *Scophthalmus maximus* shows an intermediate pattern, with a single suspected geographical barrier between the Black Sea–Egean Sea and the rest of its area of distribution (Blanquer et al. 1992). The temperature tolerances for turbot eggs and larvae span the interval 10 to 14.5°C (Jones 1972), hence they are more tolerant to warmer temperatures than are flounder larvae. The comparison of sole, flounder and turbot suggests that water temperature during the reproductive period, which is critical for the survival of offspring during their pelagic stage, is a major factor affecting gene flow and consequently the population genetic structure of a species.

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