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Allozyme variation in turbot (*Psetta maxima*) and brill (*Scophthalmus rhombus*) (Osteichthyes, Pleuronectoformes, Scophthalmidae) throughout their range in Europe

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Two species of coastal flatfish (brill and turbot, Scophthalmidae) were analysed electrophoretically at 17 common enzymatic loci in samples taken from 11 sites representing the species ranges in Europe. Brill showed a mean heterozygosity (H) of 0·11 while that of turbot was 0·02. The virtual absence of genetic diversity in turbot is probably due to a very low evolutionary rate, and provides little evidence for population substructure even if various signs indicate the possibility of a hidden differentiation (presence of the taxon *maoetica* in the Black Sea and differentiation of a species-specific cestode parasite on either side of the Strait of Gibraltar). On the other hand, the weak geographic structure in brill seems to result from rapid recolonization following the last ice age.

Key words: genetic diversity, paleohistoric migrations, allozymes, marine biogeography; Scophthalmidae, *maoetica* taxon.

I. INTRODUCTION

Turbot, *Psetta maxima* (Linnaeus, 1758) Swainson, and brill, *Scophthalmus rhombus* (Linnaeus, 1758) Rafinesque, have very similar morphologies. An immediate criterion of distinction is the presence or absence of bony tubercles, which are scattered over the top of turbot (Quéro, 1984) and are absent in brill. The two species are sympatric over a large portion of their range, i.e. the Atlantic coasts from Norway to Morocco (only turbot occur in Morocco), and the northern coasts of the Mediterranean, becoming rare in the eastern basin. In the Black Sea, *P. maxima* is replaced by *P. maoetica* (Pallas, 1811) Bonaparte, which is also found in the eastern Mediterranean (Norman, 1934). It should be noted that, although many authors raise the latter taxon to the rank of species, Tortonese (1971) considers it to be a subspecies: *P. maxima maoetica*. This taxon is characterized by bony tubercles that are larger and more abundant.

During a genetic study of allozymes in cestode parasites specific to these two species, Renaud *et al.* (1986) demonstrated that *Bothriocephalus gregarius*, a parasite of the turbot, shows considerable differentiation between the Atlantic and the Mediterranean. The two forms separate in southern Portugal (between Lisbon and Faro), whereas the brill parasite, *B. barbatus* is identical from the English Channel to the Mediterranean.

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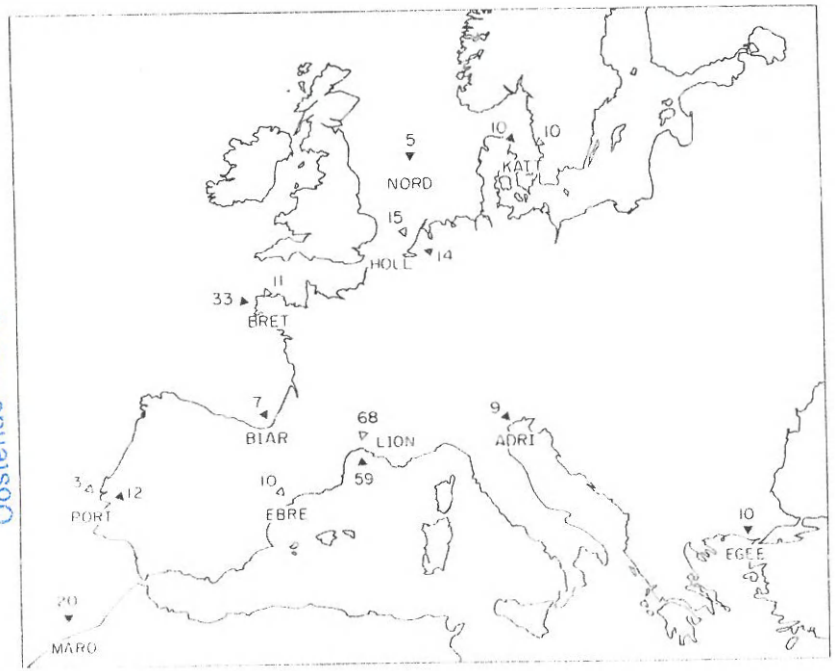

FIG. 1. Geographic ranges of turbot (▼) and brill (▽), and sites of fishings. The numbers indicate the size of each set of samples.

The objective of the present study was to analyse allozyme variation in the two species by electrophoresis of enzymes from turbot and brill captured over their whole range (Fig. 1). We compare interspecific genetic variability and analyse geographic differentiation in each of the species. An additional objective of the study was to determine whether enzyme markers corroborate the parasitological markers.

II. MATERIALS AND METHODS

Fish were caught by bottom trawling at 11 sites on the Atlantic and Mediterranean coasts (Fig. 1) and were either frozen whole and transferred to the laboratory or dissected at the site and the organs were transported to the laboratory in liquid nitrogen.

According to classical taxonomic criteria based on morphology, the turbot specimens from the Aegean Sea belonged to the taxon *maoetica*. All the others were classified as *P. maximus*.

The genetic study was carried out on skeletal muscle and liver extracts using starch gel (12%) electrophoresis adapted from the methods of Selander *et al.* (1979) and Pasteur *et al.* (1987). Enzyme loci are described here using the Shaklee *et al.* (1990) nomenclature. In both species, reference allele 100 designates, by convention, the most frequent electromorph in turbot. Table I shows the enzymes studied and the buffers used.

Electrophoretic data were analysed using classical parameters, i.e. heterozygosity H and enzymatic polymorphism P. Nei distances (1972) were calculated within and between species, and the results are given in the form of a dendrogram constructed according to the method of Sneath & Sokal (1973).

TABLE I. Qualitative description of the 17 enzymatic loci analysed

Enzyme name	E.C.	Loci	Organs of analysis	Buffers used	Turbot/ brill diagnostic
Aspartate aminotransferase	2.6.1.1.	*AAT-1**	Muscle and liver	Poulik 1/2	
		*AAT-2**	Muscle	Poulik 1/2	
		*AAT-3**	Liver	Poulik 1/2	*
Acid phosphatase	3.1.3.2.	*ACP**	Liver	PC 6·3	*
Alcohol dehydrogenase	1.1.1.1.	*ADH**	Liver	Poulik 1/2	*
Creatine kinase	2.7.3.2.	*CK**	Muscle	Poulik 1/2	
Alpha glycero phosphate dehydrogenase	1.1.1.8.	*aGPDH**	Muscle	TME 6·9	
Glucose 6-phosphate isomerase	5.3.1.9.	*GPI-1**	Muscle	Poulik 1/2	
		*GPI-2**	Muscle and liver	Poulik 1/2	
Isocitrate dehydrogenase	1.1.1.42.	*IDH-1**	Muscle	TC 8·0	
		*IDH-2**	Liver	TC 8·0	
Lactate dehydrogenase	1.1.1.27.	*LDH**	Muscle	TC 8·0	*
Malate dehydrogenase	1.1.1.37.	*MDH**	Muscle	TC 8·0	
Malic enzyme	1.1.1.40.	*ME-2**	Muscle	TME 6·9	
6-Phosphogluconate dehydrogenase	1.1.1.44.	*6PGDH**	Liver	TC 8·0	
Phosphoglucomutase	5.4.2.2.	*PGM**	Muscle and liver	Poulik 1/2	
Superoxide dismutase	1.15.1.1.	*SOD**	Liver	Poulik 1/2	

Buffers: Poulik 1/2: 18·5 g l^{-1} boric acid, 2·4 g l^{-1} NaOH, pH 8·2 for the electrodes; 2·3 g l^{-1} Tris, 0·27 g l^{-1} citric acid, pH 8·7 for the gel.

PC 6·3: 44·1 g l^{-1} trisodic citrate, 37·4 g l^{-1} dihydrogenated monosodic phosphate, pH 6·3 for the electrodes; identical buffer diluted 40-fold for the gel.

TME 6·9: 12·1 g l^{-1} Tris, 9·8 g l^{-1} maleic anhydride, 3·7 g l^{-1} EDTA, 2 g l^{-1} MgCl2, pH 6·9 for the electrodes; identical buffer diluted 10-fold for the gel.

TC 8·0: 75·6 g l^{-1} Tris, 30 g l^{-1} citric acid, pH 8·0 for the electrodes; identical buffer diluted 30-fold for the gel.

The allelic frequencies (inter-sample comparisons) and the genotypic frequencies (panmixia) were compared by χ^2 tests. These were performed using the following conventions in the case of an expected number less than 5: if the difference between expected and observed numbers was not significant, this result was accepted. If the test showed a significant heterogeneity, it was repeated by pooling the less frequent alleles. If the χ^2 test was for a square matrix 2×2 in which one or more expected number was less than 5, Yates' correction was applied (Heller, 1968, adapted from Yates, 1934).

Correspondence analysis was carried out according to the method of Benzécri (1973) using the BIOMECO program (Lebreton *et al.*, 1990) with the modifications described by She *et al.* (1988). This analysis makes it possible to represent individuals on different planes of a multidimensional space according to the set of allelic variables. Each allele was coded as follows: 0 = individual without the allele, 1 = individual with the allele in a heterozygous state, and 2 = individual with the allele in a homozygous state. Thus, there are as many variables as there are alleles.

Only individuals characterized at all enzyme loci were used in the analysis.

III. RESULTS

ENZYME POLYMORPHISMS

Thirteen enzyme systems corresponding to 17 loci were interpreted in both species (Table I). Four of these were found to be diagnostic for the two species, i.e. *AAT-3**, *ACP**, *ADH** and *LDH** (Table II).

In turbot only six polymorphic loci (*ADH**, *CK**, *a-GPDH**, *GPI-2**, *IDH-2** and *PGM**) were found, out of the 17 analysed. The percentage of polymorphism for the species was $P = 27\%$ (criterion 0·95) and the heterozygosity was $H = 0{\cdot}019$. Brill showed 10 polymorphic loci (*AAT-1**, *AAT-2**, *AAT-3**, *ADH**, *a-GPDH**, *GPI-1**, *IDH-2**, *MDH**, *PGM** and *SOD**) with a polymorphism rate of 44% and heterozygosity of 0·107.

PANMIXIA

Tests of significance on the divergence from Hardy–Weinberg equilibrium were performed following the above conventions. In all the samples, no significant deviation from the expected proportions of genotypes were observed.

GENETIC DISTANCES (TABLE III)

The UPGMA dendrogram (Fig. 2) clearly confirmed the difference in heterozygosity separating the two species. Although turbot and brill were well differentiated from one another, no important difference can be seen within each species, even in the taxon *maoetica* whose distance from the other form was practically zero ($0{\cdot}002 < D < 0{\cdot}003$).

COMPARISONS BETWEEN SAMPLES

In view of the small size of some samples, geographically neighbouring samples were pooled: North Atlantic coasts: KATT + NORD + HOLL (total of 25 brill and 29 turbot); West Atlantic coasts: BRET + BIAR + PORT (14 brill and 52 turbot); West Mediterranean coasts: EBRE + LION + ADRI (78 brill and 68 turbot). These pooled samples themselves showed no heterogeneity. The turbot samples EGEE and MARO were too isolated to be grouped.

The χ^2 tests were performed using the grouped samples (Table IV) and weak geographic structure appeared at some loci: in turbot, pairs of comparisons including EGEE were generally significantly heterogeneous at the *IDH-2** locus, showing the only structure given the low polymorphism of the species; in brill, four tests were significant out of a total of 23 (17%). It shows a weak differentiation of the Mediterranean region and homogeneous population in the two Atlantic regions.

CORRESPONDENCE ANALYSIS

In turbot, the five polymorphic loci were used for correspondence analysis encompassing 14 variables (i.e. alleles) and 164 individuals. In the projection of individuals on the principal plane (axes 1 and 20) most clustered in the central zone. This included fish from the Greek population (*maoetica*), even though that population had only allele 80 at locus *IDH-2** at a frequency of 0·20. No structure could be found, but the low level and number of detectable polymorphisms deprived us of an adequate tool for analysis.

Although the heterozygosity of brill was five times higher than that of turbot, no differentiation could be found on the main axes.

IV. DISCUSSION

VARIATION BETWEEN SPECIES

Various hypotheses have been proposed in the literature to explain differences in polymorphisms between species, as observed here between turbot and brill. They include differences in the seasonal aspect of the food supply (Ayala, 1975; Valentine *et al.*, 1976), heterogeneity of the environment (Levins, 1968), population sizes (Nei *et al.*, 1975) and mode of reproduction. However, as turbot and brill have essentially the same feeding habits and live sympatrically over vast geographic areas, it is difficult to use adaptation arguments. Although there is a difference in the behaviour of brill in that some young individuals enter lagoons, this in itself seems an unlikely explanation of such a large difference in the level of polymorphism.

Historical causes, associated with drastic reductions in populations (recent bottleneck) can give rise to low levels of polymorphism (Nei *et al.*, 1975; Chakraborty & Nei, 1977). However examples of this are rare in marine environments since there are fewer effective barriers to migration than in the case of inland animals (Grant & Ståhl, 1988). The bottleneck hypothesis has been proposed by Grant & Ståhl (1988), who found a large difference in polymorphism between Atlantic *Gadus morhua* L. and Pacific cod *Gadus macrocephalus* Tilesius, and by Kotulas (1990) to explain the decrease in genetic diversity observed in *Solea vulgaris* in the Aegean Sea. However, there is no reason why brill should not have undergone the same population reduction. If it did, it remains to be explained how the latter species regained a high level of polymorphism while the turbot did not.

Alternatively, a hypothesis based on intrinsic genetic mechanisms such as different rates of evolution can also be proposed. Possibly this is consistent with the fact that, although the taxon *maoetica* shows clear morphological differences which must have taken a long time to appear, it has not diverged generally with regard to biochemical markers.

VARIATION WITHIN SPECIES

Some small-sized samples led us to group samples in large regions such as the North Atlantic coasts, West Atlantic, Morocco, West Mediterranean and Aegean Sea. A weak differentiation was found in brill along European coasts, the Mediterranean region showing frequency divergences at *AAT-2**, *a*GPDH* and *GPI-1** loci as compared with the two homogeneous Atlantic regions.

In turbot, only those of the Aegean Sea were distinguished from the others, by having allele 80 at locus *IDH-2** at a frequency of 0·2, but this difference only gave rise to a negligible genetic distance among locations.

Why is there a weak genetic differentiation in turbot and brill over such a large range? Flatfishes of the families Scophthalmidae and Pleuronectidae are considered to be of northern origin (Greenwood *et al.*, 1973). The two species studied

TABLE II. Allele frequencies of the 10 geographic sets of *P. maxima* samples and the six sets of *S. rhombus* samples

		Turbot										Brill					
Loci	Alleles	KATT	NORD	HOLL	BRET	BIAR	PORT	LION	ADRI	EGEE	MARO	KATT	HOLL	BRET	PORT	EBRE	LION
N		10	5	14	33	7	12	59	9	10	20	10	15	11	3	10	68
H		0·011	0·021	0·008	0·015	0·000	0·021	0·027	0·017	0·024	0·021	0·067	0·078	0·098	0·193	0·084	0·121
*ATT-1**	070	0·00	0·00	0·00	0·00	0·00	0·00	0·00	0·00	0·00	0·00	0·00	0·00	0·05	0·00	0·00	0·01
	100	1·00	1·00	1·00	1·00	1·00	1·00	1·00	1·00	1·00	1·00	1·00	1·00	0·95	1·00	1·00	0·99
*AAT-2**	100	1·00	1·00	1·00	1·00	1·00	1·00	1·00	1·00	1·00	1·00	NT	NT	0·41	0·33	0·65	0·68
	110	0·00	0·00	0·00	0·00	0·00	0·00	0·00	0·00	0·00	0·00	NT	NT	0·59	0·67	0·35	0·32
*AAT-3**	075	0·00	0·00	0·00	0·00	0·00	0·00	0·00	0·00	0·00	0·00	0·05	0·12	0·09	0·17	0·05	0·17
	099	0·00	0·00	0·00	0·00	0·00	0·00	0·00	0·00	0·00	0·00	0·70	0·76	0·82	0·50	0·90	0·75
	100	1·00	1·00	1·00	1·00	1·00	1·00	1·00	1·00	1·00	1·00	0·00	0·00	0·00	0·00	0·00	0·00
	130	0·00	0·00	0·00	0·00	0·00	0·00	0·00	0·00	0·00	0·00	0·25	0·12	0·09	0·33	0·05	0·08
*ACP**	100	1·00	1·00	1·00	1·00	1·00	1·00	1·00	1·00	1·00	NT	0·00	0·00	0·00	NT	0·00	0·00
	110	0·00	0·00	0·00	0·00	0·00	0·00	0·00	0·00	0·00	NT	1·00	1·00	1·00	NT	1·00	1·00
*ADH**	002	0·00	0·00	0·00	0·00	0·00	0·00	0·00	0·00	0·00	0·00	NT	NT	0·00	0·00	0·11	0·04
	030	0·00	0·00	0·00	0·00	0·00	0·00	0·00	0·00	0·00	0·00	NT	NT	0·80	0·50	0·66	0·65
	090	0·00	0·00	0·00	0·00	0·00	0·00	0·00	0·00	0·00	0·10	NT	NT	0·00	0·00	0·00	0·00
	100	1·00	1·00	1·00	1·00	1·00	1·00	1·00	1·00	1·00	0·90	NT	NT	0·00	0·00	0·00	0·00
	105	0·00	0·00	0·00	0·00	0·00	0·00	0·00	0·00	0·00	0·00	NT	NT	0·20	0·50	0·22	0·22
	140	0·00	0·00	0·00	0·00	0·00	0·00	0·00	0·00	0·00	0·00	NT	NT	0·00	0·00	0·00	0·08
	160	0·00	0·00	0·00	0·00	0·00	0·00	0·00	0·00	0·00	0·00	NT	NT	0·00	0·00	0·00	0·01
*CK**	100	0·95	0·90	1·00	0·98	1·00	1·00	0·96	0·83	0·95	NT	1·00	1·00	1·00	1·00	1·00	1·00
	105	0·05	0·10	0·00	0·02	0·00	0·00	0·04	0·17	0·05	NT	0·00	0·00	0·00	0·00	0·00	0·00
*aGPDH**	080	0·00	0·00	0·00	0·00	0·00	0·00	0·00	0·00	0·00	NT	0·05	0·39	0·00	0·33	0·05	0·10
	090	0·00	0·00	0·00	0·02	0·00	0·12	0·03	0·00	0·00	NT	0·00	0·00	0·00	0·00	0·00	0·00
	100	0·95	0·90	1·00	0·98	1·00	0·88	0·97	1·00	1·00	NT	0·95	0·61	1·00	0·67	0·95	0·90
	120	0·05	0·10	0·00	0·00	0·00	0·00	0·00	0·00	0·00	NT	0·00	0·00	0·00	0·00	0·00	0·00
	130	0·00	0·00	0·00	0·00	0·00	0·00	0·00	0·00	0·00	0·00	0·00	0·00	0·05	0·17	0·00	0·00
*GPI-2**	100	1·00	1·00	0·93	1·00	1·00	1·00	0·93	1·00	1·00	1·00	1·00	1·00	1·00	1·00	1·00	1·00
	105	0·00	0·00	0·07	0·00	0·00	0·00	0·07	0·00	0·00	0·00	0·00	0·00	0·00	0·00	0·00	0·00
*IDH-1**	100	1·00	1·00	1·00	1·00	1·00	1·00	1·00	1·00	1·00	1·00	1·00	1·00	1·00	1·00	1·00	1·00
*IDH-2**	080	0·00	0·00	0·00	0·00	0·00	0·00	0·00	0·00	0·20	0·00	0·00	0·10	0·00	0·00	0·00	0·00
	100	1·00	1·00	1·00	0·95	1·00	0·96	0·96	1·00	0·80	0·90	0·95	0·80	0·95	1·00	0·95	0·91
	110	0·00	0·00	0·00	0·05	0·00	0·04	0·04	0·00	0·00	0·10	0·05	0·10	0·05	0·00	0·05	0·09
*LDH**	070	0·00	0·00	0·00	0·00	0·00	0·00	0·00	0·00	0·00	0·00	1·00	1·00	1·00	1·00	1·00	1·00
	100	1·00	1·00	1·00	1·00	1·00	1·00	1·00	1·00	1·00	1·00	0·00	0·00	0·00	0·00	0·00	0·00
*MDH**	080	0·00	0·00	0·00	0·00	0·00	0·00	0·00	0·00	0·00	0·00	0·00	0·06	0·00	0·00	0·00	0·00
	090	0·00	0·00	0·00	0·00	0·00	0·00	0·00	0·00	0·00	0·00	0·00	0·00	0·09	0·17	0·00	0·02
	095	0·00	0·00	0·00	0·00	0·00	0·00	0·00	0·00	0·00	0·00	0·00	0·00	0·00	0·00	0·00	0·01
	100	1·00	1·00	1·00	1·00	1·00	1·00	1·00	1·00	1·00	1·00	0·95	0·94	0·91	0·83	1·00	0·97
	120	0·00	0·00	0·00	0·00	0·00	0·00	0·00	0·00	0·00	0·00	0·05	0·00	0·00	0·00	0·00	0·00
*ME-2**	100	1·00	1·00	1·00	1·00	1·00	1·00	1·00	1·00	1·00	NT	1·00	1·00	1·00	1·00	1·00	1·00
*6PGDH**	100	1·00	1·00	1·00	1·00	1·00	1·00	1·00	1·00	1·00	1·00	1·00	1·00	1·00	1·00	1·00	1·00
*PGM**	080	0·00	0·00	0·00	0·00	0·00	0·00	0·00	0·00	0·00	0·00	0·10	0·00	0·05	0·00	0·05	0·04
	090	0·00	0·00	0·00	0·00	0·00	0·00	0·00	0·00	0·00	0·00	0·15	0·00	0·00	0·00	0·00	0·00
	100	1·00	1·00	1·00	0·96	1·00	0·96	0·94	1·00	1·00	1·00	0·75	1·00	0·95	0·83	0·95	0·92
	110	0·00	0·00	0·00	0·00	0·00	0·00	0·00	0·00	0·00	0·00	0·00	0·00	0·00	0·17	0·00	0·02
	120	0·00	0·00	0·00	0·04	0·00	0·04	0·06	0·00	0·00	0·00	0·00	0·00	0·00	0·00	0·00	0·02
*SOD**	050	0·00	0·00	0·00	0·00	0·00	0·00	0·00	0·00	0·00	0·00	0·00	0·00	0·00	0·33	0·00	0·07
	100	1·00	1·00	1·00	1·00	1·00	1·00	1·00	1·00	1·00	1·00	1·00	1·00	1·00	0·67	1·00	0·93

The size (*N*) and the heterozygosity (*H*) of samples in each case are indicated at the top of the columns. NT = enzyme not tested. For key to the abbreviations, see Fig. 1.

TABLE III. Nei genetic distances between each sample

	Turbot										Brill						
	KATT	NORD	HOLL	BRET	BIAR	PORT	LION	ADRI	EGEE	MARO	KATT	HOLL	BRET	PORT	EBRE	LION	
KATT	0	0·000	0·001	0·000	0·000	0·001	0·001	0·001	0·003	0·001	0·290	0·304	0·290	0·271	0·266	0·261	KATT
NORD		0	0·001	0·001	0·001	0·002	0·001	0·001	0·003	0·002	0·293	0·306	0·293	0·273	0·269	0·264	NORT
HOLL			0	0·001	0·000	0·001	0·000	0·002	0·003	0·001	0·290	0·305	0·289	0·272	0·265	0·261	HOLL
BRET				0	0·000	0·001	0·000	0·002	0·002	0·001	0·290	0·304	0·290	0·272	0·266	0·261	BRET
BIAR					0	0·001	0·001	0·002	0·002	0·001	0·288	0·303	0·287	0·270	0·264	0·259	BIAR
PORT						0	0·001	0·003	0·003	0·002	0·292	0·304	0·292	0·272	0·268	0·263	PORT
LION							0	0·002	0·003	0·001	0·293	0·308	0·293	0·275	0·269	0·264	LION
ADRI								0	0·003	0·003	0·294	0·309	0·293	0·276	0·269	0·265	ADRI
EGEE									0	0·003	0·296	0·307	0·295	0·279	0·271	0·266	EGEE
MARO										0	0·285	0·298	0·284	0·272	0·260	0·255	MARO
KATT											0	0·013	0·004	0·034	0·010	0·010	KATT
HOLL												0	0·012	0·033	0·015	0·014	HOLL
BRET													0	0·037	0·006	0·008	BRET
PORT														0	0·045	0·038	PORT
EBRE															0	0·002	EBRE
LION																0	LION
	KATT	NORD	HOLL	BRET	BIAR	PORT	LION	ADRI	EGEE	MARO	KATT	HOLL	BRET	PORT	EBRE	LION	

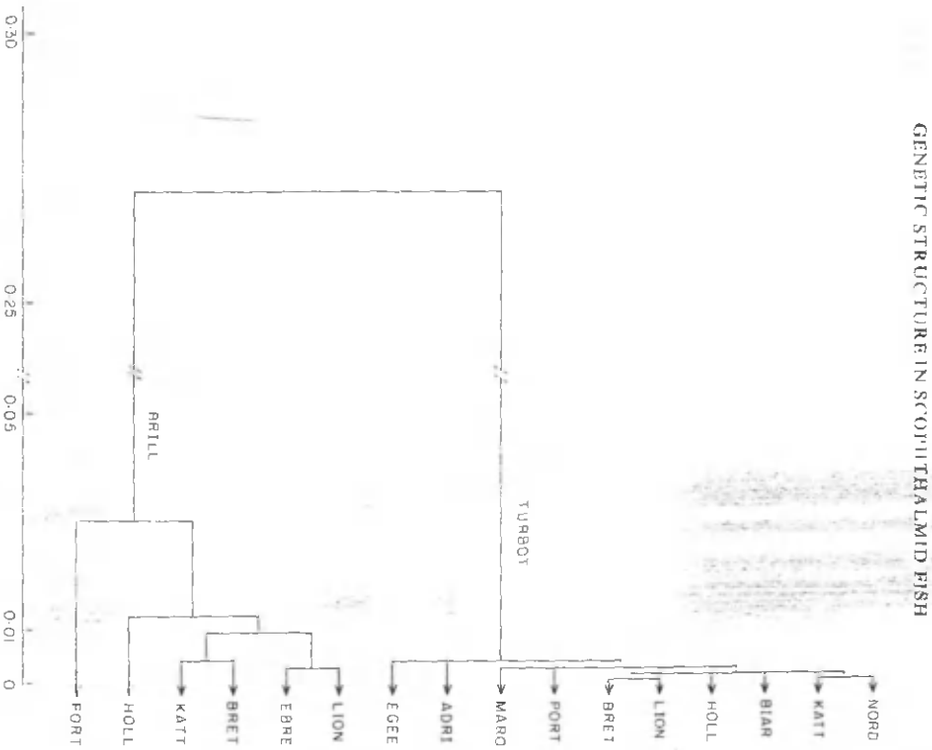

FIG. 2. UPGMA dendrogram of Nei genetic distances obtained from the 14 sets of samples analysed.

are sympatric over most of their current geographic range (except on the Moroccan coasts where only turbot occurs) allowing us to assume that they probably underwent the same intensity of climatic events and constraints related to ice ages. According to paleohistorical hypotheses concerning *Platichthys flesus* L. (Borsa *et al.*, 1987; Berrebi, 1988) and *P. maxima* (Renaud *et al.*, 1990), the Mediterranean populations originated from an Atlantic parent population, subunits of which had already penetrated the Mediterranean during various cold episodes. The absence of genetic differentiation between distant Atlantic populations can be explained by very rapid recent south–north recolonization (post-glacial). The weak differentiation between Atlantic and Mediterranean brill populations could be due to an invasion of the Mediterranean during the last ice age. Even if gene flow between the two areas was interrupted, the process has been too recent to allow much differentiation.

TABLE IV. χ^2-tests between samples

Brill				Turbot										
	1/2	1/3	2/3		1/2	1/3	1/4	1/5	2/3	2/4	2/5	3/4	3/5	4/5
*AAT-3**	–	+	–	*CK**	–	–	–	NT	–	–	NT	–	NT	NT
*AAT-2**	NT	NT	++	*aGPDH**	–	–	–	NT	–	–	NT	–	NT	NT
*ADH**	NT	NT	–	*GPI-2**	–	–	–	–	+	–		–	–	
*aGPDH**	–	++	–	*IDH-2**	++	–	++		–	+	–	++	–	–
*GPI-1**	–	–	++	*PGM**	–	–	–	–	–	–	–	–	–	–
*IDH-2**	–	–	–											
*MDH**	–	–	–											
*PGM**	–	–	–											
*SOD**	–	–	–											

In brill, 1 = KATT + HOLL (North Atlantic coasts); 2 = BRET + PORT (West Atlantic); 3 = EBRE + LION (W. Mediterranean). In turbot, 1 = KATT + NORD + HOLL (North Atlantic); 2 = BRET + BIAR + PORT (West Atlantic); 3 = LION + ADRI (West Mediterranean); 4 = EGEE and 5 = MARO.

+: significant ($P < 0{\cdot}05$); ++: highly significant ($P < 0{\cdot}01$); –: not significant ($P > 0{\cdot}05$); NT = not analysed.

An alternative hypothesis is that the persistence of current genetic exchanges has been large enough to oppose the effects of genetic drift. In fishes, the extent of exchange depends on the capacities of adults to migrate and particularly on passive exchanges during pelagic stages.

In the case of turbot, it should be stressed that although geographic homogeneity appears to be demonstrated, the low genetic diversity does not allow definitive confirmation of the absence of geographic differentiation except for the *maoetica* taxon. Other markers would have to be used (such as mitochondrial DNA). Moreover, this absence of structure appears to conflict with parasitological data obtained by Renaud *et al.* (1986) according to which cestodes of the species *Bothriocephalus gregarius*, which are turbot parasites, show significant differentiation in southern Portugal. This differentiation, indicating a break in gene flow in the parasites, is thus not parallelled by a similar phenomenon in the host. Its cause is more likely to be found in variations of climatic conditions, producing a local interruption of the parasite cycle (such as the absence of an intermediate host), which separates the parasites but not the hosts (Renaud *et al.*, 1990).

What is the origin of the differentiation of the Greek population? The presence of allele 80 at locus *IDH-2** at a frequency of 0·20, as well as the clear morphological difference described above are proof of differentiation of populations in the Aegean Sea relative to other areas (Mediterranean and Atlantic). It shows that gene flow is interrupted or very limited between the Aegean and the Adriatic. This leads us to propose other hypotheses to explain the absence of contrasted differentiation of these species as well as the differentiation of the taxon *maoetica*. Our argument is based on examples of other Pleuronectiformes. The colonization of the Mediterranean by flounder (Borsa *et al.*, 1987; Berrebi, 1988) probably occurred in at least two phases. Firstly, an ancient invasion of Atlantic origin affecting the whole Mediterranean basin and the Black Sea. Probably this was followed by a more recent colonization (last ice age, 20 000 years ago) affecting only the western Mediterranean. An analogous pattern of invasion and withdrawal, with a persistence of refuge zones, is possible in the case of turbot.

V. CONCLUSION

The present study allows a comparison of results obtained by different methods:

parasitological markers suggest general homogeneity in brill and a division of turbot on either side of Portugal;
morphology indicates homogeneity within the species, except for the taxon *maoetica* of turbot, which is sometimes considered to be a species; and
enzyme markers show only weak differentiation and tend to indicate that the taxon *maoetica* is a mere local adaptation.

These results and comparison with observations in a similar species (flounder) lead to two hypotheses: the first attributes homogeneity within species to persistent gene flow during the pelagic phase, and the second takes into account the history of colonization in the large maritime regions.

The persistence of gene flow in certain areas is not in conflict with the explanation based on colonization history. The formation of a morphological entity such as the taxon *maoetica* may be related to different waves of colonization followed by geographic isolation, whereas the absence of differentiation between other localities may be due to locally large gene flows. Over such long coastlines, the two kinds of hypothesis can be proposed simultaneously and complementarily.

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Stimulatory effectiveness of amino acids on the olfactory response in an algivorous marine teleost, the rabbitfish *Siganus fuscescens* Houttuyn

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The detection of threshold concentrations and relative stimulatory effectiveness (RSE) for 19 amino acids were studied by olfactory bulbar electroencephalogram (EEG) in the algivorous rabbitfish. The threshold concentrations for 19 amino acids ranged from 10^{-10} to 10^{-1} M. L-Alanine was the most effective amino acid, and the threshold concentration was estimated to be from 10^{-10} to 10^{-9} M. These results were compared with the olfactory response in herbivorous and carnivorous fishes previously reported. The comparison of RSE for rabbitfish with those for other fishes showed correlation coefficients ranging from $r = 0{\cdot}97$ to $r = 0{\cdot}50$, but high similarities did not correspond with difference in feeding habits for herbivores and carnivores. The role of amino acids in fish chemosensory behaviour and the olfactory response of the rabbitfish was compared with the gustatory response reported previously. It was found that L-serine was the most potent stimulant in both chemoreceptors, L-proline and L-glutaminic acid were the most potent gustatory stimulants, and L-alanine, L-glutamine, L-arginine and L-lysine were the most potent olfactory stimulants.

Key words: amino acid; algae; herbivore; olfaction; taste.

I. INTRODUCTION

In fishes, water-soluble chemical compounds are detected by olfactory and gustatory receptors. The olfactory system plays an important role in fish behaviour, i.e. feeding, schooling, courtship, migration, recognition of individuals and their own broods (Hara, 1975; Liley, 1982) and discrimination of odours of aquatic plants (Walker & Hasler, 1949), while the gustatory system is directly associated with feeding behaviour (Atema, 1977). In recent years, olfactory and gustatory studies in fish have focused largely on feeding problems in aquaculture.

Electrophysiological studies have shown that the receptors in both olfaction and taste are effective and sensitive for amino acids in many fish species (Caprio, 1988). Behavioural studies demonstrated that amino acids are the most potent stimuli and cause fish to be attracted to food and to eat food (Carr *et al.*, 1977). These results have provided evidence that amino acids act as chemical cues, such as attractants, stimulants or repellents in feeding behaviour. However, the question remains—what is the functional difference of the role of amino acid stimuli between olfaction and taste in feeding behaviour?

It has been considered that olfaction is the more sensitive distant chemoreceptor and taste is the contact chemoreceptor. However, Caprio (1978) found that the gustatory receptor of the channel catfish, *Ictalurus punctatus* Rafinesque is more sensitive to L-cysteine and L-alanine than the olfactory receptor. Furthermore,

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