Marine Biology 43, 109-116 (1977)

MARINE BIOLOGY

Genetic Control and Variability of Phosphoglucose Isomerase (PGI) in Eels from the Atlantic Ocean and the Mediterranean Sea

A. Comparini, M. Rizzotti and E. Rodinò

Institute of Animal Biology, University of Padua; Padua, Italy

Abstract

There is evidence in the scientific literature of differences in the genetic control of the enzyme phosphoglucose isomerase (PGI) in the European eel *Anguilla anguilla* and the American eel *A. rostrata*. Two PGI loci have been described in the former species, while 3 PGI loci have been reported in the latter. There is also evidence of differences in the tissue distribution of PGI isoenzymes in European eels from the English Channel and the Mediterranean Sea which suggest genetic diversity between the two populations. Studies carried out on two fairly large samples of Mediterranean (coast of Tuscany) and Atlantic (coast of Wales) *A. anguilla*, and on a small sample of *A. rostrata*, have permitted a clearer and more complete interpretation of the complex PGI electrophoretic patterns present in the tissues of eels. No difference, however, was observed in PGI genetic control between *A. anguilla* and *A. rostrata*: two loci encode PGI in both species. The patterns of tissue activity of PGI isoenzymes in the two samples of *A. anguilla* were essentially the same. There were, also, no significant differences in the PGI allele frequency between the two samples of European eels. These results, which support the genetic homogeneity of eels from different parts of Europe, are discussed in relation to the classical and more recent theories on the origin of the Atlantic and Mediterranean eels.

Introduction

The systematic study of Avise and Kitto (1973) on the electrophoretic patterns of phosphoglucose isomerase (PGI: E.C. 5.3.1.9) in 53 species of bony fishes has shown that this enzyme, which has a dimeric structure, is generally controlled in teleosts by 2 loci with differential tissue expression. Only in some species of Salmonidae and Cyprinidae are 3 or 4 PGI loci present, reflecting the tetraploid origin of these species (Ohno, 1970; Schmidtke *et al.*, 1975). Dando's (1974) study of PGI electrophoretic patterns in 23 species of teleosts confirmed the wide occurrence of 2 PGI loci and their different tissue specificities in this group.

Among teleosts, the presence of 2 PGI loci has also been described in the Anguilliformes *Congrina flava* (Avise and Kitto, 1973), *Conger conger* and *Anguilla anguilla* (Dando, 1974). In contrast, Williams *et al.* (1973) have reported 3 PGI loci in the American eel *A. rostrata*.

In the course of studies designed to verify the presence of genetically distinct populations of the European eel *Anguilla anguilla*, in the Mediterranean Sea, we have used PGI (with other enzyme systems) as genetic markers (Comparini *et al.*, 1975). Our observations confirmed the presence of two loci in these eels, a result which agrees with that of Dando (1974) on eels from the English Channel. Some clear differences exist, however, between the electrophoretic patterns we obtained and those reported by Dando. This observation is interesting because it suggests that a real genetic distinction may exist between Atlantic and Mediterranean eels.

The classic theory of Schmidt (1922) on the origin of the Atlantic eels assumed the presence of two distinct species, *Anguilla anguilla* (European eel) and *A. rostrata* (American eel), both spawning in contiguous areas in the Sargasso Sea only. On the basis of the difficulties inherent in the migration of silver eels,

the presence of small leptocephali of 50 mm in the central Mediterranean Sea, and biometrical differences between groups of elvers of different origin, some Italian authors (Grassi, 1914; Mazzarelli, 1914; Sanzo, 1928) have proposed the existence of one or more eel spawning areas in the Mediterranean Sea as well. This hypothesis found recent support in the discovery of differences in the phenotypic distribution of transferrin variants, between eels from different locations in the Mediterranean Sea and between these and eels from the Atlantic coasts of Europe (Drilhon and Fine, 1971).

Another controversy regarding the origin of the Atlantic eels arose from the paper of Tucker (1959). In Tucker's opinion, the mature European eels die during migration to the spawning area in the Sargasso Sea. The eels which spawn there come only from the nearer American coasts. The European eels originate therefore from the same gene pool as the American eels. This hypothesis received considerable attention in the following years (see D'Ancona, 1959; Bruun, 1963; Vladykov, 1964; Pantelouris *et al.*, 1971; Koehn, 1972), but recently the controversy was resolved in favour of the existence of two distinct species. In fact, De Ligny and Pantelouris (1973) detected significant differences in MDH allozyme frequencies between samples of American and European eels, and Ohno *et al.* (1973) demonstrated that the chromosome complement of *Anguilla anguilla* is strikingly different from that of *A. rostrata*.

The presence of 2 PGI loci in one species (*Anguilla anguilla*) while 3 are reported in the other (*A. rostrata*) would, if confirmed, also be conclusive in rejecting Tucker's hypothesis. Moreover, since the existing data exclude the possibility of a tetraploid origin of *A. rostrata* (see Ohno *et al.*, 1973), it appears that an early duplication, involving one of the two PGI loci, may have occurred in the genome of the American eel. Evidence of a similar duplication was reported by Kuhl *et al.* (1976) in the characid *Cheirodon axelrodi*.

Because of the widespread interest shown in these problems, we have extended our observations on the genetic control of PGI in eels by comparing the electrophoretic patterns observed in samples of the Mediterranean eel and of the two Atlantic species, and by studying the activities of the PGI loci in different tissues. In order to verify the possible genetic difference between Mediterranean and Atlantic populations of *Anguilla anguilla*, we have also compared the genetic variability of some enzyme systems in samples of eels from the Tyrrhenian coast of Italy and from the coast of Wales (UK); data on the genetic variability of the two PGI loci in these samples are reported in this paper.

Materials and Methods

Materials

The eel is a catadromous fish. The young unpigmented *Anguilla anguilla* (elvers or glass-eels) move into the rivers along Italian coasts mainly during the winter months. In inland waters elvers become pigmented, reaching through the stage of "yellow eel" the mature stage of "silver eel".

The Tyrrhenian sample of elvers was captured, by close-mesh net, in the locality of Marina di Pisa (mouth of the River Arno) during the winter of 1976. Specimens of more advanced stages ("yellow eel" and "silver eel") were taken from the eel's ponds ("valli") of Comacchio and from the fish market of Chioggia (Adriatic Sea). A number of elvers were reared in our laboratories for several months, until they were pigmented and large enough to be utilized for studying the tissue distribution of PGI isoenzymes. We refer to these as "little eels". The English sample of "little eels" (from 61 to 75 mm lengths) and some grown individuals were captured in a stream near Swansea during the summer of 1976, and stored frozen at -70°C. We also obtained two specimens of "yellow" *Anguilla rostrata* captured by angling in the Hudson river near New York City (USA). Excised livers and portions of skeletal muscle were stored on ice and returned to our laboratory for tissue preparation.

Liver, heart, skeletal muscle, intestine and brain of representative "little eels" and more advanced stages of *Anguilla anguilla* were examined by electrophoresis. With elvers, because of their small size, we have routinely used the post-branchial segment (a body segment extending about 1 cm behind the gill opening) which includes the heart, liver and intestine: it is possible to obtain a complete electrophoretic pattern of all PGI isoenzymes using this segment alone. However, we have also examined excised livers, other body segments and whole elvers.

Sample Preparation and Electrophoresis

In Mediterranean individuals, extracts for electrophoresis were obtained by homogenizing tissue samples newly dis-

sected out from freshly killed individuals. In some cases, however, tissue samples were stored frozen (-30°C) for some months until needed; no significant differences in electrophoretic patterns were observed between frozen and fresh tissues.

The tissues were finely minced with scissors in 2 to 3 volumes of 0.05 M Tris-HCl, pH 7.2, at 4°C. The homogenates were centrifuged at 10,000 x *g* for 5 min, at 4°C, and the supernatant solution used for electrophoresis.

Horizontal electrophoresis was performed in 11.5% starch gel, using a discontinuous, pH 8.2, buffer system (lithium hydroxide-borate; Tris-citrate) described by Shaw and Prasad (1970). A voltage gradient of 15 to 20 V/cm was applied for 7 to 10 h at 4°C. Following electrophoresis, the gels were sliced horizontally and the lower halfs were incubated at 37°C in a PGI staining solution, as described by Shaw and Prasad (1970). In some cases the extracting solution and electrophoretic buffer (Tris-borate, pH 8.7) used by Dando (1974) were used; the PGI electrophoretic patterns were completely comparable to that obtained by our usual method.

The upper slices of the gels were used to stain specifically for glucose-6-phosphate dehydrogenase (G6PD: E.C. 1.1.1.49) and 6-phosphogluconate dehydrogenase (6-PGD: E.C. 1.1.1.44) enzymes, which often appear secondarily to the PGI specific staining, mainly in liver samples. Specific identification of G6PD and 6-PGD was done by the method of Shaw and Prasad (1970). The stained bands corresponding to these two enzymes were clearly distinguishable, in position and appearance, from those corresponding to PGI isoenzymes.

Results

Electrophoretic Patterns of PGI in Anguilla anguilla and A. rostrata

Phosphoglucose isomerase is a complex system in fishes, and its expression is different in different tissues. To obtain a complete electrophoretic pattern of all the PGI isoenzymes in *Anguilla anguilla*, it was useful to homogenize the whole post-branchial segment of elvers and "little eels". Fig. 1 shows some of the patterns observed, together with a schematic representation of the molecular and genetic interpretation of these patterns. This interpretation agrees substantially with that reported for other teleosts (Avise and Kitto, 1973; Dando, 1974; Schmidtke *et al.*, 1975), and proposes the existence of two PGI loci, PGI-1 and PGI-2, whose polypeptide products interact to form functionally active homodimers and heterodimers. Both loci are variable with several alleles at each locus. Therefore, the patterns showing the minimum number of three isoenzymatic bands (excluding the weaker satellite bands) belong to individuals homozygous at both loci. In the heterozygotes at one or both loci, patterns with 6, or 10 isoenzymatic bands, respec-

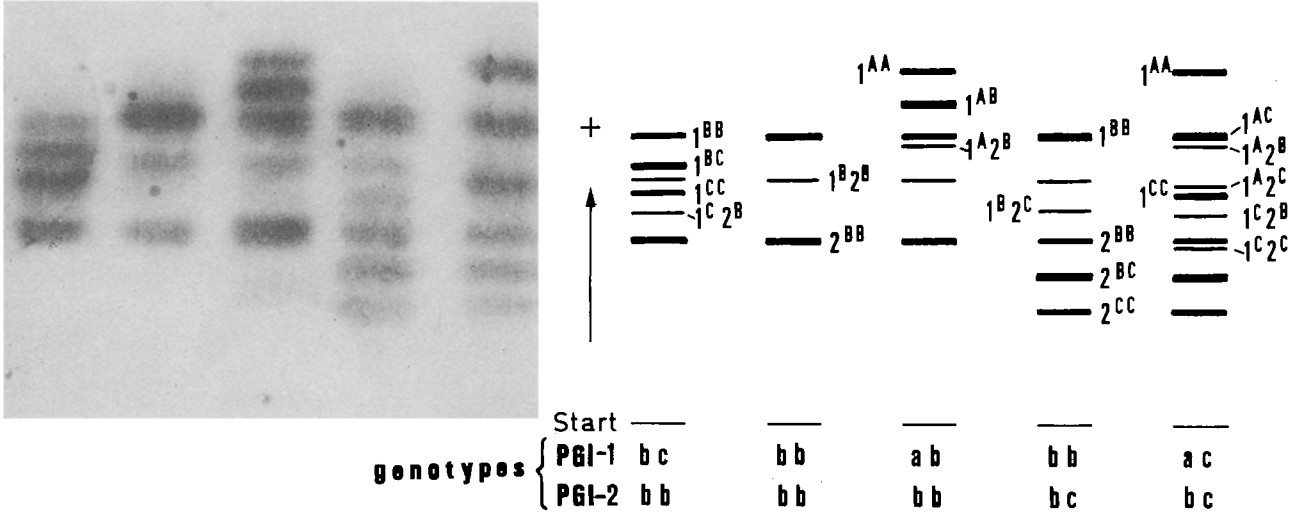

Fig. 1. *Anguilla anguilla*. Photograph of some PGI electrophoretic patterns observed in extracts of the post-branchial segments of elvers and "little eels" (Mediterranean and Atlantic populations) with a schematic representation of the molecular and genetic interpretation of these patterns. A, B, C indicate polypeptide products of the a, b, c, alleles of the PGI-1 or PGI-2 loci

tively, occur; they are produced by random combination of the polypeptide products to form homodimers (e.g. 1^{AA}, 1^{CC}, 2^{BB}, 2^{CC}, etc.) and intralocus (e.g. 1^{AC}, 2^{BC}, etc) and interlocus heterodimers (e.g. $1^{A}2^{B}$, $1^{A}2^{C}$, $1^{C}2^{B}$, $1^{C}2^{C}$, etc.). In practice, however, the number of visible bands is often smaller, due to the overlapping or closeness of some of them (see Fig. 1). The lower staining intensity of the interlocus heterodimer bands depends, at least partially, on the fact that the two loci have different activity in different tissues (see below and Fig. 3) and, therefore, their polypeptide products are in part spatially separated.

When liver, or samples containing liver, are used in electrophoresis and the gel is overstained, another more anodal zone presenting 1 or 3 bands with weak enzymatic activity appears in the gel (Fig. 2). By specific staining, it was possible to demonstrate that this anodal zone represents the activity of the 6-PGD locus.

Analysis of tissue samples of two specimens of *Anguilla rostrata* showed that the PGI electrophoretic pattern was practically indistinguishable from that described for *A. anguilla*. On the basis of this observation, it was possible to conclude that there were 2, and not 3, PGI loci in the two American eel specimens. The two individuals were apparently homozygous at both loci; the mobility of the electrophoretic bands corresponded perfectly to that of a European eel of the most common genotype: PGI-1 bb, PGI-2 bb.

Genetic Variability at the PGI Loci in European Eels

As reported in a previous paper (Comparini *et al.*, 1975), there is considerable variability at both loci in the Mediterranean eels. We observed 4 alleles (a-d) at the PGI-1 locus and 3 (a-c) at the PGI-2 locus. The analysis of the new sample of elvers from the Tyrrhenian coast (Pisa) has substantially confirmed our previous results; we have found, however, a new rare allele (d) at the PGI-2 locus, while the very rare allele, d, at the PGI-1 locus was lacking in this sample. By more prolonged electrophoresis and accurate analysis of the patterns, moreover, we have clearly resolved another PGI-1 allele (a'). The same kind of variability, with the same alleles at both loci (with the exception of the rarest two, PGI-1 d and PGI-2 d), was found in the sample of "little eels" from the Welsh coast (Swansea). The cor-

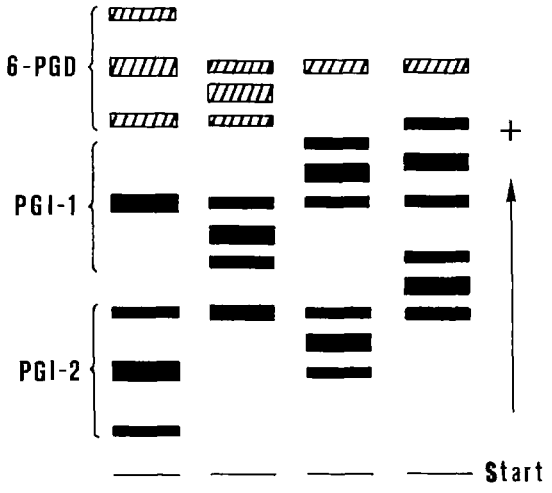

Fig. 2. *Anguilla anguilla*. Diagrammatic representation of some overstained electrophoretic patterns of the post branchial segment of elvers and "little eels" (Mediterranean and Atlantic populations). Positions of principal PGI-1 and PGI-2 bands are compared with the 6-PGD zone of activity. PGI genotypes, from left to right, are: PGI-1 bb, PGI-2 bd; PGI-1 bc, PGI-2 bb; PGI-1 ab, PGI-2 bc; PGI-1 a'b, PGI-2 ab

Table 1. *Anguilla anguilla*. Observed and expected distribution of PGI-1 and PGI-2 genotypes in samples from Pisa (Tyrrhenian coast) and from Swansea (Wales). To estimate χ^2 we have pooled classes with expected values <5

Genotypes	Pisa		Swansea	
	Observed	Expexted	Observed	Expected
PGI-1				
a a	2	1.5	0	0.4
b b	134	134.4	74	73.2
c c	4	1.9	1	0.6
a'b	4	3.2	1	1.7
a b	30	28.3	11	10.2
a'c	0	0.4	1	0.2
a c	1	3.3	1	1
b c	30	31.6	12	13.6
others	0	0.4	0	0.1
	205	205	101	101
	$\chi^2_3 = 0.18$		$\chi^2_3 = 0.26$	
	P >0.95 ns		P >0.95 ns	
PGI-2				
b b	185	185	91	90.3
c c	1	0.6	0	0.2
a b	5	4.6	2	2.8
a c	0	0.3	1	0.1
b c	21	21.4	7	7.6
b d	2	1.9	0	0
others	0	0.2	0	0
	214	214	101	101
	$\chi^2_2 = 0.03$		$\chi^2_2 = 0.05$	
	P >0.95 ns		P >0.95 ns	

Table 2. *Anguilla anguilla*. Observed number and frequencies of PGI-1 and PGI-2 alleles in samples from Pisa and from Swanses

Alleles	Pisa		Swansea	
	No.	Frequency	No.	Frequency
PGI-1				
a'	4	0.010	2	0.010
a	35	0.085	12	0.060
b	332	0.810	172	0.851
c	39	0.095	16	0.079
	410		202	
PGI-2				
a	5	0.012	3	0.015
b	398	0.930	191	0.945
c	23	0.054	8	0.040
d	2	0.005	0	0.000
	428		202	

Table 3. *Anguilla anguilla*. Comparison of allelic distribution, between Pisa and Swansea samples, by contingency tables. Rare alleles are pooled with the common allele closest to them

Alleles	Pisa		Swansea	
	Observed	Expected	Observed	Expected
PGI-1				
a'+ a	39	35.5	14	17.5
b	332	337.6	172	166.4
c	39	36.9	16	18.1
	$\chi^2_2 = 1.71$		P >0.30 ns	
PGI-2				
a + b	403	405.6	194	191.4
c + d	25	22.4	8	10.6
	$\chi^2_1 = 0.98$		P >0.30 ns	

respondent mobility of the various alleles in the two samples was proven by electrophoretic runs containing homogenates of representatives of both samples.

The distribution of the genotype frequencies at each locus, for each sample, is given in Table 1. In both cases, the observed values are very close to those expected according to the Hardy-Weinberg distribution (chi-square non-significant for both loci); this provides evidence as to the reliability of our genetic interpretation.

The observed number of alleles and their frequencies for both loci, are shown in Table 2. Comparison of allelic distribution by contingency tables (see Table 3) shows a substantial genetic similarity between the two samples.

Tissue Expression of the Two PGI Loci in Mediterranean Eels

We have studied the patterns of PGI isoenzymes in brain, heart, liver, intestine and skeletal muscle of Mediterranean eels (Fig. 3). Repeated analyses, on eels at different stages of growth, have shown that the tissue distribution of PGI isoenzymes is substantially the same in all length classes. Patterns illustrating the characteristic distribution of the PGI isozymes in various tissues in the more frequently observed homozygotes at both loci are presented diagrammatically in Fig. 4. The PGI-2 band is strongly expressed in skeletal muscle and almost absent elsewhere; the PGI-1 band is weak in muscle and very strong in liver, heart and the other tissues. The interlocus heterodimer fraction is weak, but clearly present in all the tissues, even when one of the two homodimers is almost absent. Each homodimer band displays some weak and anodal satellite bands; the fastest satellite band of the PGI-2 homodimer partially overlaps the heterodimer.

Tissue Expression of the Two PGI Loci in Atlantic Eels

Analysis of a sample of eels from the Welsh coast has shown a pattern of PGI tissue expression identical to that of Mediterranean eels. This pattern differs somewhat, however, from the one described by Dando (1974) in eels from the English Channel (see Table 4). The major differences are the presence, in our specimens, of interlocus heterodimer bands in all the tissues and the presence of PGI-1 bands in skeletal muscle. These bands are lacking in Dando's individuals.

The analysis of liver and skeletal muscle of the two specimens of *Anguilla rostrata* has also shown a PGI tissue expression identical to that observed in *A. anguilla*.

Discussion

By studying differences in electrophoretic patterns, it is often possible to deduce the genetic control of an enzyme system, even when laboratory breeding is impossible or impracticable. All the complex PGI patterns observed in this study, which included two fairly large samples of *Anguilla anguilla* at dif-

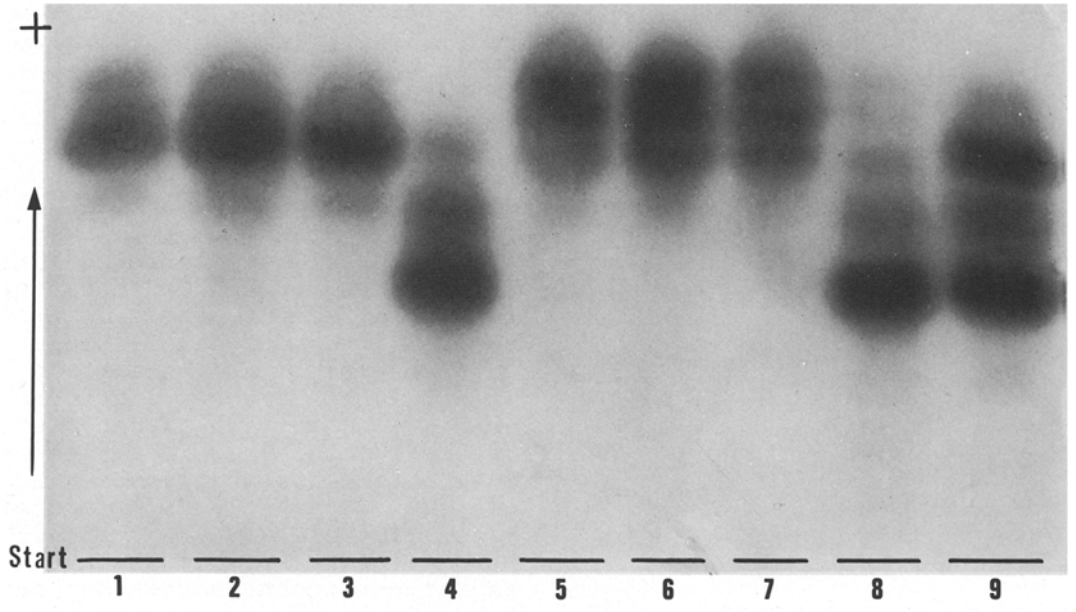

Fig. 3. *Anguilla anguilla*. PGI electrophoretic patterns in some tissues of eels from the Mediterranean. 1-4: brain, heart, liver and skeletal muscle extracts, respectively, of "silver eel" of genotype PGI-1 bb, PGI-2 bb. 5-8: intestine, heart, liver and skeletal muscle extracts, respectively, of "yellow eel" of genotype PGI-1 ab, PGI-2 bb. 9: whole-body extract of elver of genotype PGI-1 bb, PGI-2 bb

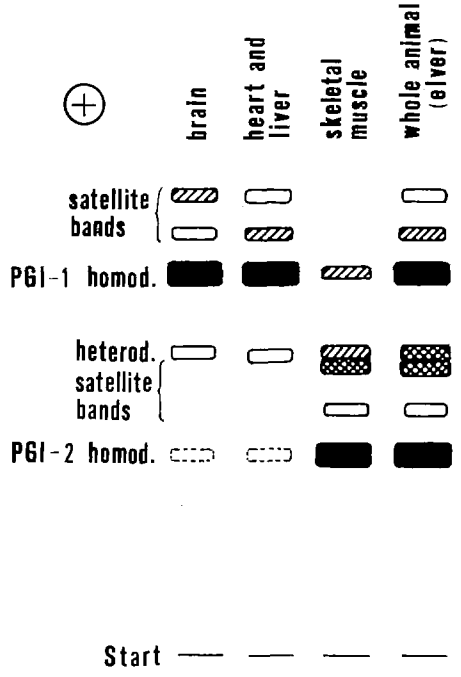

Fig. 4. *Anguilla anguilla*. Diagrammatic representation of PGI electrophoretic patterns observed in some tissues of Mediterranean and Atlantic eels. The same patterns have been observed in liver and skeletal muscle of *A. rostrata*. Degree of shading indicates strength of bands. homod: homodimer; heterod: heterodimer

ferent stages of growth and a small sample (two individuals) of *A. rostrata*, can be correctly explained on the basis of the presence of two loci (PGI-1 and PGI-2) with different tissue expressions and with several alleles at each locus.

The purpose of this work was to verify the reported differences in genetic control and tissue distribution of PGI in samples of Atlantic and Mediterranean eels and to analyse allelic variability at the PGI loci in different samples of these eels. In fact, no difference in the electrophoretic pattern between the samples of *Anguilla anguilla* and *A. rostrata*, nor between the two populations of European eels, were observed. Moreover, there were no significant differences in the frequencies of the various PGI alleles between the Atlantic and Mediterranean samples of *A. anguilla*.

The presence of 2 PGI loci in *Anguilla anguilla* is not surprising; in *A. rostrata*, on the other hand, the occurrence of only 2 loci is in sharp contrast with the report by Williams *et al.* (1973) that there are 3 PGI loci in this species. Apart from the possibility (never reported) that American eels comprise a heterogeneous population of eels of different origin, we may provide a possible interpretation of these contrasting results as follows: the more anodal PGI

Table 4. *Anguilla anguilla*. Schematic representation of PGI isoenzyme activity in some tissues. Present study: data on Mediterranean and Welsh-coast samples; Dando's study: data from Englisch Channel sample (Dando, 1974). Relative activity of electrophoretic bands is denoted as follows: +++: strong activity; +: moderate or weak activity; (+): very weak activity; -: no activity. Sk.muscle: skeletal muscle

Isoenzymatic bands	Present study			Dando's study		
	Heart	Liver	Sk.muscle	Heart	Liver	Sk.muscle
PGI-1 homodimer	+++	+++	+	+++	+++	-
Interlocus heterodimer	+	+	+	-	-	-
PGI-2 homodimer	(+)	(+)	+++	+	-	+++

zone reported by Williams *et al.* (1973) in liver (or in tissue samples containing liver) of *A. rostrata* corresponds possibly to the zone of activity of another enzyme system, most probably to that of 6-PGD (see above and Fig. 2).

The contrast between our findings and Dando's (1974) observations on the PGI tissue patterns in *Anguilla anguilla* (of which he examined 4 individuals only) may possibly be explained on the basis of a different interpretation of the electrophoretic patterns, particularly the presence-absence of the interlocus heterodimer bands that often overlap or are very close to other isoenzymatic bands (see Figs. 1, 3, 4). The lack of PGI-1 activity in the skeletal muscle of Dando's eels is less easily explainable. It must be remembered that Pantelouris *et al.* (1970) detected some differences in the frequencies of transferrins and liver esterase phenotypes among samples of eels from the Eastern North Atlantic coasts; the significance of this data with regard to the existence of genetic differences within the European eel population, has, however, been strongly challenged (see Koehn, 1972). Apart from this, the existence of a clear-cut genetic distinction between two groups of Atlantic eels, taken in places as near together as the English Channel (Dando's sample) and the Welsh coast (our sample), does not seem probable, especially in view of the complete correspondence in the PGI tissue patterns that we have observed between Western and Eastern Atlantic and Mediterranean eels.

The substantial similarity we have found both in tissue expression and in allele frequencies of the two PGI loci between the samples of *Anguilla anguilla* from the Mediterranean Sea and the Welsh Atlantic coasts seems to indicate that the two samples originated from a common gene pool. Data on the genetic variability of other enzyme systems, which will be discussed in another paper, seem to support such homogeneity, and agree also with the data of De Ligny and Pantelouris (1973) on MDH frequencies in samples of eels ranging from Greece to Poland. The regional differences detected by Drilhon and Fine (1971) in transferrin phenotypes in European eels from different locations do not seem to reflect with certainty real genetic diversity (see Koehn, 1972).

In conclusion, at the present time there is no certain evidence that different geographic races occur within the European population of eels.

Acknowledgements. This research has been supported by the Italian National Research Council (C.N.R.) through funds granted to the Institute of Marine Biology, C.N.R., Venice. Part of the work was done at the University College of Swansea with the use of the facilities of the Department of Genetics. A special thanks to Professor J.A. Beardmore for providing hospitality and also constructive criticism and helpful suggestions, and to Drs.R.D.Ward and D.O.F.Skibinski for help in the capture of eels and for technical assistance. We wish to thank also Professor B. Battaglia for reading and commenting on the manuscript; Drs. V. Scali and M. Masetti, University of Pisa, for furnishing Tyrrhenian elvers; and Mr. Mazzuccato for technical assistance. We gratefully acknowledge the kind help of Dr. N.P. Wilkins for critical reading the manuscript and for revision of the English text.

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Dr. A. Comparini
Istituto di Biologia Animale
Università di Padova
Via Loredan, 10
I-Padova 35100
Italy

Date of final manuscript acceptance: April 29, 1977. Communicated by B. Battaglia, Padua