

## Panmixia in the European eel: a matter of time...

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The European eel (*Anguilla anguilla* L.) has been a prime example of the panmixia paradigm because of its extraordinary adaptation to the North Atlantic gyral system, semelparous spawning in the Sargasso Sea and long trans-oceanic migration. Recently, this view was challenged by the suggestion of a genetic structure characterized by an isolation-by-distance (IBD) pattern. This is only likely if spawning subpopulations are spatially and/or temporally separated, followed by non-random larval dispersal. A limitation of previous genetic work on eels is the lack of replication over time to test for temporal stability of genetic structure. Here, we hypothesize that temporal genetic variation plays a significant role in explaining the spatial structure reported earlier for this species. We tested this by increasing the texture of geographical sampling and by including temporal replicates. Overall genetic differentiation among samples was low, highly significant and comparable with earlier studies ( $F_{ST}=0.0014$ ;  $p<0.01$ ). On the other hand, and in sharp contrast with current understandings, hierarchical analyses revealed no significant inter-location genetic heterogeneity and hence no IBD. Instead, genetic variation among temporal samples within sites clearly exceeded the geographical component. Our results provide support for the panmixia hypothesis and emphasize the importance of temporal replication when assessing population structure of marine fish species.

**Keywords:** *Anguilla anguilla*; conservation; genetic structure; microsatellites; temporal variation

### 1. INTRODUCTION

European eel recruitment is currently very low, at less than 1% of 1970s levels. Therefore, the biological status of this species has been set outside safe limits and precautionary actions must be taken immediately (Dekker 2003). Crucial knowledge about the biology and the life cycle of the eel is lacking, but genetic markers should help in assessing the partitioning of genetic stocks. Such information is necessary in order to develop a global management plan for this dangerously declining species.

According to the panmixia hypothesis, all European eels (*Anguilla anguilla* L.; Anguillidae; Teleostei) migrate to the Sargasso Sea for reproduction, and constitute a single, randomly mating population. This hypothesis is supported by early genetic studies using allozyme and mitochondrial DNA markers (DeLigny & Pantelouris 1973; Comparini *et al.* 1977; Comparini & Rodinò 1980; Yahyaoui *et al.* 1983; Lintas *et al.* 1998), which found no evidence for a spatial genetic structure. Similar results were obtained for the American eel (*A. rostrata*; Avise *et al.* 1986) and the Japanese eel (*A. japonica*; Sang *et al.* 1994), with the exception of clinal allozyme variation putatively imposed by selection (Williams *et al.* 1973; Koehn & Williams 1978; Chan *et al.* 1997). Therefore, panmixia in the European eel was widely accepted until three independent genetic studies recently reported evidence for a weak but

significant population structure (Daemen *et al.* 2001; Wirth & Bernatchez 2001; Maes & Volckaert 2002), with two of them finding evidence for isolation-by-distance (IBD) (Wirth & Bernatchez 2001; Maes & Volckaert 2002). The development and maintenance of such a structure requires temporal and/or spatial separation in the Sargasso Sea of spawning adult eels originating from different locations in Europe. This has to be followed by a non-random return of larvae to their parents' freshwater habitat through active swimming, seasonal changes in hydrodynamics or different pathways of the Gulf Stream (Wirth & Bernatchez 2001; Maes & Volckaert 2002).

A limitation of previous genetic studies on eels is the lack of temporal replication. For species with overlapping generations, such as the European eel, random allele frequency shifts among cohorts (year-classes) and sampling years are expected (Jorde & Ryman 1995). If not accounted for, temporal genetic heterogeneity may incorrectly be interpreted as true population differentiation (Waples 1998), particularly in situations of a suspected weak differentiation (Palm *et al.* 2003a). Since marine fishes are believed to have huge population sizes, temporal genetic heterogeneity has frequently been overlooked as a potential confounding factor when assessing population structure. This view may not apply universally because their genetically effective size ( $N_e$ ), an important parameter determining temporal shifts in allele frequency (Jorde & Ryman 1995), may be much lower than the census size (Hedgecock 1994; Hauser *et al.* 2002).

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Figure 1. Sampling locations of European eel. Large dots represent localities from which temporal samples were obtained. Details for each sampling location are listed in [table 1](#).

To correct for this source of bias in the European eel, we need to combine more extended geographical coverage, additional sampling points on the European coast and the assessment of the temporal stability in genetic structure.

Here, we report results from the most extensive genetic study, with respect to the number of locations and individuals analysed, of the European eel to date. We explore whether temporal genetic variation plays a significant role in explaining the structure reported recently, and whether any stable, geographical component remains after correction for this source of bias. We do this on the basis of hierarchical *F*-statistics accounting for temporal genetic variation and tests for IBD. If confirmed, assessment of the true cause of genetic differentiation is needed to develop sustainable management options for this threatened species.

## 2. MATERIAL AND METHODS

### (a) Sampling procedures

Eel larvae start to metamorphose into ‘glass eels’ as soon as they reach the continental shelf. When the glass eels migrate into coastal and inland waters to enter the main growth stage of the life cycle, they acquire pigments and are known as

‘yellow eels’. The yellow eels then undergo a final metamorphosis into ‘silver eels’ before they start their migration back to the Sargasso Sea for reproduction. Samples of glass, yellow and silver eels were collected from rivers along the European and African coasts between 1994 and 2002. In total, 2626 eels (62 samples) were collected at 41 locations, and we obtained temporal replicates at 12 of these sites ([figure 1](#)). In addition, 77 American eels were sampled at two locations and used as outgroups in a phenogram (see §2c). Detailed information about samples (year of sampling, life stage and sample size) is listed in [table 1](#). Glass eels sampled in a given year were considered to belong to a single cohort ([Arai \*et al.\* 2000](#)). In contrast, samples of yellow and silver eels consisted of multiple cohorts. We were unable to classify these individual eels by cohort because otoliths were not collected for age determination. The inclusion of samples consisting of multiple cohorts may result in an underestimation of the temporal genetic component ([Palm \*et al.\* 2003b](#)).

### (b) Genotyping procedures

We analysed the following six nuclear microsatellite loci, which all have been used to study population structure in the European eel previously: Aan01, Aan03, Aan05 ([Daemen \*et al.\* 1997, 2001](#); GenBank, accession numbers

Table 1. Sampling list (in alphabetical order) of the American eel (the first two samples) and the European eel, including sample code (temporal samples indicated by lower-case letters), country, sampling location, sampling year, life stage (G, glass eel; Y, yellow eel; S, silver eel) and sample size.

code	country	sampling location	sampling year	life stage	sample size
AR1	Canada	Musquash	1995	G	47
AR2	USA	St John's River	1999	S	30
BE a,b	Belgium	IJzer	1994–2001	G–G	48–54
DE1	Denmark	Guden å	2001	G	24
DE2	Denmark	Kolding å	2001	G	24
DE3	Denmark	Vester Vedsted	2001	G	24
EN1	England	Chelmer	2002	G	24
EN2	England	Parret	1994	G	48
EN3	England	Severn	2002	G	24
EN4	England	Stour	2002	G	24
FI	Finland	Kokemäenjoki	2001	Y	45
FR1 a,b	France	Loire	2001–2001	G–S	60–50
FR2	France	Arzal	1994	G	96
FR3 a,b	France	Frémur	2000–2000	G–S	24–24
FR4	France	Gironde	2002	G	46
FR5	France	Salses Leucate	2002	G	22
FR6 a,b,c	France	Tour-du-Valat	1999–2001–2001	S–G–S	45–60–51
GR	Greece	Sagiada	2001	G	48
IC	Iceland	Ölvusá	1999	S	60
IR1 a,b,c	Ireland	Burrishoole	1999–2001–2001	S–S–G	60–60–60
IR2 a,b,c	Ireland	Erne	2001–2001–2001	G–Y–S	48–24–24
IR3	Ireland	Feale	1994	G	20
IT1	Italy	Po	1999	S	28
IT2 a,b	Italy	Martha	2001–2002	G–G	48–45
IT3	Italy	Tibern	2002	G	48
LI	Lithuania	Curonian lagoon	2001	Y	48
MO1	Morocco	Moulouya	2001	G	38
MO2	Morocco	Oued Lockkos	1994	G	48
MO3 a,b,c	Morocco	Sebou	1999–2001–2001	G–S–G	60–60–60
NE a,b,c,d,e,f,g	The Netherlands	Den Oever	1994–1999–2000– 2000–2000– 2001–2001	G–S–G–Y–S–G–S	48–60–48–24–22– 60–60
NO a,b	Norway	Imsa	2000–2000	G–S	24–24
PO1	Portugal	Minho	2001	G	60
PO2	Portugal	Mira	1995	G	24
PO3 a,b	Portugal	Sisandro	1994–1995	G–G	24–24
SP	Spain	Asturias	1994	G	48
SW1	Sweden	Dalälven	2001	Y	24
SW2	Sweden	Ellenösjön	1998	Y	23
SW3	Sweden	Lagan	2001	G	24
SW4	Sweden	Motala	2001	Y	41
SW5	Sweden	Ringhals	2001	G	47
SW6	Sweden	Viskan	2000	G	48
TU	Tunisia	Médierda	2002	G	69
YU a,b	Yugoslavia	Bojana	2000–2001	G–G	48–48

U67163, U67165 and AY028638, respectively), Aro054, Aro095 and Ang151 (Wirth & Bernatchez 2001; GenBank, accession numbers AF237896, AF237897 and AF237902, respectively). DNA was extracted from frozen- or ethanol-preserved fin or muscle tissue using a chelex protocol (Walsh *et al.* 1991). Microsatellite analyses were conducted using multiplex polymerase chain reactions (PCR) at two laboratories following slightly different methods. At the Institute of Freshwater Research (Sweden), multiplex 1 included Ang151, Aro054 and Aro095, and multiplex 2 included Aan01, Aan03 and Aan05. The co-amplifications of loci were made in 25 µl volumes using Pharmacia Ready-To-Go PCR beads (Amersham Pharmacia Biotech Incorporated, NJ, USA) and approximately 100 ng of template DNA. Primers were end labelled with fluorescent dyes. The multiplex PCR

amplifications were initiated with a denaturation step at 94 °C for 5 min, followed by 27 cycles of 30 s at 94 °C, 30 s at an annealing temperature of 57 °C, and 1 min at 72 °C, ending with an 8 min elongation step at 72 °C. Electrophoresis and size determination of alleles were made on an ABI Prism 310 Genetic Analyser (Applied Biosystems, Foster City, CA, USA) used according to the manufacturer's recommendations.

At the Laboratory of Aquatic Ecology (Belgium), multiplex 1 included Aro095, Aro054, Aan05 and Ang151, and multiplex 2 included Aan03 and Aan01. The co-amplifications of loci were made in 25 µl volumes including 1× PCR buffer, 10–100 ng of template DNA, 1.5 mM MgCl<sub>2</sub>, 80 µM of dNTP, 0.125–0.80 µM of fluorescent labelled forward and non-labelled reverse primer, and 0.5 U of Goldstar Taq

Table 2. *F*-statistics (Weir & Cockerham 1984) from non-hierarchical analyses of European eel samples collected in Europe and North Africa (detailed sample information is given in table 1). (The analyses were based on six loci or a restricted dataset with four loci (see §3a). Temporal samples were pooled within locations in analyses 2–4. Analyses 5–8 refer to comparisons between locations within given cohorts. Analyses 9–20 refer to comparisons between temporal samples within given locations.)

analysis	comparison	no. samples	no. individuals	<i>F</i> -statistic six loci	<i>F</i> -statistic four loci
1	all samples	62	2626	0.0014***	0.0012**
2	all locations	41	2626	0.0010***	0.0006*
3	locations (small samples excluded)	23	2100	0.0012***	0.0013***
4	locations (temporal material)	12	1479	0.0012***	0.0012**
5	locations (glass eels 1994)	8	380	−0.0002	−0.0007
6	locations (glass eels 2000)	5	192	0.0001	0.0031
7	locations (glass eels 2001)	17	787	0.0006	−0.0002
8	locations (glass eels 2002)	8	302	0.0045***	0.0048**
9	France (Loire)	2	110	−0.0002	−0.0011
10	France (Frémur)	2	48	0.0080*	0.0187**
11	France (Tour du Valat)	3	156	0.0020	−0.0025
12	Netherlands (Den Oever)	7	322	0.0027**	0.0046**
13	Ireland (Burrishoole)	3	180	0.0017	−0.0009
14	Ireland (Erne)	3	96	−0.0003	0.0001
15	Morocco (Sebou)	3	180	0.0027*	0.0030
16	Norway (Imsa)	2	48	−0.0101	−0.0113
17	Belgium (IJser)	2	102	0.0024	0.0037
18	Italy (Martha)	2	93	−0.0053	−0.0038
19	Yugoslavia (Bojana)	2	96	−0.0011	0.0011
20	Portugal (Sisandro)	2	48	−0.0004	−0.0024

\* $p < 0.10$ , \*\* $p < 0.05$ , \*\*\* $p < 0.01$ .

polymerase (Eurogentec, Seraing, Belgium). The multiplex PCR amplifications were initiated with a denaturation step at 95 °C for 5 min, followed by 25 cycles of 30 s at 95 °C, 30 s at an annealing temperature of 57 °C and 30 s at 72 °C, and ended with an 8 min elongation step at 72 °C. Electrophoresis and size determination of alleles were made on a LICOR automatic sequencer (Model 4200, Westburg, Leusden, The Netherlands), using a 6% acrylamide 7 M urea sequencing gel. A molecular ladder (supplied by the manufacturer) was run along with the PCR products, and allele lengths and genotypes were assessed with the GENE-IMAGIR v. 4.03 software (Scanalytics, Inc., Fairfax, USA). A large number of randomly sampled individuals were analysed at both laboratories to calibrate the methods used for electrophoresis and size determinations of alleles.

### (c) Statistical procedures

Diversity statistics were calculated using the software GENETIX v. 4.02 (Belkhir *et al.* 2000). Deviations from expected Hardy–Weinberg equilibrium (HWE) were calculated using GENEPOP v. 3.1d (Raymond & Rousset 1995). The presence of null alleles was tested with the software MICRO-CHECKER v. 2.2.0 (van Oosterhout *et al.* 2004). Population structure was studied using non-hierarchical and hierarchical *F*-statistics (Weir & Cockerham 1984) calculated using GENETIX and ARLEQUIN v. 2.001 (Schneider *et al.* 2000), respectively. The partitioning of genetic variance into a spatial and a temporal component was performed using the hierarchical ‘locus by locus AMOVA’ as implemented in ARLEQUIN in combination with the ‘individual level’ option to include also the genotypic information. Significances of *F*-statistics were evaluated through 10 000 permutations.

Tests for IBD were performed using  $F_{ST}/(1-F_{ST})$  as a genetic distance (Rousset 1997). The significances of correlations between genetic distance and nearest sea distance, or

difference in nearest distance to the Sargasso Sea, which is a geographical distance roughly mimicking the recruitment route of the European eel, among pairwise comparisons of sampled locations, were evaluated using Mantel tests (Mantel 1967) as implemented in GENETIX. A phenogram based on an unbiased genetic distance (Nei 1978) was constructed using a neighbour-joining procedure in PHYLIP v. 3.6 (Felsenstein 1993). Support for the nodes was evaluated using a bootstrap procedure with 1000 randomizations. We used two samples of American eel (table 1) as outgroups.

## 3. RESULTS

### (a) Genetic variability and Hardy–Weinberg equilibrium

All six microsatellite loci analysed were highly polymorphic, with a mean number of alleles per locus, per sample ( $\pm$  s.d.), ranging from 7.83 ( $\pm$  3.06) to 12.67 ( $\pm$  5.57). Observed and expected mean heterozygosities per sample ranged from 0.53 ( $\pm$  0.04) and 0.66 ( $\pm$  0.13) to 0.76 ( $\pm$  0.04) and 0.74 ( $\pm$  0.10), respectively. Among 372 tests (62 samples  $\times$  6 loci) for HWE, 12 (3.2%) showed significant deviations from expected genotype frequencies after sequential Bonferroni (Sokal & Rolf 1995) correction ( $\alpha = 0.05$ ,  $k = 62$ ); all represented heterozygote deficiencies at the loci Aro054 and Aro095. The risk of encountering heterozygote deficiencies as a result of large-allele dropouts has been noted to increase when multiplexing primers, especially for highly variable loci (O’Connell & Wright 1997). To test this possibility, we reamplified the two deviating loci separately for two locations, but found identical genotypes, ruling out this reason for deviation.

Samples of yellow and silver eels consisted of multiple cohorts, and allele frequency differences among these cohorts may have generated a slight heterozygote

Table 3. Tests for spatial and temporal genetic variation in the European eel using  $F$ -statistics from hierarchical analyses (AMOVA) based on either six or four loci ( $p$ -values within parentheses). (The '12 locations' analyses include all locations from which temporal samples were obtained, whereas samples with fewer than 48 individuals were excluded in the 'seven locations' analyses to check for potential sampling errors associated with small sample sizes (see §3b).)

dataset	no. samples	no. individuals	no. loci	F-statistic	
				among locations	among temporal samples
12 locations	33	1479	6	0.0007 (0.11)	0.0012 (0.05)
			4	0.0007 (0.21)	0.0015 (0.11)
7 locations	19	1055	6	0.0006 (0.16)	0.0017 (0.03)
			4	0.0001 (0.44)	0.0020 (0.15)

deficiency (temporal Wahlund effect; cf. Waples 1990). However, only 33% (4 out of 12) of the deviations were found in silver or yellow eel samples. Another potential reason for deviations from HWE is the presence of null alleles. After testing all 62 samples with MICRO-CHECKER, loci Aro054 and Aro095 showed evidence for potential null alleles (assuming HWE within samples). Therefore, we did all analyses (except the neighbour-joining phenogram) with and without these loci to rule out the possibility that null alleles might have affected the results obtained (see §3b).

#### (b) Spatio-temporal genetic structure

There was a low but highly significant global genetic differentiation among all 62 European eel samples ( $F_{ST}=0.0014$ ;  $p<0.01$ ) and among the 41 locations (temporal samples pooled within sites:  $F_{LT}=0.0010$ ;  $p<0.01$ ; table 2). The global genetic differentiation was evident also in analyses excluding the two loci that deviated from HWE (table 2). The between-location differentiation remained after excluding samples with fewer than 48 individuals (table 2), indicating that the subtle heterogeneity observed was not owing to random sampling errors associated with small sample sizes (Waples 1998). Genetic heterogeneity between locations within individual cohorts could be studied for glass eels collected in the years 1994, 2000, 2001 and 2002. We observed no significant differentiation between locations for the 1994, 2000 and 2001 cohorts, whereas a significant differentiation between locations was observed for the 2002 cohort (table 2).

There were also indications of genetic heterogeneity among temporal samples within locations (table 2), prompting a hierarchical analysis to account for this variation when evaluating spatial genetic structure. When including only the 12 locations for which temporal samples were available, genetic differences between temporal replicates collected at individual sites explained a larger proportion of the total genetic variance than did differences between geographical locations (table 3). In fact, the between-location source of variance was not significantly different from zero. The temporal component was even stronger when excluding samples with fewer than 48 individuals, but only approached significance in analyses based on four loci (table 3).

#### (c) IBD and cluster analysis

We found no correlation between genetic distance and nearest sea distance or difference in distance to the

Sargasso Sea among pairwise comparisons between sampled locations, either for the complete dataset (all 41 locations), or for a dataset in which only glass eels were included to avoid potential biases owing to secondary movements of adults and translocation activities (table 4). Also, there was no correlation between genetic and geographical distances among pairwise comparisons of glass eel samples within individual cohorts, except for cohort 2000 (table 4). The distant Yugoslavian sample was the only contributor to the IBD pattern observed for cohort 2000, as this sample was involved in the few significant pairwise comparisons within this cohort (data not shown). No significant differences were observed in pairwise comparisons involving the other glass eel samples from cohort 2000. The absence of a consistent IBD pattern was supported by an AMOVA, in which locations (temporal samples pooled) were grouped according to three main ocean basins (North Atlantic/Baltic Sea, Atlantic Basin and Mediterranean Sea). These groups have been mentioned previously as possible genetic units responsible for the IBD pattern observed (Wirth & Bernatchez 2001, 2003; Maes & Volckaert 2002). No genetic differentiation was observed between the three groups ( $F_{GT}=-0.0001$ ;  $p=0.695$ ), whereas a weak but significant heterogeneity (most probably attributed to temporal genetic variation, see §3b) was observed among locations within groups ( $F_{LG}=0.0010$ ;  $p=0.020$ ).

Further, a neighbour-joining phenogram based on Nei's (1978) unbiased genetic distance between all samples did not show any clustering of temporal samples within locations, or locations within regions (figure 2). The European eel separated from the two American eel samples in all bootstrap replicates, but no nodes within the European eel were supported in more than 57% of the bootstrap replicates.

## 4. DISCUSSION

The results presented here clearly show that temporal replication is crucial in the study of genetic differentiation in marine organisms. On the basis of hierarchical  $F$ -statistics accounting for temporal genetic variation and tests for IBD, we conclude that European eels sampled along the coasts of Europe and Africa most probably belong to a single, spatially homogeneous population. Hence, our study re-opens the debate about panmixia in this species, and emphasizes the need for a deeper look into European eel using a standardized sampling approach and multiple markers.

Table 4. Tests for isolation-by-distance in the European eel.

(Results from correlations between genetic distance ( $F_{ST}/1 - F_{ST}$ ) and (1) nearest sea distance or (2) difference in nearest distance to the spawning area in the Sargasso sea, for pairwise comparisons between all sampled locations (temporal samples pooled), between glass eel locations (temporal samples pooled), and between glass eel samples collected in 4 years. The analyses were carried out using all six loci and a restricted dataset with four loci (see §3a).)

dataset	no. samples	analysis	six loci		four loci	
			Pearson's $r$	$p$	Pearson's $r$	$p$
complete data	41	1	-0.09	0.84	-0.02	0.64
		2	-0.09	0.88	-0.05	0.75
only glass eels	34	1	0.04	0.32	0.06	0.22
		2	0.01	0.45	0.03	0.32
cohort 1994	8	1	0.03	0.43	-0.05	0.57
		2	-0.31	0.88	-0.13	0.70
cohort 2000	5	1	0.58	0.05	0.53	0.05
		2	0.12	0.37	0.09	0.51
cohort 2001	17	1	-0.13	0.83	-0.11	0.83
		2	-0.01	0.53	0.04	0.35
cohort 2002	8	1	0.04	0.42	-0.01	0.48
		2	-0.02	0.50	-0.01	0.52

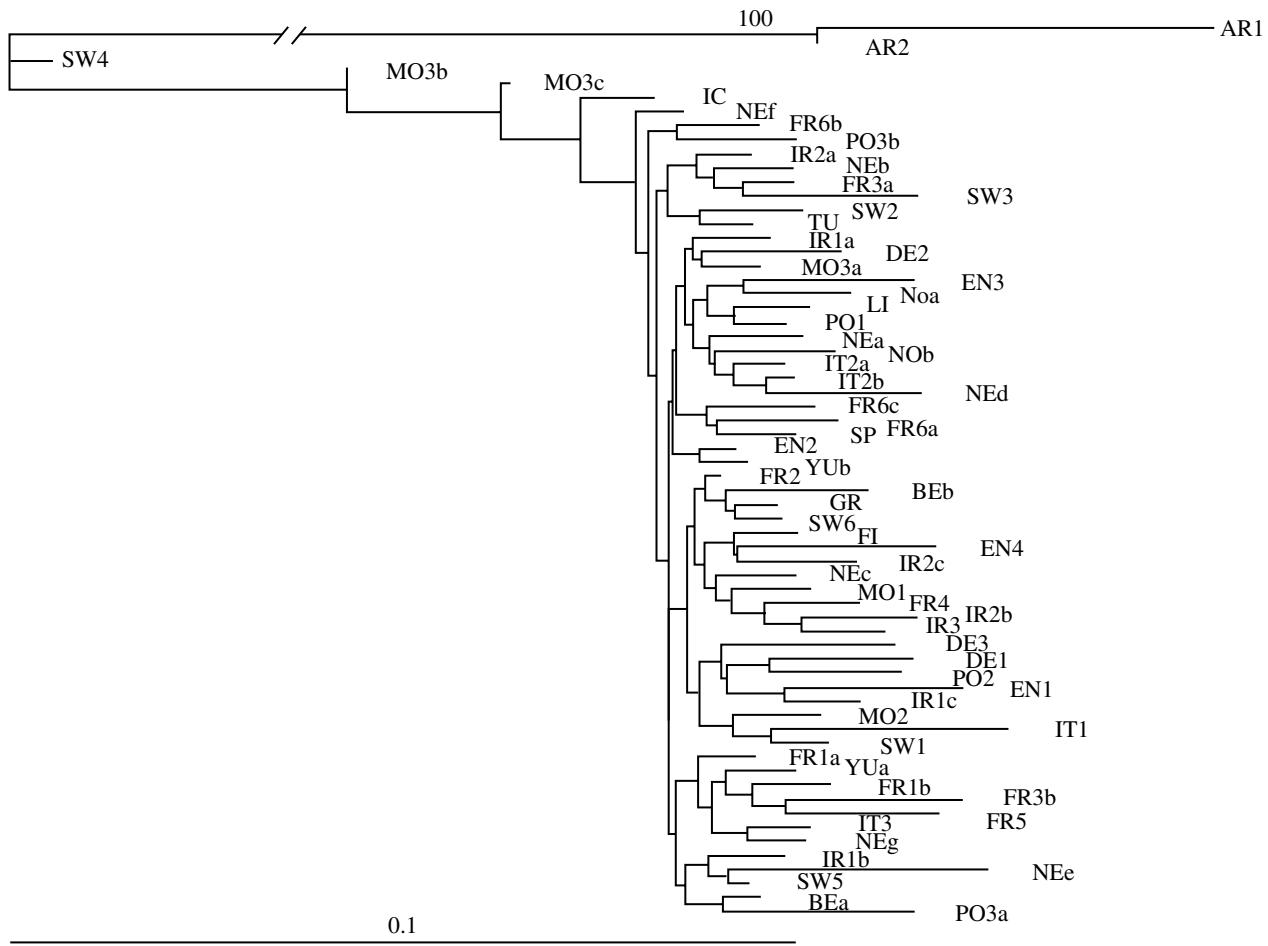


Figure 2. Neighbour-joining phenogram based on Nei's (1978) unbiased genetic distance among two samples of American eel and 62 samples of European eel. The designations of samples refer to those listed in table 1 (temporal samples within locations are identified by lower case letters). Note the broken branch separating the two species.

#### (a) Sources of genetic variation

We found a significant global genetic differentiation between all European eel samples ( $F_{ST}=0.0014$ ), which is close to the values previously reported using the same type of markers (Daemen *et al.* 2001; Wirth & Bernatchez

2001). When pooling temporal samples within locations, the overall differentiation decreased, albeit remaining significant ( $F_{LT}=0.0010$ ). This is an indication that temporal genetic variation might explain a significant amount of the total genetic variance. In addition, splitting

the samples into cohorts lowered the differentiation value and increased the *p*-value above significance for most groups analysed, emphasizing again the temporal nature of the global differentiation observed among samples.

In the hierarchical analysis of molecular variance, the proportion of the total genetic variance explained by differences between temporal samples within locations was at least twice as high as the proportion owing to spatial differences. For individual locations, the strongest indications of temporal genetic variation were found in those cases where temporal samples were relatively large and were separated in time by many years or consisted of eels of different life stages (large differences in age), which is in close agreement with previous theoretical and empirical work (Jorde & Ryman 1995; Palm *et al.* 2003b). However, because of small sample sizes, the statistical power in the within-location analyses becomes low, which might explain the many non-significant comparisons despite relatively high differentiation values.

It is important to note that our findings do not preclude the presence of a spatial genetic structure in the European eel. However, the results suggest that the global genetic differentiation that may possibly exist must be extremely weak. The significant differentiation between glass eel samples in 2002 may point to a spatial structure. However, the surface of the Sargasso Sea is huge ( $5.2 \times 10^6 \text{ km}^2$ ), and owing to its heterogeneous hydrographical structure (McGillicuddy *et al.* 2001; Knights 2003), the mating patterns and the dispersal of larvae to the coasts of Europe may not be completely random. Thus, the differences observed among glass eel samples from 2002 may well be the consequence of the eels caught at different sites in Europe being derived from different (finite) sets of parents (Allendorf & Phelps 1981; Waples 1998). This might also explain the IBD signal for cohort 2000, which was mainly a result of the fact that the distant Yugoslavian sample differed slightly from the other samples belonging to this cohort. Nevertheless, the lack of a global differentiation among glass eels collected in 1994, 2000 and 2001, in combination with the non-significant hierarchical *F*-statistic between geographical locations, and the absence of an IBD pattern in the total material and in three out of four cohorts, strongly indicates that the occasional differences observed are not consistent over time.

#### (b) Comparison with previous studies

Temporal delay in the arrival of adults from different latitudes on the East-Atlantic continental shelf to the breeding site in the Sargasso Sea, possibly in conjunction with spatial separation of spawning sites, has been proposed as an explanation for the genetic structure observed previously (Wirth & Bernatchez 2001; Maes & Volckaert 2002). If true, this temporal allopatry in combination with non-random return of larvae may generate an IBD pattern among eels caught in Europe, with some degree of genetic exchange between neighbouring populations owing to some overlap in spawning time, but restricted gene flow between distant populations. In sharp contrast to previous studies, we found no evidence for IBD. When we applied a geographical clustering into three groups as suggested in earlier studies (Wirth & Bernatchez 2001, 2003; Maes & Volckaert 2002), no significant differentiation could be found between

the groups (North Atlantic/Baltic Sea, Atlantic Basin and Mediterranean Sea). In addition, we could not detect any geographical grouping of European eel samples in a neighbour-joining phenogram. Wirth & Bernatchez (2001, 2003) presented similar analyses in which Mediterranean samples and samples from the North Sea and the Baltic Sea were proposed to form distinct clades, but these conclusions were based on very low bootstrap values. In this study, no nodes within the phenogram were supported in more than 57% of the bootstrap replicates, and no obvious structure was evident, even if bootstrap values were not taken into account.

The contrasting results between this and previous studies regarding the IBD pattern is puzzling. One reason could be differences between studies in statistical power. However, we find this explanation quite unlikely, as the number of samples is higher in the present study, and the sample sizes in many cases exceed those in previous studies. The splitting of samples into cohorts will of course decrease statistical power in subsequent analyses of IBD. Also, the use of only four loci in the restricted dataset probably reduces power to detect IBD. However, the absence of even a slight signal of IBD (see Pearson's correlations in table 4) in three out of four cohorts indicates that differences in statistical power is a less plausible explanation for the contrasting results. Also, if it exists, IBD would have been discovered in the analyses including all 41 locations and all glass eel locations.

Instead, we argue that differences in sampling procedures might explain the discrepant results of this and previous studies. We note that samples included in the study by Wirth & Bernatchez (2001) were collected in the same year, but consisted of eels of different ages. The five northernmost samples included older yellow and silver eels, whereas the eight southern samples consisted of newly recruited glass eels. In the presence of even a slight temporal genetic heterogeneity, such a sampling scheme may produce a spurious correlation between genetic and geographical distance. However, an additional analysis in which the northernmost samples were excluded also indicated the presence of IBD (T. Wirth, personal communication), although the correlation was weaker and only approached significance. Inclusion of one or a few distant samples that deviate from the others because they derived from different (finite) sets of parents (see §4a) could also result in a spurious correlation between genetic and geographical distance. Similar artefacts may explain the observations of Maes & Volckaert (2002), as their study also relied on samples collected in different years, and only one distant sample (consisting of eels that differed in age from all other sampled eels) was the main contributor to the observed IBD pattern.

One caveat applies to our conclusion of no genetic substructuring within the European eel, namely that the absence of, or a very weak, geographical differentiation at neutral loci does not preclude the existence of more pronounced differences at loci affected by selection (Cousyn *et al.* 2001; Koskinen *et al.* 2002). Since selected and non-selected genes can have different effective migration rates, adaptive differences could in theory persist in spite of significant neutral gene flow. Thus, there is always a risk that neutral markers, like microsatellites, may fail to document population differences.

**(c) Effective population size and temporal genetic variation**

Although we cannot properly estimate  $N_e$  from our temporal genetic data without basic demographic information (Jorde & Ryman 1995), the observed temporal genetic heterogeneity indicates that the effective size of the European eel stock might not be as large as previously thought. Wirth & Bernatchez (2003) used the procedure of Beaumont (1999), which estimates several genealogical and demographic parameters from microsatellite data using Bayesian statistics, and presented values of the current effective size of the European eel stock that ranged between 1050 and 6000. These values correspond to remarkably low numbers of spawning eels that succeed in reproduction each year. However, results obtained using this method should be viewed with caution as the analysis assumes knowledge about the mutation rate, which may differ considerably between loci, and that the microsatellite loci used have evolved according to a strict stepwise mutation model (Beaumont 1999).

On the other hand, these figures may not be unrealistic, as the estimated effective size of marine fishes can be several orders of magnitude less than the actual number of adults present in the population (Hauser *et al.* 2002). The genetic 'sweepstake' hypothesis (Hedgecock 1994), which has affinities with the ecological match/mismatch hypothesis (Beaugrand *et al.* 2003), was proposed to explain extremely low effective- to census-size ratios in highly fecund marine species, and states that many families do not recruit because their larvae do not end up in the right environment at the right time to survive critical life stages. Given the very distant larval migrations in an environment where currents and primary production vary seasonally and interannually (Desaunay & Guérault 1997; McGillicuddy *et al.* 2001), the European eel may qualify for such a scenario.

**(d) Implications**

Our findings have implications for the sustainable management of the European eel, whose abundance has declined steadily since the late 1970s (Dekker 2003). Many factors might be involved, such as long-term oceanic and climatic changes (Wirth & Bernatchez 2003), overfishing, pollution, diseases and the destruction and overexploitation of freshwater habitats (Knights 2003). A global management action developed for the Eastern-Atlantic shelf (including the Mediterranean) is supported by the present results, rejecting a strictly local approach. Future genetic studies on eels should focus on the collection of data necessary for a proper estimation of  $N_e$ . Also, genetic monitoring may facilitate the detection of changes in population genetic dynamics, such as an increase in the magnitude of temporal allele frequency shifts resulting from a reduction in  $N_e$ . Our results also have implications for marine fishes in general. To avoid extinction of local populations, the successful management of commercial marine fish species requires sufficiently large spawning stocks and a thorough understanding of the dynamics of their population genetic structure (Nielsen 2001; Myers and Worm 2003). The detection of temporal genetic variation in a presumably panmictic species emphasizes the need to control for this source of variation when evaluating the subtle population structure of threatened marine fishes.

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