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Panmixia in European eel revisited: no genetic difference between maturing adults from southern and northern Europe

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Previous studies of genetic structure in the European eel have resulted in seemingly conflicting results, ranging from no detectable heterogeneity to small but statistically significant differences and isolation by distance patterns among eels sampled across the continental range. Differences with respect to sampling design and choice of molecular markers, combined with a lack of power estimates, complicate comparisons of existing results. In this study we have used six microsatellite markers and, for the first time, compared maturing silver eels of known age from southern and northern Europe (Italy and Baltic Sea). In comparison with previous studies, our data may give a better representation of potential spawning stocks because eels were sampled when having begun their migration toward the presumed spawning area in the Sargasso Sea. Despite large sample sizes (404 and 806 individuals) we could not observe any signs of genetic differentiation (average $F_{ST} = -0.00003$, $P = 0.61$), and a power analysis showed that the true level of heterogeneity (if existing) must be exceedingly small to have remained undetected (say, $F_{ST} < 0.0004$). A tendency for slightly increased genetic differences between cohorts over time could be seen, but the amount of temporal change was minor and not statistically significant. Our findings reiterate the notion that previous reports of continental genetic differentiation in the European eel may be largely explained by uncontrolled temporal variation between juvenile glass eel samples.
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Introduction

To study population structure in marine organisms by means of molecular markers can be a challenging task. Samples for genetic analysis should preferably be taken during reproduction, but the vastness of the sea may complicate identification of spawning areas and collection of reproductive individuals. In such cases, samples of larvae, juveniles or nonmature adults collected from nursery or feeding areas may have to be used as a proxy. In addition, gene flow in combination with large effective population sizes can depress the extent of genetic differentiation between subpopulations that nevertheless may function largely independently from a demographic perspective (Waples and Gaggiotti, 2006; Palsböll *et al*., 2007). On the other hand, high fecundity, together with pronounced mortality, may also result in spurious observations of genetic differentiation that reflect random events associated with reproduction and survival rather than presence of a true genetic population structure (Allendorf and Phelps, 1981; Hedgecock, 1994).

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The European eel *Anguilla anguilla* (L.), with its complex and largely unknown catadromous life history, fulfills several of the above characteristics. Reproducing individuals have never been observed in the Sargasso Sea, where spawning is presumed to take place as indicated by findings of the smallest larval stages (Schmidt, 1923). Following hatching, the planktonic larvae (leptocephali) are transported with ocean currents across the North Atlantic Ocean for an extended but poorly known time period (McCleave, 2008) to continental feeding areas in fresh and coastal waters ranging from northern Africa to the White Sea. After metamorphosing at arrival, first into 'glass eels' and soon after to 'yellow eels', they feed and grow for a highly variable time period (about 2–20 years on average, depending on sex and latitudinal position; Vøllestad, 1992) until turning into sexually maturing 'silver eels' that undertake a long spawning migration back to the Sargasso Sea, where they are presumed to die after reproduction.

During the past few decades, recruitment in the European eel has decreased dramatically to fractions of pre-1970s levels. A number of reasons responsible for the decline have been proposed, including both oceanic (for example, climate change and altered ocean currents) and continental processes (for example, pollution, over fishing, and hydroelectric exploitation), although the relative importance of these factors is still not fully understood (Dekker, 2003; Friedland *et al*., 2007).

A long-standing question of importance for conservation and management is whether the European eel consists of a single panmictic population. Spurred by the lack of direct ecological data on oceanic migration and reproductive behavior, a number of molecular studies have been conducted over the years. Using different types of genetic markers, samples from various parts of the continental range have been compared as an indirect way of testing for population subdivision. To date, however, no real consensus regarding genetic structure has been reached (see review by Maes and Volckaert, 2007).

Using seven microsatellites, Wirth and Bernatchez (2001) were the first to report a small but statistically significant overall spatial difference across the continental range ($F_{ST} = 0.0017$; $P = 0.0014$) including evidence for isolation by distance (IBD). Similar indications of IBD and genetic differences at allozyme loci between eels from northern and southern Europe were soon after reported by Maes and Volckaert (2002). In brief, maintenance of a continental genetic structure would require temporal or spatial reproductive separation in the Sargasso Sea of mature adults returning from different feeding areas, followed by nonrandom return of larvae to the same continental areas. The possibility for such nonrandom transportation of larvae across the North Atlantic Ocean has gained indirect support from modeling work based on oceanographic data (Kettle and Haines, 2006). In contrast, Dannewitz *et al*. (2005), in the hitherto most comprehensive study with respect to geographic covering and sample sizes, found no evidence for population structuring or IBD when accounting for significant temporal genetic differences at six microsatellite loci between eels sampled from the same localities in different years.

The occurrence of significant temporal genetic variation has been highlighted further in subsequent detailed studies of glass eels, where genetic (and phenotypic) heterogeneity has been detected between arrival waves, that is, pulses of glass eel recruits arriving at a continental sampling site throughout a season, exceeding the genetic differences observed among different sites (Pujolar *et al*., 2006, 2007). Although the genetic differences between arrival waves within single years do not seem to show any temporal trend, subtle increasing differentiation over time has been found between glass eel samples from consecutive years (Maes *et al*., 2006).

Two main mechanisms have been discussed to explain previous observations of continental spatial and temporal heterogeneities, in addition to the presence of a genetic structure. Dannewitz *et al*. (2005) suggested that the observed temporal variation in their study could represent genetic differences between cohorts, as expected in a single population of finite effective size (compare Palm *et al*., 2003). Secondly, temporal and spatial heterogeneities between glass eel arrival waves within a year has been proposed to reflect differences between offspring groups produced by separate (finite) sets of parents spawning at a certain place or time (Dannewitz *et al*., 2005; Pujolar *et al*., 2006, 2007). The latter explanation further requires, though, that the larvae have traveled across the ocean largely without admixing with other such groups.

Altogether, the dominance of glass eel samples in previous studies, combined with lack of statistical power estimates and differences with respect to sampling design and choice of markers, makes it difficult to evaluate the relative importance of the above results and suggested mechanisms. In this paper we have revisited the issue of a geographic (continental) population structure in the European eel through a microsatellite analysis focused entirely on adult silver eels of known age from northern and southern Europe. The chosen sampling strategy is anticipated to result in a better representation of potential spawning stocks than in previous studies, by reducing confounding 'noise' stemming from genetic differences between glass eel arrival waves. At the same time, access to age data has made it possible to control for variation between cohorts when testing for spatial differentiation and to search for indications of temporal genetic change.

Materials and methods

Samples

The total material consists of 1210 adult eels sampled in October–December 2003 from the brackish Lago di Lesina lagoon on the Italian Adriatic Coast (Mediterranean Sea), and two localities (Kullen and Køge) from the Swedish and Danish side of the Öresund strait, which connects the Baltic Sea with the North Sea (Table 1). Sex and stage of maturation was determined using standard morphological criteria (for example, Tesch, 2003), with

Table 1 Number of analyzed silver eels divided on sampling locality and cohort, as determined from otolith readings

Cohort	*Baltic sea*			*Italy (Lago di Lesina)*	*Grand Total*
	Kullen	*Køge*	*Both localities*		
1975	1	0	1	0	1
1976	0	0	0	0	0
1977	1	0	1	0	1
1978	0	0	0	0	0
1979	0	0	0	0	0
1980	0	0	0	0	0
1981	1	0	1	0	1
1982	1	0	1	0	1
1983	0	1	1	0	1
1984	2	2	4	0	4
1985	10	1	11	0	11
1986	10	1	11	0	11
1987	20	2	22	0	22
1988	32	3	35	0	35
1989	59	11	70	0	70
1990	72	37	109	0	109
1991	81	42	123	1	124
1992	80	50	130	0	130
1993	56	59	115	0	115
1994	27	31	58	1	59
1995	9	19	28	1	29
1996	9	9	18	1	19
1997	2	1	3	3	6
1998	0	5	5	19	24
1999	0	2	2	29	31
2000	0	0	0	213	213
2001	0	0	0	122	122
2002	0	0	0	1	1
No age data	34	23	57	13	70
Grand Total	507	299	806	404	1210

Cohort refers to the year of arrival of an individual as a glass eel, not the actual year of birth (a few years earlier).

proportions of maturing silver eels assessed to between 97 and 100% in the three samples. Otoliths (sagittae) were collected and analyzed with respect to age and subsequent cohort assignment (see Svedäng *et al*., 1998, for technical details). It should be noted that the age determined refers to the time that has passed from the glass eel stage, not including the larval phase. In a few cases, no reliable age could be determined (Table 1); when possible, those individuals of unknown age have still been included in analyses presented below.

Although the silver eels from Kullen and Køge displayed some differences with respect to life history and morphological traits, the combined sample from these localities is presumed to be fairly representative for out-migrating eels from inland and coastal feeding habitats in the Baltic Sea area (Clevestam and Wickström, 2008). As we have found no genetic difference between the eels from Kullen and Køge ($F_{ST} = 0.0004$; $P = 0.29$), those are here onwards always analyzed in combination (sample named 'Baltic Sea').

Microsatellite genotyping

DNA was extracted from ethanol-preserved fin tissue using the Chelex protocol described by Walsh *et al*. (1991). We have scored the following six dinucleotide microsatellite markers, which have been used in previous studies of the European eel: *Aan01*, *Aan03*, *Aan05* (Daemen *et al*., 1997, 2001), *Ang151*, *Aro054* and *Aro095* (Wirth and Bernatchez, 2001).

All loci were coamplified in the same 25 µl PCR reaction (multiplex PCR) using GE illustra puReTaq Ready-To-Go PCR Beads (GE Healthcare UK Limited, Buckinghamshire, UK) and approximately 100 ng of template DNA. Primers were end-labeled with fluorescent dyes to enable comigration of all loci in the same capillary during electrophoresis, that is, loci labeled with the same dye had nonoverlapping size ranges. Uniform signal intensity among loci was achieved by adjusting primer concentrations; from 10 µM primer solutions (forward and reverse) we took 90 µl *Aan01*(yellow), 45 µl *Aan03*(red), 40 µl *Aan05*(blue), 30 µl *Ang151*(green), 190 µl *Aro054*(yellow), 30 µl *Aro095*(blue), and added water up to 2500 µl total volume. For each reaction, we used 23 µl of the primer mix with 2 µl added template DNA.

The PCR amplification was initiated with a denaturation step at 94 °C for 5 min followed by 26 cycles of 30 s at 94 °C, 30 s at an annealing temperature of 55 °C and 1 min at 72 °C. The process was terminated with a 10 min elongation step at 72 °C. Electrophoresis and size determination of alleles was made with the Liz 600 sizer on an ABI Prism 310 Genetic Analyzer (Applied Biosystems, Foster City, CA, USA) according to the manufacturer's recommendations (http://www.appliedbiosystems.com).

Statistical analyses

The program HP-RARE (Kalinowski, 2005) was used to compute estimates of unbiased heterozygosity and allelic richness. We used HIERFSTAT (Goudet, 2005) for estimating F-statistics and testing for genetic differentiation, and FSTAT (Goudet, 1995) when evaluating deviations from Hardy–Weinberg proportions and genotypic equilibrium between loci. All statistical tests were based on permutations using 10 000 randomizations. For comparison, we also performed exact tests for conformance to H-W proportions and genetic differentiation as implemented in GENEPOP (Rousset, 2008). GENEPOP was also used when analyzing correlations between linearized genetic and temporal distance between cohorts (Mantel test and bootstrapped 95% confidence interval (CI) for *b*, the estimated linear slope).

The reliability of the genotypic data was checked with MICROCHECKER (van Oosterhout *et al*., 2004) that applies permutations to identify potential problems with stuttering, large allele dropouts and null alleles. Some indications of null alleles were found (see below), and we, therefore employed the program FREENA (Chapuis and Estoup, 2007) to estimate frequencies of those putative null alleles using the EM algorithm (Dempster *et al*., 1977), and further to calculate unbiased F_{ST} estimates adjusted for presence of nulls by the so-called ENA method (Chapuis and Estoup, 2007).

Statistical power to detect genetic heterogeneity at various true levels of divergence was evaluated for the present sample sizes, number of loci and allele frequencies using the program POWSIM (Ryman and Palm, 2006). As described in further detail by Ryman *et al*. (2006), POWSIM simulates sampling of genes from a specified number of populations having diverged due to random drift to a predefined expected level of divergence (quantified as F_{ST}). Samples from the simulated populations are used for testing for genetic homogeneity at each locus separately using Fisher's exact test (and traditional χ^2-test), and in combination by Fisher's method. The proportion of significances (for example, $P < 0.05$) obtained after having repeated the above simulation procedure a large number of times (here always 1000) yields an estimate of power (or α-error, when $F_{ST} = 0$).

Results

Genetic variation

The samples from Italy and the Baltic Sea were very similar with respect to allelic richness, expected heterozygosity and average F_{IS} (Table 2). Two loci (*Aan03* and

Table 2 Genetic variation in the European eel: total number of alleles observed (N_A), allelic and private allelic richness (A_R and P_R), and expected heterozygosity (H_{exp})

Locus		*Baltic Sea* (n = *806*)				*Italy* (n = *404*)			
	N_A	A_R	P_R	H_{exp}	F_{IS}	A_R	P_R	H_{exp}	F_{IS}
Aan01	17	15.3	0.51	0.75	0.022	16.0	1.17	0.74	−0.014
Aan03	8	7.2	1.69	0.22	0.053*	6.0	0.52	0.20	0.056
Aan05	14	12.2	1.24	0.71	−0.016	12.0	1.03	0.72	0.008
Ang151	30	24.6	4.81	0.88	−0.003	23.0	3.15	0.88	−0.009
Aro054	21	19.4	1.74	0.87	0.004	18.0	0.30	0.88	0.029
Aro095	26	21.2	2.50	0.89	0.050***	21.0	2.25	0.89	0.039*
Average (6 loci)	19.3	16.7	2.1	0.72	0.014*	16.0	1.4	0.72	0.014
Average (4 loci)	22.4	19.2	2.6	0.82	0.002	18.4	1.8	0.82	0.004

*$P < 0.05$; ***$P < 0.001$.
F_{IS} quantifies deviations from Hardy–Weinberg proportions. Levels of significance (H_0: $F_{IS} = 0$) were assessed through permutation tests (10 000 randomizations). The averages over four loci were calculated without *Aan03* and *Aro095*.

Aro095) displayed statistically significant heterozygote deficiencies (that is, positive F_{IS} estimates), and the diagnostic routines in MICROCHECKER indicated potential occurrence of null alleles at *Aro095*. Accordingly, exclusion of these two loci resulted in decreased average F_{IS} estimates approaching zero within both samples (Table 2). When testing for pairwise deviations from genotypic equilibrium, only one fairly weak significance could be seen in the total material (*Aan01*/*Aro095*; $P = 0.015$) that did not remain after Bonferroni correction for multiple tests ($k = 15$).

Spatial and temporal genetic homogeneity

The results of a hierarchical analysis, quantifying genetic heterogeneity among the Italian and Baltic Sea samples while accounting for temporal variation (between cohorts), are presented in Table 3. We found no indications of spatial genetic differentiation ($F_{\text{Locality/Total}} = -0.00003$; $P = 0.61$), and removal of the two loci with suspected null alleles did not change the result more than marginally. Likewise, no temporal variation between cohorts could be detected ($F_{\text{Cohort/Locality}} = -0.00051$; $P = 0.74$).

As power to detect genetic heterogeneity at higher levels in a hierarchical analysis may be limited, we also performed a nonhierarchical comparison of our geographic samples (thereby taking the risk of obtaining spurious significances due to uncontrolled temporal variation; compare Dannewitz *et al*., 2005). Not even in that case could any evidence for spatial genetic differentiation be found ($F_{ST} = -0.00020$; $P = 0.53$).

Null alleles

Applying the EM-algorithm (implemented in FREENA) resulted in point estimates of putative null allele frequencies at the two suspected loci (*Aan03* and *Aro095*) in the two analyzed samples around 0.02 (range 0.018–0.022), whereas the corresponding average frequency at the other four loci was 0.005 (range 0.000–0.015). In line with these minor null allele frequencies and the apparent lack of differentiation, the unbiased F_{ST} values obtained using the ENA method became virtually identical to the uncorrected ones; the average ENA estimate over all six loci was negative ($F_{ST\,(ENA)} = -0.00017$; 95% CI: −0.00037 to 0.00006), only differing on the fifth decimal from the nonhierarchical F_{ST} average presented above.

Table 3 Hierarchical analysis of spatial (continental) and temporal genetic variation in European eel

Locus	$F_{Locality/Total}$	$F_{Cohort/Locality}$	$F_{Individual/Cohort}$	$F_{Iindividual/Total}$
Aan01	0.00024	−0.00052	0.0296	0.0293
Aan03	−0.00049	0.00093	0.0801	0.0805
Aan05	−0.00003	−0.00257	−0.0069	−0.0095
Ang151	−0.00052	−0.00006	0.0007	0.0001
Aro054	0.00045	0.00030	0.0237	0.0244
Aro095	−0.00012	−0.00043	0.0548***	0.0543***
Average (6 loci)	−0.00003	−0.00051	0.0243**	0.0237**
Average (4 loci)	0.00003	−0.00063	0.0119	0.0113

**$P < 0.01$; ***$P < 0.001$.

$F_{\text{Locality/Total}}$ and $F_{\text{Cohort/Locality}}$ quantify allele frequency differences between sampling localities (Baltic Sea and Italy) and temporal variation within localities (between cohorts), whereas $F_{\text{Individual/Cohort}}$ and $F_{\text{Individual/Total}}$ represent measures of deviations from Hardy–Weinberg proportions within cohorts and in the total material, respectively. Levels of significance were assessed using permutations (10 000 randomizations). The averages over four loci were calculated without *Aan03* and *Aro095* (loci with suspected null alleles; see text).

Selective neutrality

None of the loci or alleles assayed showed signs of potential directional selection (compare Larsson *et al*., 2007). When comparing our two continental samples in a nonhierarchical manner, each single locus F_{ST} estimate was very close to zero (range −0.00046 to 0.00022) and all but one of the observed 116 alleles had an associated F_{ST} estimate smaller than 0.004 (only exception was a rare allele, **195* at *Ang151*, with $F_{ST} = 0.008$). The striking homogeneity across loci and alleles suggested that a formal testing procedure for selective neutrality (for example, Beaumont and Nichols, 1996) was not warranted.

Statistical power

Estimates of the power to detect various levels of true genetic heterogeneity, based on the present number of loci, average allele frequencies and sample sizes, are presented in Figure 1. We have assessed power both with respect to comparisons of our two spatial samples and for temporal variation among the consecutive cohorts 1985–2001 (compare Table 1). Clearly, the true degree of differentiation between eels from Italy and the Baltic Sea must be very small (say, $F_{ST} < 0.0004$) to have a high likelihood of having remained 'undetected' using the present six microsatellites and sample sizes. In comparison, power when testing for temporal homogeneity is somewhat lower, but the true genetic difference between the studied cohorts must nevertheless be minor (Figure 1).

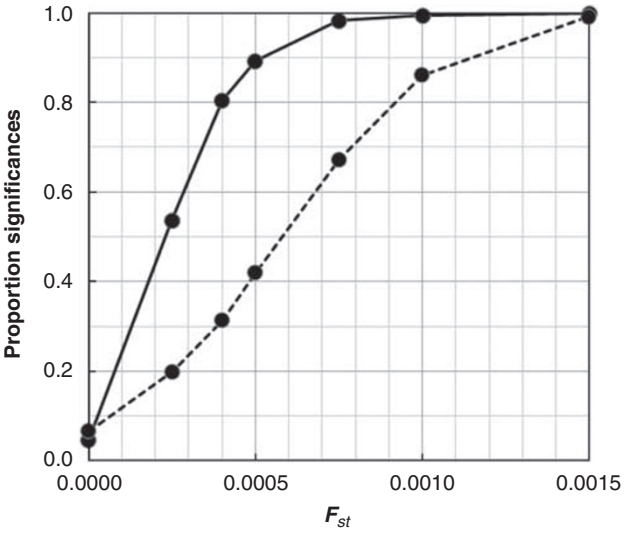

Figure 1 Simulated estimates (average of 1000 runs) of power and α-error for the present set of microsatellite loci and alleles at different true levels of divergence (F_{ST}). The solid and stippled lines indicate the probability of obtaining a significance when comparing 2 geographic samples (Italy and Baltic Sea) and 17 consecutive cohorts (1985–2001; data for Italy and Baltic Sea combined), respectively. Corresponding sample sizes are given in Table 1. See text for details.

Table 4 Analyses of correlations between linearized genetic [$F_{ST}/(1-F_{ST})$] and temporal distance (no. of years between cohorts): estimated linear slope (*b*, with 95% bootstrapped confidence interval) and result from Mantel test (one-sided *P* value)

Min. n	*No. of cohorts*	*No. of pairs*	b	*(95% CI)*	P *(Mantel)*
11	16	120	−0.00002	(−0.00027; 0.00018)	0.81
28	11	55	0.00011	(−0.00008; 0.00023)	0.73
58	8	28	0.00006	(−0.00007; 0.00015)	0.74
109	6	15	0.00013	(0.00000; 0.00040)	0.24

Results on different lines have been obtained using various subsets of the total material (compare Table 1), as indicated by the minimum sample size per cohort allowed (Min. *n*) with corresponding number of used cohort samples (no. of cohorts) and pairwise comparisons (no. of pairs).

Temporal genetic change

Although we did not observe any overall statistically significant heterogeneity between cohorts, all real (finite) populations are expected to change genetically over time. Hence, we looked for indications of a temporal trend (compare Maes *et al*., 2006) through testing for a correlation between linearized genetic ($F_{ST}/(1-F_{ST})$) and temporal distance, measured as the number of years lapsing between cohorts. Thus we treated the cohorts from Italy and the Baltic Sea as if belonging to the same population, which appeared justified considering the lack of any detectable spatial genetic divergence.

No trend of increasing genetic differences over time could be seen when including all cohorts with a sample size larger than $n = 10$ (Table 4). As random error variation due to small sample sizes (and few loci) may mask a true positive relationship, we tested to successively remove the smallest cohorts while repeating the linear regression analysis. As shown by the estimated slopes (*b*) in Table 4, a tendency toward an increasing (positive) correlation between genetic and temporal distance could be seen, although the estimated amount of genetic change remained minor and not statistically significant.

Discussion

This study suggests that no genetic differences exist between maturing adult silver eels sampled from contrasting ends of the continental range in Europe. Further, and in contrast to previous studies based mainly or completely on glass eel samples, we have not found a statistically significant temporal component of genetic variation.

Although few, if any, species are truly panmictic in the sense that all mature individuals mate randomly exactly at the same place and time, our results imply that the European eel could still be viewed on as panmictic from a genetic perspective, which is in line with previous findings of Dannewitz *et al*. (2005) and Pujolar *et al*. (2007). In comparison, studies of genetic structure in other *Anguilla* species with a similar life history have given mixed results. Like our present results, no deviations from apparent panmixia have been found in *A. rostrata* (Wirth and Bernatchez, 2003), *A. reinhardtii* (Shen and Tzeng, 2007a) or *A. japonica* (Ishikawa *et al*., 2001; N Yoshizawa *et al*., in preparation; but see Tseng *et al*., 2006), whereas genetically distinct subpopulations have been identified in *A. australis* (Shen and Tzeng, 2007b), *A. marmorata* (Minegishi *et al*., 2008) and *A. bicolor* (Y Minegishi *et al*., in preparation).

Absence of statistical significance can never be equated with proof against presence of a genetic structure. Our power analysis has shown, however, that even though we only studied six microsatellite loci the true level of continental genetic divergence must be exceedingly small to have remained undetected (for example, we have 80% power at $F_{ST} = 0.0004$; Figure 1). Screening of additional gene markers and (or) larger sample sizes will be needed to yield increased power. As an illustration, when doubling and tripling our set of six microsatellites using the present sample sizes, power at a simulated F_{ST} of 0.0001 increased from 19 to 27 and 33%, respectively.

Genetic effects of translocations

Power issues aside, it is possible that the present level of genetic differentiation between eels from northern and southern Europe is lower than that in historical times as an effect of large-scale translocations of mainly glass eels from western Europe (Bay of Biscay and southern England) to countries around the Baltic Sea. Such translocations were in some cases initiated already in the mid 1900s to promote fisheries (FAO/ICES, 2009), and are still used extensively as a management tool (European Commission, 2007).

To our knowledge, however, few if any large-scale translocations between the Mediterranean and northern European localities have occurred, suggesting that if a continental genetic structure (for example, IBD; Wirth and Bernatchez, 2001) existed previously that pattern should not have become completely eradicated. Furthermore, a complete 'genetic swamping' (compare Ryman *et al*., 1995) would require that (1) a significant proportion of the translocated eels have survived in their new environments and (2) later on have migrated and reproduced at another place or time in the ocean than if not translocated. Nevertheless, analyses of historical DNA (for example, extracted from old otoliths) would be a way to provide further insights into the issue of a historical continental genetic structure that may have decreased over time.

Likewise, we cannot rule out that a significant portion of our Baltic Sea sample consists of individuals that were originally translocated from localities in western Europe. If so, we may have underestimated the overall level of genetic divergence somewhat (given that a population structure exists), further suggesting somewhat larger differentiation in years when comparably fewer eels were stocked and vice versa. Hence, we have made an attempt to check whether pairwise estimates of F_{ST} between our Baltic cohorts (1985–1996) and the total Italian material (F_{ST} in range: −0.0049 to 0.0022) are negatively correlated with data on the amount of eels

translocated over the same time period. By combining available information (FAO/ICES, 2009) on yearly numbers of glass eels stocked in countries surrounding the Baltic Sea (in millions) with corresponding annual estimates of natural recruitment to the same area (scaled to the average recruitment during the 1970s), we computed a 'stocking index' that is expected to reflect the relative proportion of translocated eels per year (cohort). When comparing this yearly index to our corresponding estimates of genetic divergence (F_{ST}), no significant negative correlation could be seen (Spearman's $r = -0.14$; $n = 12$; one sided $P = 0.33$).

Temporal variation and effective size

All real populations are expected to display temporal genetic change and the lack of statistically significant differences between cohorts in this study may, therefore, be expected to reflect low power (compare Figure 1). When concentrating only on cohorts with larger sample sizes we also observed a weak (albeit not significant) tendency for increased differences over time, which is in accordance with Maes *et al*. (2006) who observed such a pattern between glass eel samples separated by a few consecutive years.

The amount of random genetic change over time at neutral loci (genetic drift) is expected to be inversely proportional to the current effective population size (N_e), which is the rationale for estimating this parameter from molecular data using the so-called temporal method. The subtle and nonsignificant temporal differences between cohorts observed herein could thus indicate that N_e has not yet become alarmingly small from a pure conservation genetic perspective, although we acknowledge that the studied time period must be short in terms of (unknown) generation intervals. It should also be noted that our observations refer mainly to the 1990s, and the present amount of genetic change between cohorts could be larger due to a decreased spawning stock size.

Furthermore, in species with overlapping generations the short-term temporal genetic dynamics is largely dependent on the age structure in the spawning stock, which should be accounted for when estimating current N_e from temporal data (for example, Waples, 1990). As that basic demographic information is lacking for the European eel, it is not evident how to translate the present observations of (minor) temporal allele frequency shifts into reliable N_e estimates, and it may be necessary to use other analytical approaches in addition to the temporal method. For example, data on linkage disequilibrium could be one way to estimate the effective number of parents behind the sampled cohorts (Waples, 2006), although preliminary results (not shown) indicate that screening of additional genetic markers and (or) larger sample sizes will be required to obtain precise such estimates.

Future studies of genetic structure

Although nothing in the present dataset seems to indicate presence of more than a single genetically homogenous (population in the European eel, we still can see some further possibilities for a genetic population structure that may have been missed.

A subdivided species with large local effective population sizes may be expected to display very little divergence at neutral loci even when the amount of gene flow is fairly restricted. In such cases, larger genetic differences between subpopulations could exist at loci subject to strong divergent selection, which in turn indicates presence of local adaptation (Conover *et al*., 2006). Future analyses using putative nonneutral markers such as EST:s and MHC-linked microsatellites (Vasemägi *et al*., 2005), or large scans of AFLP:s and SNP:s (Campbell and Bernatchez, 2004; Morin *et al*., 2004) could be a way to unravel such a potential situation in the European eel. We may foresee problems, however, when interpreting apparently nonneutral genetic differences between continental samples; it may be hard to show unambiguously that the observed differences existed already at birth, and are not a result of selective mortality in contrasting environments during the larval oceanic transportation or at later life stages. Hence, genetic analyses of larvae collected in the Sargasso Sea area may be necessary to perform as a complement (for example, ICES, 2007).

Future analyses of larvae (or adults) sampled in the oceanic environment could also be a way of identifying another type of potential population structure. Theoretically, spatial or temporal reproductive isolation could exist within the Sargasso Sea, although larvae from such spawning populations may spread more or less randomly across continental nursery and feeding areas where they occur mixed until reproduction. If the genetic differentiation associated with such an oceanic population structure is weak, it may be difficult to detect genetic signs of population admixture in continental samples, especially when analyzing a restricted number of molecular markers and individuals.

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