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Genetic patchiness among recruits in the European eel *Anguilla anguilla*

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ABSTRACT: Heterogeneity in genetic composition among recruits of marine species is mostly due to a large variance in reproductive success mediated by oceanographic processes. Temporal genetic variation in a population of the European eel was quantified over 2 time scales among glass eel (1) inter-annual samples (cohorts), and (2) intra-annual samples within cohorts ('arrival waves'). A total of 789 glass eels comprising 11 different arrival waves were collected at Den Oever in The Netherlands over the period 2001 to 2003. All samples were screened for genetic variation using 10 allozyme and 6 microsatellite loci. The main result from this study is the highly significant genetic differentiation among arrival waves, despite the low F_{ST} values (F_{ST} = 0.0036). Heterogeneity in genetic composition was observed both among cohorts and among samples within cohorts. Genetic differentiation partitioned within cohorts was more than 10-fold the differences among cohorts. Genetic heterogeneity is likely to result from a large variance in the contribution of individuals to each cohort determined by genetic drift. Although natural selection and gene flow could also play a role in the observed genetic pattern, we suggest that large variances in reproductive success are a contributing factor to the recruit differentiation. If only a subset of the adults contribute to the new recruits, effective population size in European eel might be much lower than the census size. A low effective population size combined with fluctuating oceanic conditions might have contributed to the current dramatic decline in abundance of European eel.

KEY WORDS: Allozymes · Arrival waves · European eel · Genetic patchiness · Microsatellites

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INTRODUCTION

Organisms with high fecundities and high mortalities of early life stages have the potential to exhibit temporal genetic variation. Adult populations may consist of a mixed structure built on an annual or seasonal basis due to heterogeneous recruitment (Hendry & Day 2005). When a given pattern of spatial variation is inconsistent over time, the inference of the genetic structure of the population may be inaccurate (Waples 1998). Many studies examining spatial differentiation assume temporal homogeneity and include sampling on just 1 occasion. Most genetic temporal studies of marine fish are based on the comparison of gene frequencies from 2 to 3 temporal samples, and few studies have involved systematic surveys of several cohorts (Lenfant & Planes 2002). Hence, the inclusion of temporal sampling of recruits may be an effective solution to elucidate population structure.

The term 'chaotic genetic patchiness' was introduced by Johnson & Black (1982) to describe unpatterned genetic heterogeneity among local populations on a small spatial scale. There is a consensus in most studies showing genetic patchiness on a microgeographical scale that such genetic heterogeneity is likely to result from temporal variation in the genetic composition of the recruits (Johnson & Black 1982, Hedgecock 1994, David et al. 1997, Li & Hedgecock 1998, Planes & Lenfant 2002). The unpredictability associated with reproduction in the marine environment results in a large variance of the contribution of individuals to each cohort. Under the hypothesis of 'sweepstakes reproductive success' (Hedgecock 1994), chance events determine which adults are successful in each spawning event. Many individuals fail to contribute to recruit-

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ment, which results in (1) a reduction in the effective population size, and (2) changes in allele frequencies when differences in genetic composition are present among spawning groups. Hedgecock (1994) attributed the variation in reproductive success of adults to spatio-temporal variation in oceanographic conditions, occurring within and among seasons. In each generation, a small fraction of individuals replaces the entire population by a sweepstakes-chance matching of reproductive activity with oceanic conditions linked to spawning, fertilization, larval development and recruitment. The random variation in parental contribution to the next generation leads to the variation in genetic composition of recruits observed in genetic patchiness.

There is growing evidence of temporal genetic heterogeneity among recruits of marine organisms, attributable to a large variance in reproductive success. Several studies on sea urchin populations revealed significant differences among recruit samples (Edmands et al. 1996, Moberg & Burton 2000). Li & Hedgecock (1998) reported temporal heterogeneity among larval samples in a Pacific oyster *Crassostrea gigas* population, which appeared to reflect inter-family variance in reproductive success. In fish, Planes & Lenfant (2002) observed significant differentiation among and within cohorts in a white sea bream *Diplodus sargus* population, with genetic relatedness data suggesting that temporal changes were due to large variance in parental success.

Alternatively, temporal variation among recruits may be the consequence of selection on larval populations and a differential survival of genotypes after recruitment (Johnson & Black 1982). In such a situation, genetic polymorphism often shows clines correlated with varying environmental factors such as temperature or salinity (Koehn et al. 1980, Larson & Julian 1999). Large-scale migration could be another factor leading to temporal variance in allele frequencies. Gene flow of larvae from different spawning populations with variable allelic composition might generate temporal genetic heterogeneity (Kordos & Burton 1983, Ruzzante et al. 1996, Larson & Julian 1999).

The dependence of recruitment on oceanic conditions holds particularly true for the European eel (*Anguilla anguilla* L.; Anguillidae; Teleostei), a catadromous fish species with a particularly complex life cycle, which moves between marine and continental environments. After spawning in the Sargasso Sea, larvae (leptocephali) arrive on the European and North African shores following the Gulf Stream and North Atlantic Drift Current. Upon reaching the continental shelf, larvae metamorphose into glass eels and some move into freshwater systems. The arrival of glass eels occurs throughout the year in pulses or groups known as 'arrival waves' (Boëtius & Boëtius 1989, Tesch 2003). After a period of intensive feeding of (on average) 7 to 8 yr for males and 11 yr for females, they metamorphose into silver eels and migrate back to the Sargasso Sea, where they spawn once and die (Tesch 2003).

The long spawning migration, the mating and spawning process, the development of eggs and larvae, and the long migration of eel larvae are all under the influence of oceanic factors and climate variation. Large-scale environmental fluctuations of particular interest are the North Atlantic Oscillation (NAO) and the El Niño-Southern Oscillation (ENSO), which have been shown to affect both marine and terrestrial communities (Stenseth et al. 2002). In the case of eels, significant negative correlations have been determined between glass eel recruitment abundance and the NAO winter index (NAOI) for European and American eels, and the ENSO index for Japanese eel *Anguilla japonica* (Knights 2003).

Recruitment abundance of European eel has declined dramatically in recent decades, jeopardizing the future of the species (Dekker 2003). A better understanding of crucial aspects of its biology, including partitioning of genetic stocks, may promote effective measures to protect the species. Early genetic studies using allozyme and mitochondrial DNA markers (reviewed in Dannewitz et al. 2005) reported no evidence of spatial substructuring, which supports the panmixia hypothesis, namely that all European eel comprise a single, randomly mating population. The existence of a single panmictic population was challenged by evidence for a weak but significant population structure (Daemen et al. 2001, Wirth & Bernatchez 2001, Maes & Volckaert 2002), with both allozyme and microsatellite loci showing Isolation-by-Distance (IBD) pattern. The lack of temporal replication made it difficult to test the stability of genetic structure over time in these studies. In this sense, Dannewitz et al. (2005) conducted the most extensive study to date on European eel, with a total of 41 sampling sites, 12 of which included inter-annual samples. Overall genetic differentiation among samples was low, but highly significant and comparable to earlier studies. Nevertheless, hierarchical analyses revealed no geographical or IBD pattern, with temporal genetic variation within sites clearly exceeding the geographical component.

In the present study, we examined temporal changes in a single European eel population at Den Oever (The Netherlands) using 10 allozyme and 6 microsatellite loci. We quantified temporal genetic variation over 2 time scalesamong glass eel (1) inter-annual samples (cohorts), and (2) intra-annual samples collected throughout the year in each cohort. The latter samples consisted of 'arrival waves' of glass eels. The aim of the study was to test whether small-scale temporal differentiation influences the genetic population structure of

European eel by partitioning the amount of genetic differentiation into an inter- and intra-annual component. We also tested whether the variance in reproductive success was due mostly to random drift, gene flow or selective processes.

MATERIALS AND METHODS

A total of 789 glass eels were collected at Den Oever (52° 56′ 20″ N, 05° 02′ 70″ E) in The Netherlands over the period 2001 to 2003. Samples consisted of 11 different arrival waves from 3 different cohorts: 2 arrival waves from 2001, 5 arrival waves from 2002 and 4 arrival waves from 2003. Table 1 details sampling date, sample size and morphometric characteristics of all samples, including length, weight and condition index.

All individuals were analyzed for allozyme variation using Cellulose Acetate Gel Electrophoresis (CAGE) (Harris & Hopkinson 1976, Richardson et al. 1986). Tissue extraction, electrophoresis and procedures for visualizing proteins, and buffer systems (Tris Glycine [TG] and Tris Malate [TM]) are described in Maes & Volckaert (2002). We examined 7 enzymatic systems: aspartate aminotransferase (*AAT-1**, *AAT-2**, *AAT-3**, EC 2.6.1.1, TM), alcohol dehydrogenase (*ADH**, EC 1.1.1.1, TG), glucose-6-phosphate isomerase (*GPI-1**, *GPI-2**, EC 5.3.1.9, TG), isocitrate dehydrogenase (*IDHP**, EC 1.1.1.42, TM), malate dehydrogenase (*MDH-2**, EC 1.1.1.37, TM), mannose-6-phosphate isomerase (*MPI**, EC 5.3.1.8, TG) and phosphoglucomutase (*PGM**, EC 5.4.2.2, TG). Genetic nomenclature followed the suggestions of Shaklee et al. (1990). Allele assignment was carried out comparing the relative distance with the most common allele (**100*).

The same individuals analyzed with allozymes were screened for microsatellite variation, except for those samples consisting of 100 individuals, in which a subsample of 60 individuals was used. DNA purification and PCR amplification are described in Pujolar et al. (2005). Genotypes were examined at 6 dinucleotide repeat microsatellite loci: *Aan 01*, *Aan 03*, *Aan 05* (Daemen et al. 2001); *Aro 063*, *Aro 095* and *Ang 151* (Wirth & Bernatchez 2001). PCR products were visualized on an automated sequencer (LICOR 4200), using a molecular ladder (Westburg) in order to quantify allele sizes. Fragment data were analyzed using Gene ImagIR version 4.03 (Scanalytics).

All individuals were measured for standard length (L, mm) and body weight (W, mg). Ricker's (1975) condition index factor [$CI = 1000(W/L^b)$] was calculated for each individual, where b is the slope from the log length-log weight regression for all samples. Differences in morphometric measures among inter- and intra-annual samples were tested by a nested ANOVA. Regression analyses (Spearman's correlation) were performed between morphometric measures and the NAOI (from www.cgd.ucar.edu/cas/jhurrell/indices.html) and the Den Oever glass eel recruitment abundance index (DOI) (Dekker 2004). The NAOI was lagged by 1 yr to relate spring glass eel recruitment in 1 yr to oceanic data pertaining to the preceding year.

Within-sample genetic variation was assessed by observed (H_o) and expected (H_e) heterozygosity per locus and level of polymorphism ($P_{0.95}$) using GENETIX version 4.05 (Belkhir et al. 2005) and allelic richness using FSTAT (Goudet 1995). A possible correlation between genetic variability (expected heterozygosity and allelic richness at allozyme and microsatellite loci) and the NAOI/DOI was tested using a regression analysis (Spearman's correlation). Deviations from Hardy-Weinberg equilibrium, linkage disequilibrium, and differences in allele and genotype frequencies among samples were tested using GENEPOP version 3.4 (Raymond & Rousset 1995). Partitioning of genetic differentiation into an inter- and intra-annual component was performed using a hierarchical locus-by-locus AMOVA with 10 000 permutations as implemented in ARLEQUIN version 2.001 (Schneider et al. 2000). Significance levels for multiple simultaneous comparisons were adjusted using the sequential Bonferroni technique (Rice 1989). Pairwise multilocus comparisons among samples were calculated by Cavalli-Sforza & Edwards' (1967) chord distance and a multivariate ordination was conducted by multidimensional scaling (MDS) analysis using STATISTICA version 6.0 (StatSoft).

Table 1. *Anguilla anguilla*. Summary of genetic samples including sampling date, number of individuals analyzed and mean length (L, mm), mean weight (W, mg) and mean condition (CI). Standard deviation in parentheses

Sample	Sampling date	N	L	W	CI
NL101	9 May 2001	100	69.85 (3.17)	223.00 (51.00)	0.65 (0.10)
NL102	25 May 2001	40	67.47 (2.87)	247.66 (39.95)	0.80 (0.10)
NL201	3 April 2002	100	73.30 (3.85)	310.24 (64.38)	0.78 (0.12)
NL202	10 April 2002	100	73.81 (3.59)	358.64 (71.13)	0.89 (0.14)
NL203	17 April 2002	100	72.46 (3.85)	313.46 (55.47)	0.82 (0.11)
NL204	1 May 2002	100	73.29 (4.13)	341.93 (67.36)	0.87 (0.12)
NL205	15 May 2002	40	72.90 (4.62)	346.45 (72.36)	0.89 (0.10)
NL301	2 April 2003	52	76.64 (3.91)	337.10 (65.00)	0.74 (0.08)
NL302	16 April 2003	54	75.56 (2.96)	317.06 (50.44)	0.73 (0.08)
NL303	30 April 2003	50	74.64 (3.68)	268.88 (51.42)	0.64 (0.08)
NL304	21 May 2003	53	75.15 (3.03)	278.36 (46.54)	0.65 (0.08)

Isolation by Time (IBT) was tested by correlating pairwise temporal distance (measured as the differences in days among samples) versus Cavalli-Sforza & Edwards' (1967) chord distance using a Mantel test (Mantel 1967) as implemented in GENETIX. Average relatedness of all individuals to each other (r_{xy}) (Queller & Goodnight 1989) was calculated within samples at 6 microsatellite loci using the program IDENTIX (Belkhir et al. 2002). The significance of the values of relatedness was tested using 1000 permutations of alleles among individuals to know the proportion of pair-wise relatedness values attributable to significant half-sib or full-sib relationships compared to random sharing of alleles. Regression analysis (Spearman's correlation) was performed between average relatedness and genetic variability estimators (expected heterozygosity and allelic richness at microsatellite loci) and the NAOI. All statistical analyses were performed in STATISTICA.

RESULTS

Morphometric data

Significant differences were found when comparing L and W of all samples of glass eel arrival waves in the 2001, 2002 and 2003 cohorts (Fig. 1). A nested ANOVA showed slightly larger differences in L among cohorts ($p < 0.001$) than among arrival waves within cohorts ($p = 0.001$). Highly significant differences were observed in W, both within and among cohorts ($p < 0.001$). The CI also showed significant differences within and among cohorts ($p < 0.001$). When comparing the 3 samples from 2002 and the 3 samples from 2003 with similar sampling dates, differences among cohorts exceeded differences within cohorts in L (among cohorts: $p < 0.001$; within cohorts $p = 0.034$) but not in W (among cohorts: $p = 0.018$; within cohorts: $p < 0.001$). All morphometric measures showed highly significant negative correlations with the NAOI (L: $r = -0.153$, $p < 0.001$; W: $r = -0.477$, $p < 0.001$; CI: $r = -0.530$, $p < 0.001$). The DOI showed a highly significant positive correlation with L ($r = 0.480$, $p < 0.001$) and W ($r = 0.237$, $p < 0.001$) but not with CI ($r = 0.008$, $p = 0.822$).

Allozyme data

The 7 enzymatic systems resolved were coded by 10 polymorphic loci. Overall tests for Hardy-Weinberg proportions with all polymorphic loci, and for linkage disequilibrium among all loci, showed no significant departures from expected values. Mean H_o and H_e per sample ranged from 0.123 (±0.171) to 0.166 (±0.173) and from 0.118 (±0.151) to 0.161 (±0.157), respectively. All loci were moderately polymorphic in all samples ($P_{0.95}$ = 0.4 to 0.6), and allele richness per sample ranged from 2.288 to 2.769. Differences in mean H_o and H_e, $P_{0.95}$ and allele richness among samples were not statistically significant. Regression analysis showed significant negative correlations between the NAOI and H_e ($r = -0.100$; $p = 0.005$) and allelic richness ($r = -0.388$; $p < 0.001$). The DOI was negatively correlated with H_e ($r = -0.320$; $p < 0.001$) but positively correlated with allelic richness ($r = 0.057$; $p = 0.156$).

Genetic differentiation based on the distribution of allele frequencies over all samples was low (F_{ST} = 0.0009) and not significant ($p = 0.094$). Hierarchical ANOVA showed that differences among samples within cohorts ($F_{SC} = 0.0006$) explained a larger proportion of the total genetic variation than differences among cohorts (F_{CT} = 0.0003), although values were not statistically significant. No correlation was ob-

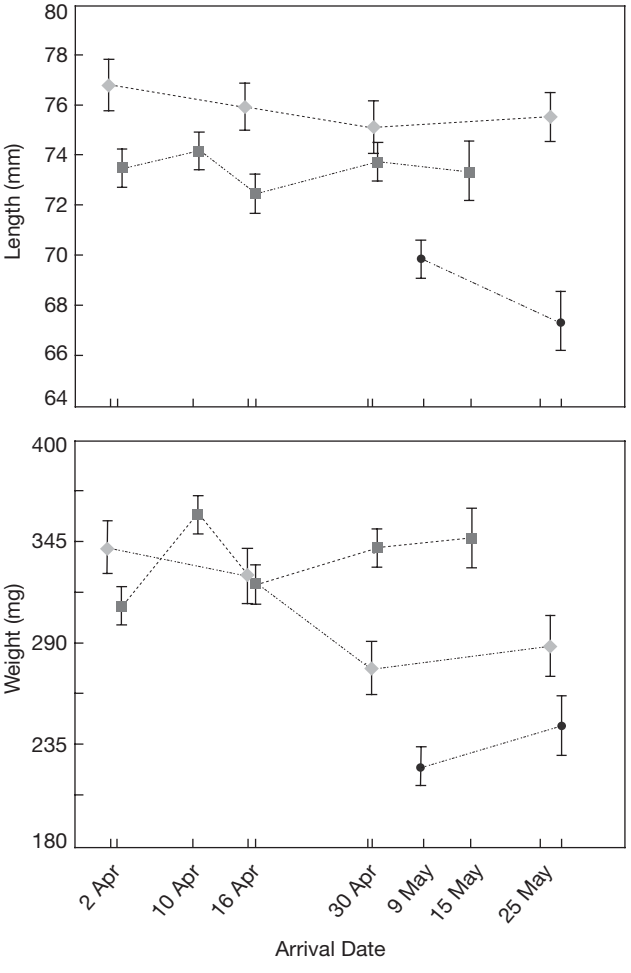

Fig. 1. *Anguilla anguilla*. Length and weight distribution for all samples in the 2001 (●), 2002 (■) and 2003 (◆) cohorts

served between temporal distance and Cavalli-Sforza & Edwards' (1967) chord distance (r = 0.238, p = 0.125). Within cohorts, a significant positive correlation between temporal and genetic distance was observed in the 2002 cohort (r = 0.636, p = 0.048), while a negative correlation was observed in the 2003 cohort (r = –0.163, p = 0.758). The absence of a consistent IBT pattern was supported by MDS (Fig. 2), which did not show any clustering of temporal samples within cohorts. When comparing the 2002 and 2003 samples with similar sampling dates (NL201 vs. NL301, NL203 vs. NL302 and NL204 vs. NL303), only samples NL201 and NL301 (corresponding to 2–3 April) clustered together.

Microsatellite data

Only 2 out of 66 (3.33%) tests for Hardy-Weinberg equilibrium revealed significant deviations at the 5% significant level after sequential Bonferroni correction

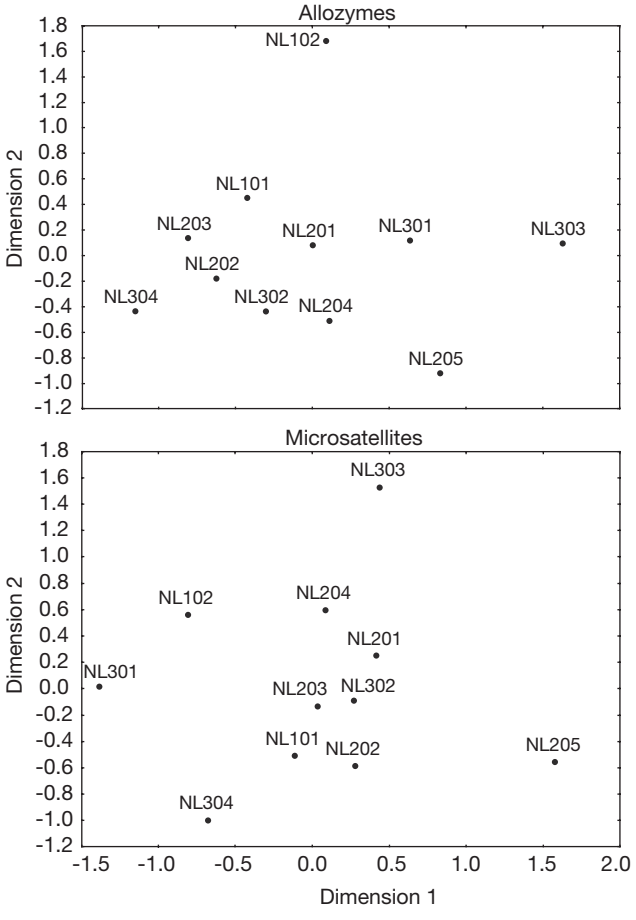

Fig. 2. *Anguilla anguilla*. Multidimensional scaling analysis of the matrix of pairwise Cavalli-Sforza & Edwards' (1967) chord distance based on 10 allozyme and 6 microsatellite loci. Sample abbreviations as in Table 1. Stress: allozymes = 0.120; microsatellites = 0.184

(k = 11). Disequilibria were attributed to a deficit of heterozygotes at locus Aro095 in samples NL301 and NL302. Dannewitz et al. (2005) suggested that locus Aro095 showed evidence for null alleles, but the authors proved that null alleles did not affect the results obtained after repeating all analyses with and without this locus. Overall, tests for linkage disequilibrium among all loci showed no significant departures from expected values. All 6 microsatellite loci were highly polymorphic in all samples. H_o and H_e ranged from 0.659 (±0.257) to 0.747 (±0.250) and from 0.702 (±0.314) to 0.747 (±0.265), respectively. Allele richness per sample ranged from 10.160 to 11.115. Values of genetic variability (heterozygosities, $P_{0.95}$ and allele richness) were not statistically significant across samples. Genetic variability was positively correlated with the NAOI (H_e: r = 0.226, p < 0.001; allelic richness: r = 0.053, p = 0.142) but negatively correlated with the DOI (H_e: r = –0.303, p < 0.001; allelic richness: r = –0.204, p < 0.001).

Overall, genetic differentiation among samples was highly significant (p < 0.001), although the overall multilocus F_{ST} value was low (F_{ST} = 0.0036). Genetic differentiation partitioned significantly among samples within cohorts (F_{SC} = 0.0033, p = 0.004), which was about 10 times larger than the differentiation among cohorts (F_{CT} = 0.0003, p = 0.603). Temporal distance showed no correlation with genetic distance (r = 0.081, p = 0.556). Within cohorts, temporal and genetic distance were positively correlated in 2002 (r = 0.435, p = 0.208) and negatively correlated in 2003 (r = –0.087, p = 0.870), although associations were not statistically significant. There was no evidence for temporal grouping based on MDS on all samples (Fig. 2), and samples from different cohorts clustered together. When comparing the 2002 and 2003 samples with similar sampling dates, only samples NL203 and NL302 (corresponding to 16–17 April) clustered together.

Average relatedness values over all individuals within each sample varied from –0.0468 to 0.0183 (Table 2). All samples showed low values of relatedness, although individuals with high values (r_{xy} > 0.5) were observed in all samples. Within samples, the values of genetic relatedness appeared significantly different from zero for the NL202 sample, for the 2002 cohort (pooling all 2002 samples) and for all pooled samples. The 2002 and 2003 cohorts showed a similar pattern, with maximum and mean relatedness decreasing throughout the season. A highly significant negative correlation was observed between temporal distance (difference in days among samples) and maximum relatedness in 2002 (r = –0.968, p = 0.006), which was not significant in 2003 (r = 0.590, p = 0.410). The association between temporal distance and mean relatedness was also negative but not significant (2002:

Table 2. *Anguilla anguilla*. Values of average relatedness (r_{xy}) within samples including maximum and minimum relatedness based on 6 microsatellite loci. Standard deviations in parentheses. $^{**}p < 0.01$, $^{***}p < 0.001$

Sample	r_{xy}	Max. r_{xy}	Min. r_{xy}
NL101	−0.0395 (0.231)	0.950	−0.522
NL102	0.0183 (0.232)	0.905	−0.448
NL201	0.0181 (0.241)	0.944	−0.593
NL202	−0.0119 (0.238)**	1.000	−0.487
NL203	−0.0133 (0.218)	0.883	−0.448
NL204	−0.0251 (0.244)	0.720	−0.798
NL205	−0.0266 (0.231)	0.590	−0.443
NL301	0.0046 (0.242)	1.000	−0.798
NL302	−0.0308 (0.221)	0.619	−0.428
NL303	−0.0032 (0.274)	0.845	−0.887
NL304	−0.0468 (0.210)	0.652	−0.421
2001 cohort	−0.0184 (0.230)	0.954	−0.548
2002 cohort	−0.0232 (0.237)***	1.000	−0.892
2003 cohort	−0.0253 (0.242)	1.000	−0.915
All cohorts	−0.0240 (0.239)***	1.000	−0.915

r = −0.442, p = 0.457; 2003: r = −0.720, p = 0.280). A highly significant negative correlation was observed between average relatedness and H_e (r = −0.756; p < 0.001), which was negative but not significant between average relatedness and allelic richness (r = −0.005; p = 0.888). Genetic relatedness was negatively correlated with the NAOI (r = −0.296; p < 0.001) and positively correlated with the DOI (r = 0.027; p = 0.453).

DISCUSSION

Evidence for genetic patchiness among recruits

Temporal genetic variation in European eel was quantified by integrating 2 time scales: (1) among cohorts sampled over 3 consecutive yr, and (2) among arrival waves within cohorts. The fundamental result is the highly significant genetic differentiation at microsatellite loci among arrival waves, despite the low F_{ST} values. Heterogeneity in genetic composition was observed both among cohorts and among samples within cohorts. Nevertheless, genetic differentiation partitioned within cohorts was over 10-fold the differences among cohorts. Hierarchical F_{ST} and MDS described a pattern of genetic patchiness among temporal samples, in which samples did not cluster either by cohort origin or arrival time within the year. In comparison with microsatellites, allozymes showed no significant genetic differentation, although differences within cohorts were higher than differences among cohorts. It might be that allozyme variation is too low for allozymes to detect genetic differentiation.

Many studies attribute genetic patchiness on a small spatial scale to the annual recruitment of genetically variable cohorts within a site (reviewed in Planes & Lenfant 2002). Genetic patchiness among annual arrival waves mostly originates from a large variance in parental contribution. Due to the unpredictability of the oceanic environment, reproduction by marine species may be viewed as a sweepstakes event, in which not all but only a fraction of the adult population actually contributes to the next generation (Hedgecock 1994). The extent of how many and which individuals are successful is determined by chance. Hedgecock (1994) proposed that genetic patchiness reflects the family structure of the larval pools. In our study, average genetic relatedness showed a similar pattern in all samples, which consisted of individuals with high values of relatedness mixed with a large group of non-related individuals. The value of genetic relatedness for the 2002 cohort and for the total samples appeared significantly different from zero, suggesting that at least some individuals within or among arrival waves are related, even if the values of relatedness observed are far from the 0.5 value expected for full siblings. As a consequence of the catadromous life-history of the European eel, larval mixing may occur either after spawning in the Sargasso Sea or during the extensive larval migration to the European continental shelf, which explains the low mean relatedness observed for most samples. Maximum and mean relatedness decreased throughout the season in both the 2002 and 2003 cohorts, suggesting that arrival waves occurring late in the season are more mixed up and include a larger proportion of individuals from other spawning events. Mixing of individuals might be accentuated in the case of glass eel arrivals in northern Europe. Boëtius & Boëtius (1989) showed that eel larvae metamorphosing in northern Europe do not ascend immediately up rivers but are forced to stay offshore, where they are supposed to starve. In The Netherlands, this period has been estimated to last about 3 mo (Desaunay & Guérault 1997), which could increase the mixing of individuals before immigration into estuarine waters.

Oceanic influence on recruitment

Hedgecock (1994) attributed the large variance in reproductive success of adults to spatio-temporal variation in oceanic conditions, occurring within and among seasons. Climate and oceanic processes influence a range of ecological processes including larval survival, which depends on primary production, zooplankton bloom and current patterns at the oceanic scale (Hurrell et al. 2003). Interannual variation in larval survival can be explained by the match between

phytoplankton production and spawning timing. The strength of the match determines the quality of food available for larvae, and in turn the number of individuals that survive in a year-class (match-mismatch hypothesis; Cushing 1990). Alternatively, physical larval retention rather than production may explain recruitment variation as argued for the member-vagrant hypothesis (Sinclair 1988).

The NAO is a large-scale environmental fluctuation which has been shown to affect terrestrial and marine ecosystems (Stenseth et al. 2002). During a positive NAOI period, less plankton is available due to higher-than-average temperatures and a delay in primary production the following spring, which in turn results in a lower food availability for larvae (Hurrel et al. 2003). A positive NAOI period is also characterized by stronger oceanic winds and currents and an increase in the amount of storms (Stenseth et al. 2002), which have the potential to displace larvae from favorable environments or retention zones. Following a positive NAOI period, a higher larval mortality would be expected due to either poor feeding conditions or changes in water circulation, which in turn would cause a lower recruitment. In marine ecosystems, the NAO has been linked to fluctuations in recruitment abundance for many species including the short-finned squid *Illex illecebrosus* in the northwest Atlantic Ocean and several fish stocks (cod *Gadus morhua*, herring *Clupea harengus*, capelin *Mallotus villosus* and sardine *Sardina pilchardus*) in the Barents Sea (Alheit & Hagen 1997, Dawe et al. 2000, Ottersen et al. 2001, Hjermann et al. 2004). Similarly, Knights (2003) reviewed a significant negative correlation between the NAOI and the DOI. In the period 1960 to 1990, the length and number of glass eels immigrating to Den Oever followed a synchronized pattern, triggering considerable speculation that the decline of the European eel stocks was caused by oceanic climate. Nevertheless, after an all-time low in 1991 for abundance and length, both the NAOI and length recovered to average values, while abundance dropped to a new all-time low in 2001 (Dekker 2004). The latest recruitment information for the period 2002 to 2003 shows a return to the former pattern, with a decrease in the NAOI associated with a slight increase in both length and abundance (Dekker 2004). This is congruent with the significant correlations observed in our study among all size estimators (L, W and CI) and the DOI and NAOI.

The 2001 cohort (associated with the lowest DOI/highest NAOI) showed the lowest genetic variability at allozymes and the largest variance in genetic variability at both allozyme and microsatellite loci. Similarly, the 2001 cohort showed the largest variance in genetic relatedness, so that sample NL101 presented the overall lowest relatedness of all samples, while sample NL102 presented the overall highest relatedness. It can be argued that a higher mortality in the larval phase following unfavorable oceanic conditions (after spawning in the Sargasso Sea or in the migration across the Atlantic Ocean) may cause a large variance in the number of individuals constituting each larval wave, which in turn could lead to a large variance in genetic variability (depending on the larval wave size) and genetic relatedness (depending on the proportion of high- and low-related individuals eliminated). At the moment, although highly speculative, the DOI/NAOI link points to a direct (larval retention) or indirect (food availability) oceanic contribution to the genetic heterogeneity observed among recruits. Further validation is needed in the coming years to confirm the synchronicity of ocean climate, feeding conditions and recruitment abundance.

Alternative hypothesis

As pointed out in previous studies, together with a high variance in reproductive success, natural selection and gene flow might account for the genetic heterogeneity among recruits. Most likely, all 3 processes might play a role. In the case of natural selection, different selective histories of the larval pools might explain differences in the genetic composition of recruits (David et al. 1997). In the case of eel larvae, the larval pool could be completely mixed and genetically homogeneous in the Sargasso Sea, and then spatially or temporally diverse selective forces could result in genetic differences among recruits. Under selection, genetic composition mostly shows clines in allele frequencies that parallel environmental gradients. This has been shown in the fruit fly *Drosophila melanogaster*, where latitudinal clines in allozyme frequencies at ADH and G6PDH provided evidence for temperature-derived selection (Oakeshott et al. 1982, 1983). In the blue mussel *Mytilus edulis*, an allele frequency cline at the LAP locus was observed between high and low salinity locations (Koehn et al. 1980). In fish, LDH in mummichog *Fundulus heteroclitus* showed a steep latitudinal cline along the Atlantic coast that appeared to result from natural selection and historical subdivision (Bernardi et al. 1993). Genetic polymorphism may also show homogeneity of allelic frequencies attributable to balancing selection, as suggested in oysters (Karl & Avise 1992) and Atlantic cod *Gadus morhua* (Pogson et al. 1995). Thus, the temporal genetic patchiness observed in a single European eel population, with recruit composition varying over time, seems to suggest a lack of consistent selection.

Gene flow between genetically differentiated populations could also produce temporal genetic changes

among recruits. Kordos & Burton (1993) reported a high genetic variation among annual blue crab *Callinectes sapidus* recruitments from the Texas coast. The observed homogeneity among winter samples contrasted with a marked differentiation among summer samples from the same locations, which was explained in part from changes in larval-source populations caused by seasonal changes in oceanographic conditions. Flowers et al. (2002) argued that distinguishing between genetic patchiness due to gene flow among populations, or genetic drift within populations, is often impossible because the 2 processes occur simultaneously in most marine species' planktonic larval dispersal. The gene flow hypothesis implies the existence of different source populations, which contradicts field observations of a single reproductive area for European eel (Tesch 2003).

Many populations are composed of a mixture of individuals that reproduce at different times, which may often be heritable (Hendry & Day 2005). Temporal assortative mating may limit gene flow between early and late spawners, producing an IBT pattern. In our study, no significant correlation was observed by plotting pairwise genetic distance values against temporal distance measured as difference in days between samples, suggesting that differentiation among arrival waves was not attributable to an IBT pattern. Similarly, no IBT pattern was observed within cohorts, although an intra-annual IBT pattern would have been difficult to detect since the maximum difference between early and late arrival waves within a season was 2 mo. Failure to observe an IBT pattern does not preclude its existence on a broader geographical scale after correcting for spatial variation.

CONCLUSIONS

In summary, the pattern of temporal genetic patchiness observed among arrival waves in a population of European eel suggests that each arrival wave represents an isolated reproductive event involving a restricted number of adults. Although natural selection and gene flow might also play a role, we suggest that large variance in reproductive success is a contributing factor to recruit differentiation. Our results corroborate previous observations on the mediating influence of oceanic conditions on eel population dynamics. A direct implication of our results is that a large variance in reproductive success can limit effective population numbers to fractions of actual abundances. If only a subset of the adults contribute to spawning, the effective population size in European eel should be considerably lower than the census size. Using a Bayesian approach to infer demographic parameters from microsatellite data, Wirth & Bernatchez (2003) suggested a contemporary effective population size of about 5×10^3 to 10^4 eels. Together with oceanic factors, a low effective population size might have contributed to the current decline in the abundance of European eel. Fisheries management should integrate long-term monitoring of eel recruitment and adult catches with oceanic-climate studies. Replication of the current study on a broader geographical scale would allow us to shape the pattern of population structure reported. This example of genetic heterogeneity in European eel glass eels highlights the importance of studying early-life stages as a basis for understanding patterns of genetic variation in adults.

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