Genetic patchiness in European eel adults evidenced by molecular genetics and population dynamics modelling

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ABSTRACT

Disentangling the demographic processes that determine the genetic structure of a given species is a fundamental question in conservation and management. In the present study, the population structure of the European eel was examined with a multidisciplinary approach combining the fields of molecular genetics and population dynamics modelling. First, we analyzed a total of 346 adult specimens of known age collected in three separate sample sites using a large panel of 22 EST-linked microsatellite loci. Second, we developed a European eel-specific model to unravel the demographic mechanisms that can produce the level of genetic differentiation estimated by molecular markers. This is the first study that reveals a pattern of genetic patchiness in maturing adults of the European eel. A highly significant genetic differentiation was observed among samples that did not follow an Isolation-by-Distance or Isolation-by-Time pattern. The observation of genetic patchiness in adults is likely to result from a limited parental contribution to each spawning event as suggested by our modelling approach. The value of genetic differentiation found is predicted by the model when reproduction occurs in a limited number of spawning events isolated from each other in time or space, with an average of 130–375 breeders in each spawning event. Unpredictability in spawning success may have important consequences for the life-history evolution of the European eel, including a bet-hedging strategy (distributing reproductive efforts over time) which could in turn guarantee successful reproduction of some adults.

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1. Introduction

One major paradox concerning the population genetics of marine organisms is the observation of small but statistically significant genetic differences among local populations despite high rates of gene flow (Burton, 1983; Hedgecock, 1994). While the apparent absence of real barriers for fish/larvae displacement in marine environments could lead to a depression of the extent of genetic differentiation between subpopulations (Ward et al., 1994), several studies have detected significant heterogeneity among recruits on a small spatial scale in marine species dispersing via pelagic larvae, including gastropods (Johnson and Black, 1982), bivalves (Hedgecock, 1994; David et al., 1997; Li and Hedgecock, 1998), echinoderms (Edmands et al., 1996; Moberg and Burton, 2000; Flowers et al., 2002) and fish (Ruzzante et al., 1996; Planes and Lenfant, 2002; Pujolar et al., 2006, 2007). Hedgecock (1994) proposed that such genetic heterogeneity is likely to result from a large variance in reproductive success of

parents. Under the sweepstakes hypothesis, chance events determine which adults are successful in each spawning event. The random variation in parental contribution to the next generation leads to unpatterned variation in genetic composition of recruits (genetic patchiness). Many individuals fail to contribute to recruitment, and in each generation the entire population is replaced by a small fraction of individuals by a sweepstakes-chance matching of reproduction activity with oceanic conditions. Reproduction of marine organisms is mediated by spatio-temporal variation in oceanographic processes that affect spawning, fertilization, larval survival and successful recruitment. Despite high fecundities, marine organisms show great variability in gamete quantity and quality (Levitan, 2008), suggesting that the potential for variance in reproductive success exists prior to fertilization.

The dependence of recruitment on oceanic conditions holds particularly true for the European eel (Anguilla anguilla), a catadromous fish species with a complex life cycle that includes two migrations across the North Atlantic, one feeding migration at the beginning of its life and a spawning migration at the end (Van den Thillart et al., 2009). After spawning in the Sargasso Sea, larvae (leptocephali) cross the Atlantic Ocean following the

Gulf Stream and North Atlantic Drift Current and metamorphose into glass eels upon reaching the edge of the continental shelf off Europe and North Africa (Kettle and Haines, 2006). Arrival of glass eels occurs in pulses or groups known as arrival waves (Boëtius and Boëtius, 1989). Glass eels complete the migration into continental growth habitats as yellow eels, and after an highly variable feeding period of 2–20 years depending on sex and geographic latitude (Vøllestad, 1992), they metamorphose into silver eels that migrate back to the Sargasso Sea, reproduce once and die (Van den Thillart et al., 2009).

The stock of the European eel has shown a decline over the last decades and is considered to be outside safe biological limits (ICES, 2008). Recruitment has shown a decline of 90–99% in different areas since the 1970s (Dekker, 2003). Most data sets also show a decline in fishing yield and landings of yellow and silver eels (Dekker, 2003), with catches reported to FAO showing a gradual drop to about 25% of the level of the mid 1960s (ICES, 2008). The crash seems to have affected in a similar fashion European, American and Japanese eel, with no single apparent cause (Dekker et al., 2003). Several hypotheses have been put forward concerning the causes of the eel stock decline, including human and natural causes operating during the different stages of the life cycle. On the one hand, there are several anthropogenic factors mainly affecting eels on their continental phase, such as overfishing, migration barriers (dams and hydroelectric power plants), habitat loss, pollution (PCBs and heavy metals) and human-introduced diseases (EVEX virus) and parasites (the swimbladder nematode *Anguillicoloides crassus*) (Van den Thillart et al., 2009). On the other hand, climatic and oceanic events might contribute to the decline as they influence silver eel migration, mating and spawning success of adults in the Sargasso Sea, egg and larvae development, and larval survival during migration to the continental feeding grounds (Knights, 2003; Friedland et al., 2007; Bonhommeau et al., 2008; Miller, 2009; Miller et al., 2009; Martin et al., 2010).

A question of fundamental importance to conservation and management is whether the European eel consists of a single panmictic population. While the existence of one single spawning ground in the Sargasso Sea could lead to individual mixing and homogenization of the gene pool, genetic differences between populations could arise if temporal or spatial segregation occurs between spawning groups followed by non-random larval dispersal (Kettle and Haines, 2006). In this sense, panmixia in the European eel is widely accepted, following the study of Dannewitz et al. (2005) that proved previously observed Isolation-by-Distance (IBD) patterns to be unstable over time when temporal replicates of the geographical sampling were included in the analysis. Recent studies focusing on the genetic composition of glass eels have observed a subtle pattern of genetic patchiness among intra-annual samples within cohorts (arrival waves) (Pujolar et al., 2006, 2007, 2009a). Panmixia on a broader scale and genetic patchiness on a local scale are not contradictory but possibly the consequence of the long migration loop of the European eel notably affected by fluctuating oceanic conditions (Pujolar et al., 2009a).

Previous genetic studies on eels present many limitations. First, the low number of genetic markers used, with all recent studies being based on 6–7 microsatellite loci (Wirth and Bernatchez, 2001; Dannewitz et al., 2005; Maes et al., 2006; Pujolar et al., 2006, 2007). Second, neutral loci (microsatellites) might fail to detect differences at loci affected by selection, which could lead to underestimating adaptive variation between populations (Conover et al., 2006). Third, previous genetic studies have focused entirely on glass eel samples, which might not be an adequate representation of potential spawning stocks because eels were sampled after completion of their trans-Atlantic migration and before commencing their continental growth stage. The only exception in the use of adults is the recent study of Palm et al. (2009), which had the

caveat of using only six microsatellite loci. Despite large sample sizes, no signs of genetic differentiation were observed, with an average $F_{ST} = -0.00003$ ($p = 0.61$). To correct for the bias caused by a limited number of neutral markers, in the present study we used a total of 22 EST-derived microsatellite loci, which were previously used to successfully screen for genetic variation in glass eel arrival waves (Pujolar et al., 2009a). Expressed Sequence Tags or ESTs are single-read sequences produced from partial sequencing of a mRNA pool and have the advantage of being putatively under the influence of selection, either directly or indirectly through genetic linkage, because of their location in gene regions. Ecologically important traits are likely to have been shaped by natural selection, which means that examining patterns of molecular evolution in EST-linked microsatellites could provide a way of screening numerous genetic loci for signatures of adaptive evolution. In this sense, the recent study of Gagnaire et al. (2009) suggested that spatial distribution of allele frequencies displayed by some AFLP loci in *A. anguilla* and *Anguilla rostrata* hybrids in Iceland were possibly shaped by natural selection associated with ecological conditions. To correct for the dominance of glass eels samples in previous studies, our analysis focused entirely on adult eels of known age. We expect that by analyzing adults only, our sampling will be a better representation of the spawning stock.

Finally, understanding of the genetic structure of the European eel can greatly benefit from the development of integrated genetic/demographic models that take into consideration the complex life cycle of this catadromous species. Thus far, modelling studies on eel population dynamics have focused on quantifying the recruitment-stock relationship, thus providing new knowledge on factors affecting the continental phase (e.g. overfishing, migration barriers) that can impair spawner production at local level (Vøllestad and Jonsson, 1988; De Leo and Gatto, 1995; Dekker, 2000a; Feunteun, 2002; Bevacqua et al., 2007). In this sense, modelling studies on eel have always neglected the oceanic phase, including reproduction and an overall stock–recruitment relationship. The only exception is the model of Åström and Dekker (2007), which was aimed at estimating potential recovery time for the overall eel stock and thus disregarded the estimation of key aspects of eel demography (e.g. effective number of breeders) and omitted any evolutionary or genetic component.

The goal of this study is to bring together molecular genetic and population dynamics in order to gather new knowledge on the role of demographic processes that determine the genetic structure of the European eel. First, we analyzed the population structure of the European eel using adult specimens of known age that will allow estimating genetic differentiation among samples. Second, we developed a European eel-specific model to unravel the demographic mechanisms that can produce the level of genetic differentiation estimated by molecular markers. The demographic model considers both continental and oceanic phases of the eel life cycle and encompasses particular life history features of the European eel such as sexual dimorphism, sexual maturation, body growth, larval and adult migration, glass eel and adult survival, and reproduction. The information obtained by molecular markers and a genetic–demographic model will ultimately allow setting up an integrated conservation framework that takes into account contemporary evolution.

2. Material and methods

2.1. Sampling collection

A total of 1234 European eel *A. anguilla* individuals were caught using fyke nets in three separate geographic sites in the Mediterranean Sea (Fig. 1): (1) the low course of the Tiber river (41°48′N;